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PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/);

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Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English evaluation) towards the study should appear at the end of the article.

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Editorial

Dear JPR Readers,

We are proud and happy to announce the third issue of "The Journal of Pediatric Research" in 2020 has been published. Troubled days are experienced in the world due to crucial coronavirus pandemic that is still going on. Since the beginning of the pandemic, science has been at the heart of all efforts to save lives. Almost every day there is more news about research into vaccines, and therapeutics. Once again, we have understood that doing scientific research have great importance in our lives.

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In this issue, we present you 14 articles including 12 original researches, one meta-analysis study and one case report from different disciplines. In this issue the readers will find the opportunity to update their knowledges about the effect of fatigue-reducing interventions on fatigue levels of children with cancer with a meta-analysis study.

In one of the article in this issue is about the correlation of faecal calprotectin (FC) levels with endoscopic and histopathological findings in 112 children who underwent upper gastrointestinal system (UGIS) endoscopy. Although a positive correlation was detected between eosinophil counts and FC levels in patients with Helicobacter pylori gastritis and esophagitis. The authors found that diagnostic value of FC levels in UGIS diseases was not sufficient to establish a definitive diagnosis in their study.

Body esteem refers to self-evaluations of one's body or appearance. We would like to recommend our readers to spend time for the association of parents' body esteem and body mass index (BMI) with children's body esteem and BMI. Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed mental disorder of children. This issue contains related articles about this topic. Also this issue includes some different topics such as the differential diagnosis between vesicoureteral reflux and urinary tract infection in children, brain death in paediatric critical care, pediatric appendicitis score in acute appendicitis in children.

We would like to acknowledge the authors, the reviewers, editorial team and Galenos Publishing House for their support in the preparation of this issue. We look forward to your scientific contributions in our future issues.

Best wishes,

Ebru Canda

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Original Article

Autoantibody Positivity in Children with Chronic Diarrhea

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ABSTRACT

Aim: We aimed to determine the frequency of autoantibody antinuclear (ANA), peripheral anti-neutrophil cytoplasmic antibody (p-ANCA), anti-saccharomyces cerevisiae antibody (ASCA), anti-pancreatic exocrine gland antibody (PAb), goblet cell antibody (GAb) positivities in children with the complaint of chronic diarrhea and inflammatory bowel disease (IBD). We also purposed to explore the role of these autoantibodies in the differential diagnosis of IBD.

Materials and Methods: In our study, serum samples of 51 patients with the complaint of chronic diarrhea and 35 healthy controls were analyzed. Clinical and laboratory data at the time of serum sampling were collected and a differential diagnosis was made as the results of performed tests were recorded. For all patients, ANA, p-ANCA, ASCA, GAb, PAb positivities were evaluated by indirect immunofluorescence. The chronic diarrhea group was divided into two groups, namely, the IBD group and non-IBD group.

Results: In the chronic diarrhea group, 11 (21.6%) patients had ANA, 3 (5.9%) had p-ANCA, 1 (2%) had PAb, 1 (2%) had Gab and 1 (2%) had ASCA positivity. From the 35 cases of the control group, 8 (22.9%) had ANA, 7 (20%) had ASCA positivity. In the control group, ASCA was found to be high (p=0.007). Six cases were diagnosed as IBD; 1 (16.7%) had ANA, 1 (16.7%) had p-ANCA, 1 (2%) had Gab and 1 (2%) had ASCA positivity. ASCA and GAb positivities were significantly more frequent in the IBD group (p=0.006, p=0.006, respectively).

Conclusion: ASCA was determined to be significantly higher in the control group. High positivity in the control group showed that the percentage of nonspecific positivity may be high for this test. ASCA and GAb of those patients with a diagnosis of IBD were found significantly higher. The serologic tests which depend on p-ANCA, ASCA, PAb, GAb can be supportive of diagnoses and differential diagnoses of IBD. Autoantibodies in IBD may be used as a supportive diagnostic tool in selected cases, rather than as the diagnosis of IBD as routine practice.

Keywords: Chronic diarrhea, inflammatory bowel disease, autoantibody, PAb, GAb

Introduction

Diarrhea is one of the most important causes of mortality and morbidity in children all over the world, particularly in developing countries. For children, diarrhea can be defined as an immediate increase in their usual frequency of defecation and a decrease in their usual stool consistency (1). Diarrhea lasting more than four weeks is defined as chronic diarrhea (1).

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Inflammatory bowel diseases (IBD), one of the chronic inflammatory diseases which can involve various areas and layers of the gastrointestinal tract, manifests with remissions and exacerbations. Its exact causative mechanisms are still obscure. Crohn's disease (CD) and ulcerative colitis (UC) are the most common subtypes of IBD. A complete classification is still not possible in 10-15% of cases despite the exclusion of other gastrointestinal diseases by endoscopic, radiological and histopathological examinations, laboratory tests, and patient and family history in the differential diagnosis of UC and CD (2). Although the differential diagnosis of UC or CD in the medical treatment of IBD is not completely crucial, these two diseases differ significantly in terms of prognosis and complications. The role of antibodies against intestinal goblet cells in IBD pathogenesis is still unclear (2). Anti-pancreatic antibodies (PAb), perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA), anti-saccharomyces cerevisiae antibody (ASCA), intestinal goblet cell antibody (GAb), antibodies against extracted nuclear antigens, or antinuclear antibodies are seen not only in CD or UC patients but also in healthy first-degree close relatives of these patients (2,3). Acute phase reactants frequently used for the diagnosis and monitoring of intestinal inflammation have a weak association with intestinal disease activity. The correlation of IBD with p-ANCA or ASCA has been demonstrated in various studies (4-9). Therefore, serological markers have become important in the diagnosis and follow-up of IBD (2).

In our study, the levels of the antinuclear antibody (ANA), serum p-ANCA, ASCA, GAb PAb were examined in order to investigate a possible increase in their frequency compared to a control group and chronic diarrhea patients without IBD.

Materials and Methods

A total of 51 patients with the diagnosis of chronic diarrhea aged between 6 months and 18 years who presented at our clinic between 2009 and 2011, and 35 healthy subjects similarly aged between 6 months and 18 years were enrolled in the study. Previous medical history reviews and physical examinations of the patients were performed after informed consent was obtained from the families during outpatient clinic visits. The presence of consanguinity between parents, duration of breastfeeding, duration of diarrhea, and accompanying symptoms such as abdominal pain, tenesmus, weight loss, fever, bleeding, and aphthous stomatitis were questioned in the medical history of the patients. Patients with underlying chronic diseases other than diarrhea were excluded from the study. Cases with chronic diarrhea were also evaluated as either IBD or non-IBD.

Information on complete blood count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal occult blood (FOB), fecal parasite and stool culture were obtained from each patient included in the study. For hemoglobin (Hb), ALT, AST, CRP and ESR, the lower and upper limit values of the measurement method of the biochemistry laboratory were considered as the standard reference range. Abdominal ultrasonography, endoscopy and colonoscopy were performed and biopsy samples were collected and evaluated for eligible cases.

ANA, GAb, PAb, ASCA and p-ANCA were studied using the immunofluorescence (IF) Titerplane technique in the pediatric immunology laboratory for both the patients and the controls. IF assays for ANA were performed using CIBD profile kits (Euroimmun AG, Lubeck, Germany). A serum dilution of 1:100 was made for ANA for the test. For the CIBD profile tests, as substrates, primate intestinal tissues were used to assess intestinal goblet cells, while primate pancreatic tissue, Saccharomyces cerevisiae and primate liver cells were used to assess acinar cells. For the CIBD profile tests, serum dilutions of 1:10 and 1:100 for IgA and serum dilutions of 1:10 and 1:1000 for IgG were used. In the study phase, titers were determined by performing a series of dilutions (1:160, 1:320 and 1:640) when the IF ANA test results were positive. Titers above 1:100 were considered significant.

The study was started after the Ethics Committee approval numbered 09-9/12 dated 10/23/2009 from the Ege University Faculty of Medicine Clinical Trials Local Ethical Committee was obtained.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). All data were given as mean ± standard deviation and median values [interquartile range (IQR)]. The distribution of the data was assessed using the Kolmogorov-Smirnov test. Student t-test and Mann-Whitney U test were used for the comparisons of the data with and without normal distribution, respectively. A chi-square test was used to compare the group's ratios. Pearson correlation analysis was used to determine the relationships between variables. An alpha error level below 0.05 was considered as statistically significant.

Results

Fifty-one cases with the complaint of chronic diarrhea and 35 healthy subjects were included in the study. Of the patients with chronic diarrhea, 30 (58.8%) were male and 21 (41.2%) were female. The mean age of the patients was 60.9±59.8 [median: 52 (87)] months. Twenty-two (62.9%) of the patients in the control group were male and 13 (37.1%) were female. The mean age of the control subjects was 65.2±52.8 [median: 52 (87)] months (Table I).

Of the 35 subjects in the control group, ANA positivity in eight (22.9%) subjects and ASCA positivity in seven (20%) subjects were determined. No p-ANCA, GAb, PAb positivity was detected. Of the 51 patients presenting with chronic diarrhea, 11 (21.6%) were determined to have ANA positivity, 3 (5.9%) had p-ANCA positivity, 1 (2%) had PAb positivity and 1 (2%) had ASCA positivity (Table I). In the ANA typing of the patients presenting with chronic diarrhea, one fine speckled (granular), one coarse granular, four cytoplasmic and two nucleolar ANA positivities were determined. Of the 11 patients with ANA positivity, 1/100 titer was determined in nine patients and 1/160 titer was determined in two patients.

There was no statistically significant difference between chronic diarrhea and control groups in terms of ANA, p-ANCA, PAb and GAb positivity (p=0.545, p=0.203, p=0.593, p=0.593 respectively) (Table I). ASCA positivity was determined to be significantly higher in the control group when compared with those patients with the complaint of chronic diarrhea (p=0.007) (Table I).

Those patients presenting with chronic diarrhea had an average diarrhea duration of 12.62±23.09 [median: 6 (10)] months. Sixteen (31.4%) patients had consanguineous parents. The clinical and laboratory characteristics of these cases are summarized in Table II.

Endoscopy was performed in 20 (39.2%) of the 51 patients with chronic diarrhea. Endoscopic biopsy findings revealed that five (25%) of 19 cases had IBD, nine (45%) had esophagitis-gastritis-duodenitis and three (15%) had non-specific findings, three (15%) had normal endoscopic findings. Six (11.7%) (4 UC, 2 CD) of the patients presenting with the complaint of diarrhea were diagnosed with IBD. The diagnosis of IBD was based on the first endoscopic biopsy findings in five of these cases and the clinical and laboratory findings obtained at follow-up in one of these cases. Diagnosis distributions other than IBD were recorded as follows: Nine (17.6%) cases with esophagitis-gastritis-duodenitis, four (7.8%) with infection, two (3.9%) with lactose intolerance, three with (5.8%) IgA deficiency, one (1.9%) with hypogammaglobulinemia, one (1.9%) with abetalipoproteinemia, one (1.9%) with cow's milk allergy, one (1.9%) with non-specific colitis, one (1.9%) with lipid malabsorption, one (1.9%) with colitis secondary to congenital cytomegalovirus infection and one (1.9%) with polyp.

The mean age of the patients diagnosed with IBD was 138.1±63 months [median: 145 (104) months] and the mean duration of diarrhea was 7.1±9.1 months [median: 2.5 (8) months]. Four (66.7%) of the six patients diagnosed with IBD were female, and two (33.3%) were male. Of

Table I. Comparison of the demographic and laboratory findings of chronic diarrhea and control groups

	Chronic diarrhea (n=51)	Control (n=35)	p
Age (months)	29 (87)	52 (87)	0.570*
Gender (M/F)	30/21	22/13	0.707**
ANA	11 (21.6%)	8 (22.9%)	0.545**
pANCA	3 (5.9%)	0	0.203**
ASCA	1 (2%)	7 (20%)	**0.007****
PAb	1 (2%)	0	0.593**
GAb	1 (2%)	0	0.593**

*Mann-Whitney U test, **chi-square test. The data were given as median (IQR) values, M: Male, F: Female, ANA: Antinuclear antibody, pANCA: Serum perinuclear anti-neutrophil cytoplasmic antibody, ASCA: Anti-saccharomyces cerevisiae antibody, PAb: Anti-pancreatic antibodies, GAb: Intestinal goblet cell antibody

Table II. Clinical and laboratory characteristics of the cases diagnosed with chronic diarrhea

n=51	n (%)
Abdominal pain	30 (58.8)
Weight loss	24 (47.1)
Growth retardation	22 (43.1)
Fever	19 (37.3)
Bleeding	11 (21.6)
Recurrent aphtha	4 (7.8)
Tenesmus	3 (5.9)
Anemia	19 (31.3)
FOB positivity	9 (17.6)
Parasite in the stool	2 (3.9)
Positive stool culture	1 (2)
CRP positivity	10 (19.6)
Elevated ESR	12 (23.5)

FOB: Fecal occult blood, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, n: Number

these six patients, all had abdominal pain (100%) and weight loss (100%), four had a fever (66.7%), and growth retardation (66.7%), and one had tenesmus (16.7%). These six patients with IBD had no recurrent aphthous stomatitis. Of the patients presenting with chronic diarrhea, the ones diagnosed with IBD had significantly higher rates of abdominal pain, weight loss and bleeding (p=0.036, p=0.007, p<0.001 respectively). There was no significant relationship between IBD and fever, growth retardation or tenesmus (p>0.05). The other demographic, clinical and laboratory findings of the 51 patients with chronic diarrhea according to the diagnosis of either IBD or non-IBD are presented in detail in Table III.

Table III. Comparison of demographic, laboratory and clinical data of the cases with inflammatory bowel disease and the other chronic diarrhea

	IBD (n=6)	Other (n=45)	p
Gender (M/F)	2/4 (33.3%/66.7%)	17/28 (37.7%/62.3%)	0.177**
Age (months)	145 (104)	22 (68)	**0.003***
Age of symptom onset (months)	174 (114)	13 (30)	**0.002***
Follow-up period (months)	2.5 (8)	0 (1)	**0.037***
Breastfeeding duration (months)	12 (6.75)	11 (7.5)	0.746*
Duration of diarrhea (months)	2.5 (14)	6 (10)	0.252*
Hemoglobin (g/dL)	9.6 (1.8)	11.60 (1.4)	**0.006***
ALT iu/L	16.0 (18.0)	17.0 (13.5)	0.977*
AST iu/L	27.5 (27.8)	30 (20)	0.471*
CRP (mg/dl)	1 (1)	0 (0)	**0.002***
ESR mm/h	35.0 (24.7)	10.0 (7.5)	**0.000***
Anemia	4 (66.7%)	15 (30%)	0.179**
FOB positivity	5 (83.3%)	4 (8.8%)	**0.000****
Parasite in the stool	1 (16.7%)	1 (2.2%)	**0.006****
Positive stool culture	1 (16.7%)	0 (0%)	**0.006****
CRP positivity	4 (66.7%)	6 (13.3%)	**0.020****
Elevated ESR	6 (100%)	6 (13.3%)	**0.000****
ANA	1 (16.7%)	10 (22.2%)	0.756**
pANCA	1 (16.7%)	2 (4.4%)	0.232**
ASCA	1 (16.7%)	0	**0.006****
PAb	0	1 (2.2%)	0.712**
GAb (n-%)	1 (16.7%)	0	**0.006****

*Mann-Whitney U test, **Chi-square test. The data were given as n (%) and median (IQR). F: Female, M: Male, IBD: Inflammatory bowel disease, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, FOB: Fecal occult blood, ANA: Antinuclear antibody, pANCA: Serum perinuclear anti-neutrophil cytoplasmic antibody, ASCA: Anti-saccharomyces cerevisiae antibody, PAb: Anti-pancreatic antibodies, GAb: Intestinal goblet cell antibody, n: Number

ANA, p-ANCA and GAb positivities were observed in one patient diagnosed with UC and ASCA positivity was seen in another patient diagnosed with CD. No autoantibody positivity was detected in the other four IBD patients. ANA positivity, p-ANCA positivity and PAb positivity were not significant in IBD (p=0.756, p=0.232 and p=0.712 respectively). ASCA positivity and GAb positivity were significantly higher (p=0.006 and p=0.006, respectively) (Table III). The ANA titer in IBD was found to be significantly higher than the ANA titer in other chronic diarrhea cases (p=0.026). Other autoantibody titers were not significantly higher in IBD (p>0.05).

Treatment and diet were recommended for 25 of the 51 patients with appropriate clinical and laboratory findings. Seven (13.7%) patients were treated with steroid and salazopyrine, nine (17.6%) patients with diet regulations, two (3.9%) cases with H2 receptor blockers and seven (13.7%) patients with antibiotics-antiparasitic agents. Polypectomy was performed for one case with polyps.

ASCA positivity was significantly higher in the control group, and GAb positivity was significantly higher in the IBD group when compared to IBD, other chronic diarrhea and control groups (p=0.001 and p=0.008, respectively) (Table IV).

There was no statistically significant relationship between ASCA, p-ANCA, PAb, GAb positivity, and the age of the patients, age of symptom onset or diarrhea duration. A positive correlation was detected between elevated CRP levels and ASCA, p-ANCA, PAb, GAb positivity (p=0.043, p=0.036, p=0.043 and p=0.043, respectively).

Table IV. Comparison of frequency of autoantibody positivity of the cases with inflammatory bowel disease, the other chronic diarrhea, and control groups

	IBD (n=6)	Other chronic diarrhea (n=45)	Control (n=35)	p
ANA	1 (16.7%)	10 (22.2%)	8 (22.9%)	0.944
pANCA	1 (16.7%)	2 (4.4%)	0	0.106
ASCA	1 (16.7%)	0	7 (20%)	**0.008**
PAb	0	1 (2.2%)	0	0.631
GAb	1 (16.7%)	0	0	**0.001**

Chi-square test. The data were given as n (%), IBD: Inflammatory bowel disease, ANA: Antinuclear antibody, pANCA: Serum perinuclear anti-neutrophil cytoplasmic antibody, ASCA: Anti-saccharomyces cerevisiae antibody, PAb: Anti-pancreatic antibodies, GAb: Intestinal goblet cell antibody, n: Number

There was no statistically significant relationship between ASCA, p-ANCA, PAb, GAb positivity and ALT, AST, Hb, ESR ($p>0.05$).

Discussion

Consistent with other studies, the mean age of those patients diagnosed with IBD in our study was found to be 138.1±63.6 months (10,11). In our study, it was found that diarrhea duration was shorter in patients diagnosed with IBD, which was statistically insignificant. This may be explained by the fact that the disease is diagnosed more rapidly when the findings are more severe despite the shorter duration of diarrhea in IBD patients.

Nowadays, acute phase reactants such as CRP and ESR are used to monitor intestinal inflammation in order to diagnose the disease, to determine its activation and to predict the treatment response. In our study, the rates of elevated ESR levels were found to be 100%, elevated CRP levels were 66.7% and anemia frequency was 66.7% in those patients diagnosed with IBD. Compared to the non-IBD chronic diarrhea group, low levels of Hb and elevated levels of ESR and CRP were determined as statistically significant. These findings were found to be consistent with other studies (12).

Today, it is known that CRP, ESR and other acute-phase reactants frequently used for diagnosis and monitoring of intestinal inflammation have poor correlation with intestinal disease activity. Therefore, serologic markers have become important in the diagnosis and follow-up of IBD. After the detection of ANCA in patients with vasculitis in the 1980s, the correlation between IBD and ANCA in recent years has been highlighted. In the late 1980s, p-ANCA was found to be positive in patients with UC and this was accepted as a subclinical indicator for UC (13). Likewise, IgA and IgG antibodies (ASCA IgA and IgG) to a protein found in the outer wall of Saccharomyces cerevisiae used in the preparation of fermented foods, beer and winemaking were found to be positive in the serum of patients with CD (14). Usually, ASCA positive and ANCA negative serology is suggestive of CD while ASCA negative and ANCA positive serology is suggestive of UC (5).

In various studies, the association between IBD and p-ANCA or ASCA has been frequently demonstrated. In UC patients, the incidence of p-ANCA has been reported to be between 50-80% (5,6,8). In a study conducted by Kovacs et al. (7), 72.3% ASCA positivity was determined in patients with CD. In various pediatric studies, ASCA positivity was found to be between 44-76% in CD (4,9). In a study conducted by Kiliç et al. (5) on the Turkish population, the prevalence of p-ANCA for UC was found to be 65%. The prevalence of ASCA was found to be 63.9% in patients diagnosed with CD and no correlation was found between ASCA and the clinical activity of the disease. ASCA positivity was 43.7% in patients with UC. In the same study, p-ANCA (+) and ASCA (-) tests were found to have a lower positive predictive value, negative predictive value and sensitivity compared to p-ANCA for UC alone. Similarly, the positive predictive value, negative predictive value and sensitivity of p-ANCA (-) and ASCA (+) association were also found to be low (5). In our study, p-ANCA positivity was 16.7% and ASCA positivity was 16.7% in those patients diagnosed with IBD. When non-IBD chronic diarrhea patients and IBD patients were compared, there was no statistically significant difference in p-ANCA positivity. Similar to other studies, ASCA positivity was found to be significantly higher in IBD cases (7,8). There was no significant difference in p-ANCA positivity when our control group and chronic diarrhea group were compared. ASCA was determined to be significantly higher in the control group. The increase in ASCA positivity of the control group suggests that the percentage of nonspecific positivity may be high and the method used may not be efficient in the measurement of this auto-antibody. The positivity in the control group compared to the diarrhea group may also be due to the low number of subjects in the healthy control group.

Although antibodies against pancreatic secretion and exocrine pancreas are also suggested as CD markers, these antibodies have not been demonstrated to be associated with the development of pancreatitis in CD, and have not been proven to have direct effects on the pathogenesis of the disease and have been considered to be the result of a cross-reaction against intestinal flora due to impaired mucosal immune response (15). In some studies, PAb positivity was found to be between 31-40% in patients with CD, and it was concluded that the presence of PAb in CD is a specific marker but its sensitivity is low (3,7,16,17). Stocker et al. (18) showed that the prevalence of pancreatic antibodies is 25% in patients diagnosed with CD within the previous 2.5 years. In addition to this, in those patients diagnosed with CD more than 2.5 years previously, the incidence of the pancreatic antibodies was determined to be 46%. Klebl et al. (16) reported that PAb is a particular marker for CD. However, Koutrabakis et al. (19) reported that PAb is also highly prevalent in UC and not only in CD (41.6 and 24.7%, respectively) and that PAb should be used to differentiate IBD from diseases that cause non-IBD intestinal inflammation rather than CD. In contrast, in a study by Zhang et al. (8), PAB positivity was higher in CD

patients compared to both UC and control patients, and PAb was stated as a specific marker that could be used to differentiate CD from UC. In other recent studies, it was determined that the specificity of PAB positivity in IBD was high but its sensitivity was low (7,20). In our study, PAb positivity was not significantly higher in the 51 patients with chronic diarrhea compared to the control group. Also, in our study, it was shown that PAb positivity was not significantly higher in those patients diagnosed with IBD compared to other chronic diarrhea patients. This may be due to the relatively small number of IBD cases in our study.

The positivity of antibodies against intestinal goblet cells in UC is determined by an indirect IF method using fetal intestinal tissue from appropriate primates. The role of the antibodies against intestinal goblet cells in IBD pathogenesis is still unclear, and quite different results on the prevalence of GAb in IBD have been reported. In various studies, it was determined to be 0-33% in CD, 29-39% in UC and 0-2% in healthy controls (21-23). In one study, it was argued that GAb should be used for the diagnosis of IBD and not IBD classification, and may indicate a genetic predisposition (21). In some studies, GAb was considered as a significant marker in the differentiation between CD and UC, whereas in other studies there was no significant difference in the prevalence of the two diseases (8,21-23). In a study by Kovacs et al. (7), 12.2% of patients with UC and 1.9% of patients with CD were GAb positive. In a study by Homsak et al. (13), 46.4% of patients with UC, 2.3% of patients with CD and 0% of healthy controls were GAb positive. These findings suggest that GAb can be used in the differential diagnosis of IBD. In our study, GAb positivity was determined in one patient with UC and GAb positivity was significantly higher in those patients with IBD compared to the non-IBD cases. When we compared those patients presenting with the complaint of chronic diarrhea with the control group, there was no statistically significant correlation between GAb positivity and chronic diarrhea.

Pancreatic and goblet cell antibodies are significant because of their organ specificity and their association with the disease. Both antibodies have direct pathogenic autoimmunity against intestinal goblet cells in UC and the secretion produced by the pancreas in CD (3). However, neither GAb nor PAb had a significant correlation with either age at diagnosis, duration of the disease, area of involvement, the activity of the disease, acute phase reactants or the drugs used (19,23).

In our study, PAb positivity was determined in a patient with non-IBD chronic diarrhea. There was no statistically significant relationship between PAb positivity and the age of the patients, age of symptom onset or diarrhea duration. The patient with PAb positivity was observed to have a long follow-up period. A positive correlation was determined between PAb positivity and elevated CRP levels. There was no statistically significant relationship between PAb positivity and ALT, AST, Hb, or ESR. There was no statistically significant relationship between GAb positivity and the age of the patients, the age of symptom onset, the duration of the follow-up period or diarrhea duration. A positive correlation was determined between GAb positivity and elevated CRP levels. There was no statistically significant relationship between GAb positivity and ALT, AST, Hb or ESR. This makes the role of both autoantibodies in disease pathogenesis disputable. All these data suggest that these two antibodies are a non-pathogenic phenomenon independent of inflammation rather than contributing to the pathogenesis of IBD.

Study Limitations

In addition, studies on various antibodies as serological markers in IBD have been performed. For example, PAb, p-ANCA, ASCA, GAb, antibodies against extracted nuclear antigens, or antinuclear antibodies have been shown not only in CD or UC patients but also in healthy first-degree close relatives of these patients. These individuals were considered to be at high risk for the development of IBD (3).

Conclusion

In conclusion, in the light of these data, we think that autoantibodies in IBD may be used as an adjunctive diagnostic tool in selected cases, rather than in the diagnosis of IBD as a routine practice.

In a study performed by Kovacs et al. (7) in 152 pediatric patients diagnosed with IBD (103 CD, 49 UC); 72.8% ASCA positivity, 33% p-ANCA positivity, 34% PAb positivity and 79.6% GAb positivity were determined for CD while 26.5% ASCA positivity, 77.5% p-ANCA positivity, 20.4% PAb positivity and 12.2% GAb positivity were determined for UC. In our study, we aimed primarily to investigate autoantibody positivity in chronic diarrhea. There is no study in the literature that evaluates all autoantibodies (p-ANCA, ASCA, GAb, PAb) in childhood chronic diarrhea in relation to IBD. In our study, ASCA positivity was significantly higher in the control group compared to the chronic diarrhea group. There was no statistically significant difference in the prevalence of ANA, p-ANCA, GAb and PAb when both of the groups were compared. ASCA and GAb of those patients with the diagnosis of IBD were found significantly higher, while they were negative in patients with chronic diarrhea.

The ANA titer in IBD was found to be significantly higher than the ANA titer in other chronic diarrhea cases. CRP, as an inflammatory marker was also positively correlated with the titrations of these antibodies.

Antibodies in this context are noninvasive and sensitive markers for disease follow-up in patients diagnosed with IBD. We hope that with the more routine use, they will serve as a useful adjunct to the diagnosis of chronic diarrhea patients suggestive of IBD in larger multicenter study groups.

Ethics

Ethics Committee Approval: The study was started after the Ethics Committee approval numbered 09-9/12 dated 10/23/2009 from the Ege University Faculty of Medicine Clinical Trials Local Ethical Committee was obtained.

Informed Consent: Informed consent was obtained from the families during outpatient clinic visits.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: N.K., G.A., F.Ç., Design: N.K., G.A., F.Ç., Data Collection or Processing: H.T., A.A., Ç.E., Analysis or Interpretation: E.A., N.K., Literature Search: E.A., N.K., Writing: H. T.

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Original Article

An Investigation of Pediatric Nurses' Oral Care Practices

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ABSTRACT

Aim: Oral care is a standard practice used to reduce ventilator-associated pneumonia in intensive care units and in the treatment of chemotherapy-induced oral mucositis. This research examines the oral care practices of pediatric nurses.

Materials and Methods: This is a descriptive and cross-sectional study. Its sample was composed of 90 nurses working in the pediatric services of a university hospital in western Turkey from March to December 2016. The research data was collected using a sociodemographic data form and the Oral Care Practices Information Form.

Results: Of the nurses, 62.2% had received oral care education. There were statistical differences in oral diagnosis in terms of clinic, work shift, the making of oral diagnoses before each oral care practice, oral care frequency and the number of patients with impaired oral mucosal integrity ($p<0.05$).

Conclusion: Nurses need training about oral care that is up to date with the literature to manage oral care practices more effectively.

Keywords: Pediatric nurses, oral care, nursing practices

Introduction

Children need oral care for strong and healthy tooth development and to reduce the risk of infections (1). Oral care is an important part of daily hygiene and is conducted to clean the mouth, prevent infections and provide a sense of comfort and hygiene (2,3).

Dryness of oral mucosa in children, especially in intubated infants who cannot be fed orally, infections due to bacterial and fungal infections of dry membranes, mucus injuries due to long-term endotracheal intubation and even ulcers may develop (2). One of the most important ways to prevent oral mucositis and thus oral infections is good, regular and consistent oral care. The number of the microorganisms can be reduced by increasing the quality and frequency of oral care, thus delaying oral mucositis and its complications (4). Oral care is a standard practice used to reduce ventilator-associated pneumonia (VAP) in intensive care units (5) and in the treatment of chemotherapy-induced oral mucositis (6) and it must be conducted by nurses or family members for infants and children who are hospitalized and cannot perform it on their own (3). Nurses have three important roles in the management of mucositis: To diagnose and monitor the oral cavity accurately, to provide the most appropriate oral care for the patients' current conditions and to educate patients (7,8).

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Using soft bristled toothbrushes to prevent mucositis, training patients and health care providers about health care protocols and using valid scales to evaluate mouth ulcers and oral pain are recommended in the guidelines prepared by the Multinational Association for Supportive Care in Cancer and the International Society for Oral Oncology based on the opinions of experts in the field of chemotherapy and limited evidence regarding basic oral care. Nurses' evaluation of patients' mouths at regular intervals using current scales will help to determine both the presence and degree of mucositis and the required oral care frequency (7,9). The Oral Assessment Guide, the Mucositis Evaluation index of the World Health Organization, the National Cancer Institute Toxicity Criteria, the International Child Mucositis Assessment scale and the Oral Assessment Guide for Children and Young People can be used to evaluate mucositis (4,10-15).

The literature includes studies of nurses' oral diagnoses, using scales for oral diagnosis, determining oral care frequency, and using toothbrushes, suction toothbrushes and sponge swabs as oral care materials (16-22). It has also been reported that the solutions that nurses used for oral care were as follows: Sodium bicarbonate, chlorhexidine, salty water, fluoride toothpaste, nystatin, tap water, hydrogen peroxide and sterile water (16,18-21,23). Some studies have conducted randomized controlled trials with oral care solutions made to prevent VAP. They emphasize that oral care is important for the prevention of VAP (24-27). Oral care is both the care practice that pediatric nurses should carry out on sick children most often and the one that is neglected most frequently (28).

The aim of this study is to examine the oral care practices of pediatric nurses.

Material and Methods

Study Population and Design

This is a descriptive, cross-sectional study. Its sample was composed of 90 pediatric nurses working in the pediatric services of a university hospital in western Turkey between March 2016 and December 2016. Having worked in a pediatric clinic for at least one month and voluntary participation were the inclusion criteria.

Ethical Considerations

Before starting the study, permission was obtained from the Ethics Committee of Ege University's Faculty of Nursing (IRB no: 2016-43) and from the health institution where the research was conducted. Those nurses who agreed to participate expressed their consent verbally.

Instruments

Instruments were created by the researchers based on the literature.

The Sociodemographic Data Form

The sociodemographic data form consists of 6 questions about the pediatric nurses' age, their work experience, their work experience in the pediatric clinic, the number of the patients given care, their education level, the clinic where they were working at the time and the number of patients with impaired oral mucosal integrity.

The Oral Care Practices Information Form

The Oral Care Practices Information Form was prepared by the researchers according to the literature. It consists of 24 questions about the pediatric nurses' use of oral diagnostic scales in oral diagnosis, the materials and solutions they use for oral care, percentages of usage and dilution ratios of sodium bicarbonate ampoule, oral care frequency, mouthwash and use of chlorhexidine.

Data Collection

The research data was collected using a Sociodemographic Data Form and the Oral Care Practices Information Form in face-to-face interviews with the pediatric nurses. Each form took approximately 15 minutes to complete by the researchers.

Statistical Analysis

SPSS for Windows 16.0 software was used for statistical analyses. Descriptive statistics (frequency distributions, means, standard deviations, etc.) were used for socio-demographic information. Normal distribution was assessed using the Shapiro-Wilk test. The chi-square and ANOVA tests were used to evaluate the differences between dependent and independent variables. The results were assessed at a 95% confidence interval and a significance level of $p<0.05$ (29).

Results

The mean age of the pediatric nurses was 32.44±7.20 years, and their mean work experience was 9.24±7.61 years. Their mean work experience in pediatric services was 6.65±6.87 years, and the mean number of the patients given care was 11.26±6.01. Of the pediatric nurses, 82.2% had bachelor's degrees, 7.8% had master's-doctoral degrees, 6.7% were graduates of vocational health schools, and 3.3% had associate's degrees. Of the pediatric nurses, 44.4% were working in pediatric services, 18.9% in pediatric

oncology-hematology, 15.6% in pediatric intensive care, 14.4% in pediatric surgery and 6.7% in neonatal intensive care (Table I).

Oral Care Practices

Of the pediatric nurses, 66.7% read the literature on oral care, and 62.2% had received education on oral care (n=56). Of the pediatric nurses who had received oral care education, 85.7% did so at in-service programs (n=48), and 14.3% did so at congresses or in courses (n=8). Of the nurses, 91.1% made oral diagnosis before doing oral care, and only 3.8% used the Oral Assessment Guide for oral diagnosis (n=3) and 52.2% carried out oral diagnosis before shift changes (n=47).

Of the pediatric nurses, 17.8% used toothbrushes in oral care, none used suction toothbrushes, all of them used tongue depressors and gauze, and 11.1% used prepackaged oral care sets. Of them, 57.8% used mouthwash and 12.2% used chlorhexidine. Of the pediatric nurses, 20.0% used nystatin (n=18), 47.8% used tantum verde (n=43), 7.8% used pheniramine mouthwash (n=7), and 6.7% used chlorhexidine mouthwash (n=6). For oral care practices with tongue depressors and gauze: 21.1% used sodium bicarbonate ampoules, 75.6% used diluted sodium bicarbonate ampoules, 4.4% used powdered sodium bicarbonate, 20.0% used saline solution, and 12.2% used distilled or boiled water (Table II). To dilute sodium bicarbonate ampoules: 35.6% of the pediatric nurses used a ratio of one-to-one (n=32), 20.0% used one glass to one ampoule of sodium bicarbonate (n=18), 14.4% used 1 mL sodium bicarbonate to 9 mL water (n=13), 4.4% used one to three (n=4), and 1.1% used 1 mL sodium bicarbonate to 4 mL of water (n=1). Of the pediatric nurses, 3.3% carried out oral aspiration after oral care.

For oral care frequency, the results were: 2.2% of the pediatric nurses performed oral care once a day, 26.7% did so twice a day, 23.3% did so 3 times a day, 24.5% did so 4 times a day, and 23.3% did so when needed.

The number of patients with impaired oral mucosal membrane integrity by clinic were: pediatric oncology-hematology (1.94±1.43), pediatric clinics (0.95±1.10), neonatal intensive care unit (0.66±1.03), pediatric surgery (0.30±0.48) and pediatric intensive care (1.07±1.20) (Table III).

Table I. Distribution of the pediatric nurses according to their descriptive characteristics (n=90)

Socio-demographic Characteristics	M ± SD (Min-Max)
Mean age	32.44±7.20 (min: 15, max: 54)
Work experience	9.24±7.61 years (min: 3 months, max: 32 years)
Work experience in the pediatric clinic	6.65±6.87 years (min: 3 months, max: 31 years)
Number of the patients given care	11.26±6.01 patients (min: 2, max: 30)

Education status	n (%)
Graduate of high school	6 (6.7)
Associate's degree	3 (3.3)
Bachelor's degree	74 (82.2)
Post-graduate degree	7 (7.8)
Units	
Pediatric clinic	40 (44.4)
Pediatric oncology-hematology	17 (18.9)
Pediatric intensive care	14 (15.6)
Pediatric surgery	13 (14.4)
Neonatal intensive care	6 (6.7)
Total	90 (100.0)

M: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum, n: Number of patients

Table II. The materials and solutions the pediatric nurses used for oral care

Materials and solutions the nurses used in oral care	Yes	No
	n (%)	n (%)
Toothbrush	16 (17.8)	74 (82.2)
Suction toothbrush		90 (100.0)
Tongue depressor	90 (100.0)	-
Gauze	90 (100.0)	-
Prepackaged oral care set	10 (11.1)	80 (88.9)
Toothpaste	3 (3.3)	87 (96.7)
Mouthwash	52 (57.8)	38 (42.2)
Glutamine	4 (4.4)	86 (95.6)
Chlorhexidine	11 (12.2)	79 (87.8)
Sodium bicarbonate ampoule	19 (21.1)	71 (78.9)
Diluted sodium bicarbonate ampoule	68 (75.6)	22 (24.4)
Powdered sodium bicarbonate	4 (4.4)	86 (95.6)
Saline solution	18 (20.0)	72 (80.0)
Distilled water/boiled water	11 (12.2)	79 (87.8)

n: Number of patients

Oral Care Practices and Socio-demographic Characteristics

There were statistically significant differences according to the clinic where the pediatric nurses worked and the practice of carrying out an oral diagnosis at each shift change (X^2=15.561, p=0.004), carrying out an oral diagnosis before each oral care practice (X^2=10.97, p=0.027), oral care frequency (X^2=51.82, p=0.000) and the number of patients with impaired oral mucosal integrity (F=4.32, p=0.003) (p<0.05).

Table III. Distribution of patients with impaired oral mucosal membrane integrity by clinic

Clinics	M ± SD (Min-Max)
Pediatric oncology-hematology	1.94±1.43 (min: 0, max: 5)
Pediatric clinic	0.95±1.10 (min: 0, max: 4)
Neonatal intensive care	0.66±1.03 (min: 0, max: 2)
Pediatric surgery	0.30±0.48 (min: 0, max: 1)
Pediatric intensive care	1.07±1.20 (min: 0, max: 4)

M: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum

There were also statistically significant differences with respect to the clinic where the pediatric nurses worked and the use of tooth brushes (X^2=10.74, p=0.030), mouthwash (X^2=38.40, p=0.000), diluted sodium bicarbonate ampoules (X^2=14.30, p=0.006), powdered sodium bicarbonate (X^2=17.97, p=0.001), saline solution (X^2=24.94, p=0.000) and distilled or boiled water (X^2=11.31, p=0.023). However, there were no statistically significant differences in terms of the clinic where the pediatric nurses worked and the use of toothpaste (X^2=1.88, p=0.757), prepackaged oral care sets (X^2=5.74, p=0.219), chlorhexidine (X^2=2.46, p=0.651) and sodium bicarbonate ampoules (X^2=6.67, p=0.154) (p>0.05).

There were no statistically significant differences in terms of the pediatric nurses' education level and the carrying out of oral diagnosis at shift changes (X^2=2.97, p=0.395), the carrying out of oral diagnosis before each oral practice (X^2=4.28, p=0.233), oral care frequency (X^2=10.83, p=0.543) and number of the patients with impaired oral mucosal integrity (F=0.67, p=0.572).

There were statistically significant differences in terms of having received education about oral care and carrying out oral diagnosis before each shift change (X^2=4.28, p=0.038) and oral care frequency (X^2=12.20, p=0.016). However, education about oral care did not make a statistically significant difference in the practice of making oral diagnosis before each oral care (X^2=2.28, p=0.131).

Discussion

One of the most basic nursing roles is to provide patients with oral care. The first stage of oral care in pediatric oncology and pediatric hematology clinics is to make oral diagnosis before and after treatment (4). Most of the nurses made oral diagnosis before oral care practice, but few of those who made oral diagnosis used the Oral Assessment Guide for oral diagnosis, and only half of them made oral diagnosis before each shift change. Özveren et al. (16) report that 77.8% of nurses made oral diagnosis, and Ganz et al. (19) report that 95.0% did so. Ganz et al. (19) found that 71.0% of the nurses made oral diagnosis before oral care, and 33.0% did so at each shift change. Feider et al. (18) and Ganz et al. (19) also found that nurses did not use a Standard Oral Diagnostic scale. However, Southern (6) found that nurses used two different oral assessment guides. Training nurses about the use of scales that are valid for oral diagnosis will Increase scale utilization rates.

The American Dental Association recommends that oral care be started a few days after birth. Infants' gums should be cleaned with a piece of gauze after they are fed to protect their teeth from plaque and bits of food. The infants' mouths should be aspirated after oral care, and cleanliness of the lips and moistness of the oral mucosa should be maintained using a sponge swab dipped into an alcohol and oxygen-free water solution (27). In this study, all the pediatric nurses used tongue depressors and gauze, few used prepackaged oral care sets and toothbrushes, and none used suction toothbrushes. Studies have reported high rates of using sponge swabs (18,20,21), gauze (17,19) and gauze with tongue depressors (16,20). Toothbrush use varied, with low use of suction toothbrushes and high use of suction (18-21). It is thought that the difference between the rates of using oral care materials is related to the institutions and clinics where the nurses worked.

In their oral care practices with tongue depressors and gauze, the nurses mostly used diluted sodium bicarbonate ampoules, sodium bicarbonate ampoules, saline solution, distilled or warm water, chlorhexidine or powdered sodium bicarbonate, in order of prevalence. In addition, more than half of them used mouthwash in oral care practices. The nurses mostly used sodium bicarbonate, chlorhexidine and salty water, in order of prevalence (16,18-21). It is thought that the difference between the rates of using oral care solutions is related to the institutions and clinics where the pediatric nurses worked.

Nurses can determine oral care frequency using scores on an oral assessment guide (12). In this study, the frequencies

were: 2 times a day (26.7%), 4 times a day (24.4%), 3 times a day (23.3%), when needed (23.3%) and once a day (2.2%). Özveren et al. (16) found these frequencies as follows: when needed (37.8%), 3 times a day (18.4%), 2 times a day (17.3%) and once a day (9.2%). However, Ibrahim et al. (17) found the results to be as follows: 2 times a day (61.0%), once a day (27.9%), more than 3 times a day (5.8%) and 3 times a day (5.2%). Feider et al. (18) found that almost all the nurses performed oral care 4 times a day or more. It is thought that oral care frequency can be defined more clearly by making the usage of the 'oral assessment guide' common practice.

Evaluation of Oral Care Practices with Respect to Some Variables

There was a statistical difference with respect to the clinic where the pediatric nurses worked and making oral diagnosis during their work shifts, making oral diagnosis before each oral care, oral care frequency and number of patients with impaired oral mucosal integrity ($p<0.05$). Feider et al. (18) reported a statistically significant difference in the frequency of oral care practices by those nurses with work experience of 7.1 years or more. Türk et al. (20) found a statistically significant difference in oral care frequency according to the clinic where the nurses worked.

Although saline solution, sodium bicarbonate and chlorhexidine mouthwashes are most commonly recommended, there is no broad consensus in the literature over which solution should be used in oral care or which solution is more effective (12). There was a statistically significant difference in terms of the clinic where the pediatric nurses worked and the use of toothbrushes, mouthwash, diluted sodium bicarbonate ampoules, powdered sodium bicarbonate, saline solution and distilled or boiled water in oral care practices, but no such difference in their use of toothpaste, prepackaged oral care sets, chlorhexidine and sodium bicarbonate ampoules. Türk et al. (20) reported a statistically significant difference in terms of the clinic where the nurses worked and use of chlorhexidine, sponge swabs, gauze wound around tongue depressors and toothbrushes. In a study of oral care practices at four different hospitals, Özveren et al. (16) reported statistically significant differences between the hospitals in terms of use of swabs, suction toothbrushes, mouthwashes and toothpaste; however, there were no statistically significant differences between the hospitals in terms of use of gauze wound around tongue depressors. They also found a statistically significant difference between the hospitals in terms of use of oral care solutions (sodium bicarbonate, chlorhexidine and distilled water).

There was a statistically significant difference between having received education about oral care and the practice of making oral diagnosis before each shift change and oral care frequency. However, the difference between having received education about oral care and carrying out oral diagnosis before each oral care intervention was not statistically significant. Educating nurses about making diagnosis and determining oral care frequency using valid scales, and also administrative encouragement to do so will be effective.

Conclusion

With regards to the pediatric nurses who took part in this study:

- All used tongue depressors and gauze in oral care practices as stated in the literature.
- Most of the pediatric nurses conducted oral diagnosis but did not use valid assessment tools to do so or to determine oral care frequency.
- Their levels of education about oral care practices were low.

With regards to the prevalence levels of patients with impaired oral mucosal membrane integrity, the clinics were ranked from high to low as follows: pediatric hematology/oncology, pediatric intensive care, pediatric clinic, neonatal intensive care and pediatric surgery.

All pediatric nurses should use valid scales for oral diagnosis before oral care practices and to determine oral care frequency. Educational programs based on standardized oral care protocols and evidence-based studies, and quasi-experimental research to increase knowledge about oral care should be organized.

Ethics

Ethics Committee Approval: Permission was obtained from the Ethics Committee of Ege University's Faculty of Nursing (IRB no: 2016-43) and from the health institution where the research was conducted.

Informed Consent: Those nurses who agreed to participate expressed their consent verbally.

Peer-review: Enternally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Y., G.Ö.G., M.G., H.B.Y., Concept: B.Y., G.Ö.G., M.G., H.B.Y., Design: B.Y., G.Ö.G., M.G., H.B.Y., Data Collection or Processing: B.Y., G.Ö.G., M.G., H.B.Y., Analysis or Interpretation: B.Y., G.Ö.G., M.G., H.B.Y., Literature Search: B.Y., G.Ö.G., M.G., H.B.Y., Writing: B.Y., G.Ö.G., M.G., H.B.Y.

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Original Article

Evaluation of the Relationship between Mucosal Diseases and Faecal Calprotectin Levels in Children who Underwent Upper Gastrointestinal System Endoscopy

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ABSTRACT

Aim: In this study, the correlation of faecal calprotectin (FC) levels with endoscopic and histopathological findings was evaluated in children who underwent upper gastrointestinal system (UGIS) endoscopy.

Materials and Methods: The patients aged 4-18 years admitted to our endoscopy unit were included in this study. Faecal samples were collected on the day participants presented for the study. A calprotectin assay was performed on the faecal samples using enzyme-linked immunosorbent assay kits. FC levels were compared with those of children with normal histopathology.

Results: The FC levels of 112 children included in this study were evaluated according to their histopathological diagnoses. Their FC levels were not found to be significantly higher than those in healthy children. No significant difference was found in the FC levels of participants with or without signs of inflammation in the esophagus, stomach and duodenum. In those participants with chronic gastritis, *Helicobacter pylori* (*H. pylori*) gastritis, reflux esophagitis or celiac disease, FC levels above cut-off values were observed. However, no significant difference was found in those participants with normal endoscopic findings. There was no significant difference in FC levels between similar regions of the UGIS with or without signs of inflammation. A positive correlation was discovered between eosinophil counts and FC levels in patients with *H. pylori* gastritis and esophagitis.

Conclusion: The diagnostic value of FC levels in UGIS diseases was not sufficient to establish a definitive diagnosis in our study.

Keywords: Calprotectin, children, gastritis, esophagitis

Introduction

Faecal calprotectin (FC) found in neutrophils is a complex protein bound to calcium and zinc, and it is resistant to enzymatic degradation and unaffected by medications or diet. It is released into the lumen as a result of alterations in the intestinal barrier induced by inflammation (1,2). In childhood, higher levels of FC, mainly in inflammatory bowel disease (IBD) and Henoch Schönlein vasculitis have

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been reported (1,3). In some studies, although slightly higher levels of FC have been reported at the time of celiac disease diagnosis, these increased FC levels have no diagnostic value (4-6).

FC has been evaluated in other upper gastrointestinal system (UGIS) diseases such as reflux esophagitis, *Helicobacter pylori* (*H. pylori*) gastritis, eosinophilic enteropathy and eosinophilic esophagitis in children, but there are an inadequate number of studies on these. It has been indicated that FC plays a role in the differentiation between organic and functional bowel diseases of the gastrointestinal system and is associated with findings of endoscopic and histopathological inflammation (1,7). In this study, endoscopic and histopathological findings and the relationship between eosinophil and neutrophil counts in biopsy materials were evaluated in pediatric patients undergoing UGIS endoscopy.

Materials and Methods

Patients aged 4-18 years admitted to Tepecik Training and Research Hospital Pediatric Gastroenterologic Endoscopy unit between June 2017 and January 2018 were included in this study. Informed consent was obtained from the family and patients. This study was approved by the local Ethics Committee (approval number: 77- 21.4.2016).

Our study population consisted of patients scheduled for UGIS endoscopy with presumptive diagnoses of gastritis-esophagitis based on complaints of chronic dyspepsia, vomiting, nausea or epigastric pain and a presumptive diagnosis of celiac disease or tissue transglutaminase (TTG) positivity (>20 RU/mL). Participants who complained of long-term diarrhea with TTG IgA negativity, TTG IgG negativity, or those with normal colonoscopy and histopathology findings were also included in the study. Cases of foreign body swallowing, varicose vein control, gastrointestinal bleeding, patients requiring endoscopic dilatation, recent users of non-steroidal anti-inflammatory drugs, those with a detected infection or IBD were not included in this study (2).

Parents were asked to provide their child's faecal sample on the morning of endoscopy after informed consent for the study was obtained. Faecal sampling and the endoscopic procedure were performed on the same day. The same pediatric gastroenterology team performed all endoscopic procedures. Biopsies were taken with endoscopic forceps from the duodenum, duodenal bulb, antrum of the stomach, and esophagus in the same session.

The native stool samples (50-100 mg) were homogenized according to the manufacturer's directions using the Smart-Prep faecal sample preparation kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). The homogenized stool samples were transferred into plastic tubes and stored at -20 °C until analyzed. Calprotectin concentrations in fecal samples were measured using enzyme-linked fCAL® immunosorbent assay kits (Bühlmann Diagnostics Corp., Amherst, NH, USA). The lowest and the highest values within the working range were considered to be 30 and 1.800 µg/G, respectively. The results were represented as µg/G. The cut-off value recommended by the manufacturer was 50 µg/G for children aged 4-17 years, regardless of gender (8).

All biopsy specimens were evaluated by the same pathology team. Gastric endoscopic biopsy samples were evaluated histopathologically with the updated Sydney classification and grading system, and the gastritis and *H. pylori* status of each patient was determined (9). Using this classification, chronic inflammation, neutrophil activity, glandular atrophy, intestinal metaplasia and *H. pylori* density were graded and evaluated. Intestinal metaplasia was histochemically assessed by pediatric appendicitis score-Alcian Blue staining in addition to histomorphologic studies. The presence of *H. pylori* was determined by Toluidine blue staining. Gastritis was classified as mild, moderate or severe according to *H. pylori* density (10,11).

In addition, eosinophils, leukocytes and neutrophils were counted in the lamina propria of gastric biopsies under 400x magnification. Diagnosis of eosinophilic gastritis was made based on the presence of more than 30 eosinophils in gastric biopsy materials under 400x magnification (12,13). Bulbus and duodenum villus to crypt ratio, crypt hyperplasia, intraepithelial lymphocyte count, neutrophil and eosinophil leukocyte counts in the lamina propria and epithelium were evaluated with hematoxylin and eosin (H&E) and CD3 stained sections of biopsy specimens (14,15).

The FC levels in participants who were diagnosed based on histopathological and endoscopic findings of the esophagus, stomach, bulbus and duodenum were compared with the FC levels of participants with normal histopathology. The relationship between FC values and eosinophil and neutrophil counts in the biopsy samples was also evaluated.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics 25 package program (IBM Corp., Armonk, New York, USA). Data are presented as count (n), percent (%), mean and standard deviation ($\bar{x} \pm sd$), minimum-maximum value and median (25%-75% quartiles). Shapiro-

Wilk's test was used and a histogram and Q-Q plot were examined to assess the data normality. Mann-Whitney U test was used to compare the differences between two groups for continuous variables. Kruskal-Wallis analysis was used for comparisons of more than two groups. The relationship between variables was evaluated by Spearman correlation analysis. Receiver operating characteristic curves were used to determine the discriminative power of the FC levels in the diagnosis of histopathological findings (normal, abnormal). A p value <0.05 was accepted as statistically significant.

Results

Of the 112 patients included in the study, 68% were female (n=77) with a mean age of 12.8±4.5 years. Endoscopy was planned based on the following indications: gastric complaints (59%; n=67), persistent reflux symptoms (12.5%; n=14), TTG IgA-IgG positivity (18%; n=20) and other causes that included chronic epigastric pain, chronic diarrhea or dysphagia (10%; n=11). As a result of UGIS endoscopy, diagnoses were as follows: chronic gastritis/*H. pylori* gastritis (67.8%; n=76), biliary reflux (2.7%; n=3), celiac enteropathy (13.4%; n=15), duodenitis-bulbitis (4.4%; n=5), reflux esophagitis (6.2%; n=7) and eosinophilic esophagitis (0.9%; n=1), while 4.5% (n=5) had normal endoscopic findings.

The FC levels in 95 cases (85%) with abnormal histopathologic findings and 17 cases (15%) with normal histopathological findings are shown in Table I. In one of the two cases with eosinophilic esophagitis, the FC level was found to be 850 µg/G, while the other case had FC levels within normal limits . In one participant with severe eosinophilic duodenitis and ulceration, the FC level was found to be 1.200 µg/G. As a result, when FC levels were evaluated according to histopathological diagnosis, there was no significant difference in the FC levels between the biopsy specimens of cases with or without abnormal findings (Figure 1, Table I).

No significant difference in FC levels between similar regions of the UGIS with or without signs of inflammation was detected (Table II). A positive correlation was found

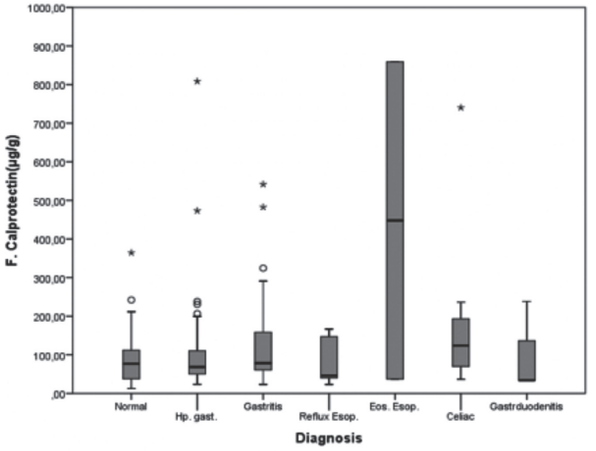

Figure 1. Fecal calprotectin levels according to histopathological diagnoses
Hp. gast.: *Helicobacter pylori* gastritis, Esop.: Esophagitis, Eos.: Eosinophilic

Table I. Comparison of fecal calprotectin levels according to histopathological diagnosis

	Diagnosis	n	M (Q_1-Q_3)	Min-max	Mean Rank	Test Statistics	p⁺
FC	Normal	17	108±94.7 76.8 (36.2-156.5)	12.7-364.4	53.09	8.933	0.348
	Hp. Gast.	36	116.2±146.5 68.4 (49.5-112.9)	23.5-808.5	51.38		
	Gastritis	33	131.3±124.4 78.5 (56.7-163.6)	23.2-541.8	60.12		
	Reflux Esop.	5	84.6±66.7 46.4 (31.6-156.8)	23.2-166.5	43.10		
	Eos. Esop.	2	448.1±581.1 448.1 (37.20 - -)	37.2-859	64.50		
	Bulbitis	1	- -	58.1-58.1	34.00		
	Duodenit-Eos.	1	- -	1264-1264	112.00		
	Celiac	14	168.3±176 124 (68.3-196.6)	36.6-740.5	69.89		
	Gastroduodenitis	3	102.1±118 34.2 (33.8 - -)	33.8-238.4	41.00		

⁺:Kruskal-Wallis test, FC: Fecal calprotectin, Hp. Gast.: *Helicobacter pylori* gastritis, Esop: Esophagitis, Eos: Eosinophilic, Min: Minimum, Max: Maximum

between eosinophil and neutrophil counts in the esophagus, antrum, bulbus and duodenum (r=0.483, 0.687, 0.392, and 0.368, respectively) (p<0.001). When the correlation between eosinophil and neutrophil counts with FC levels in the biopsy specimens was examined, positive correlation was found between eosinophil counts and FC in patients with *H. pylori* gastritis and reflux esophagitis (Table III).

Finally, normal and abnormal endoscopic and histopathological findings were compared based on the FC cut-off level of 50 µg/G. The sensitivity and specificity of this cut-off value was determined to be 75% and 35%, respectively (Figure 2).

Discussion

FC is a marker that assists in the differentiation of disease-specific and functional gastrointestinal system diseases, showing mucosal inflammation of the gastrointestinal system lumen. Although FC is resistant to bacterial enzymes and intestinal proteases, it appears that FC does not have a practical role in UGIS diseases in children (16-18).

In this study, endoscopic and histopathological findings and FC measurements of children with UGIS were evaluated in combination. In participants with UGIS pathologies, although the mean FC levels were above the cut-off value, no significant increase was observed compared to participants with normal histopathological findings. Different results have been reported in the literature regarding FC levels in different UGIS diseases (19). It has been reported that FC levels in peptic ulcer disease and erosive gastritis in adults are higher compared to normal endoscopic findings, with 60% sensitivity and 81% specificity in the prediction of endoscopic findings (20). Since we did not find gastric peptic ulcer disease and erosive gastritis in our study participants, data related to these diseases could not be presented. In another study performed in children, a significant correlation was found between FC level and gastritis (severity and

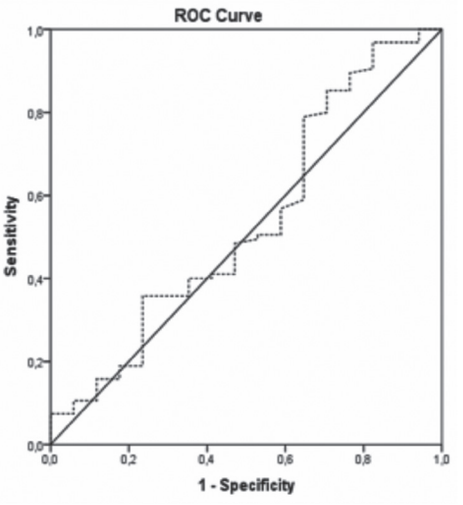

Figure 2. Evaluation of the level of fecal calprotectin and pathologic findings in histopathology and endoscopy of the upper gastrointestinal tract
Area under the ROC curve (AUC): 0.536 (95% Confidence Interval; 0.439 to 0.631); Significance level p (Area=0.50): 0.664
ROC: Receiver operating characteristic

Table II. Fecal calprotectin levels according to histopathological findings

Parts of Upper Intestine	Histopathology	n	M (Q_1-Q_3)	Min-max	Mean Rank	Test Statistics	p†
Esophagus	Normal	91	121.2±136.1 72.8 (50.3-132.8)	12.7-808	52.7	-1.018	0.309
	Abnormal	16	251.4±345.8 112 (41.6-245.4)	23.2-1264	61.3		
Antrum	Normal	25	145.3±192.5 76 (37.2-183.9)	12.7-859	51.6	-0.685	0.493
	Abnormal	85	139.4±182.8 76.8 (54.3-158.3)	23.2-1264	56.6		
Duodenum	Normal	87	129.77±150.67 76.8 (47.4-158.3)	12.7-859	55.2	-0.807	0.420
	Abnormal	25	174.7±267.3 72.8 (61.1-169.7)	33.8-1264	61.1		
Duodenal- Bulb	Normal	86	132.6±150.5 76.8 (50-160.3)	12.7-859	56.8	-0.522	0.601
	Abnormal	25	140.2±242.8 69.9 (37.2-130.7)	23.2-1264	53.1		

†: Mann-Whitney U test, min: Minimum, Max: Maximum

Table III. Correlation between eosinophil and neutrophil counts by histopathological diagnosis

	Upper GI parts		Normal -FC	Hp. Gastritis FC	Cr. Gastritis FC	Reflux Esop.-FC	Celiac- FC	Gastro-duodenitis -FC
Eosinophils	Esophagus	r_s	0.077	-	0.031	**0.894**	-	-
		p	0.802	-	0.870	**0.041**	-	-
		n	13	35	31	5	12	3
	Antrum	r_s	0.000	**0.353**	-0.157	0.289	0.559	0.500
		p	1.000	**0.044**	0.417	0.638	0.118	0.667
		n	14	**33**	29	5	9	3
	Duodenal-Bulb	r_s	-0.306	0.033	-0.140	0.200	0.258	-0.500
		p	0.310	0.854	0.486	0.800	0.471	0.667
		n	13	33	27	4	10	3
	Duodenum	r_s	-0.268	0.123	-0.164	-0.738	0.498	-1.000
		p	0.355	0.497	0.415	0.262	0.173	
		n	14	33	27	4	9	3
Neutrophils	Esophagus	r_s	0.077	0.209	-0.024	-	-	0.866
		p	0.802	0.244	0.897	-	-	0.333
		n	13	33	31	5	11	3
	Antrum	r_s	-0.047	0.072	-0.143	0.289	0.453	0.866
		p	0.881	0.697	0.478	0.638	0.221	0.333
		n	14	32	27	5	9	3
	Duodenal-Bulb	r_s	0.437	-0.202	0.010	-0.738	0.364	0.000
		p	0.136	0.284	0.960	0.262	0.301	1.000
		n	13	30	27	4	10	3
	Duodenum	r_s	0.044	-0.105	-0.001	-0.105	0.143	-0.500
		p	0.881	0.581	0.995	0.895	0.694	0.667
			11	1	1	1	1	1

r_s: Spearman correlation coefficient, GI: Gastro-intestinal, FC: Fecal calprotectin, Cr: Chronic, Esop.: Esophagitis, Hp: *Helicobacter pylori*

inflammation grade) and colonization of *H. pylori* (21). In our study, there was no significant difference between FC levels in participants with or without *H. pylori* chronic gastritis. In participants with H. pylori-associated gastritis, FC was found to be above the cut-off value. However, no statistically significant differences were observed between those patients with normal gastric mucosa and those with *H. pylori*-positive gastritis. In another study, the FC was not found to be significantly higher among participants with histologically active or non-active gastritis than among healthy controls (21).

Increased FC levels have been demonstrated in celiac disease and are also associated with villus atrophy and a gluten-free diet (5,6). However, it was reported that these high values have no diagnostic specificity (19). In our study, no significant difference was observed in the FC levels of children with a diagnosis of celiac disease compared to patients with normal duodenal histopathology. This is an expected condition when we consider that the main histopathological element in this condition is intraepithelial lymphocyte infiltration.

Higher FC levels have been reported in people with allergic colitis and cow's milk protein allergy (16,22). Our study population did not contain any cases of cow's milk protein allergy. However, higher FC levels were detected in one participant with severe eosinophilic esophagitis, and another with dense eosinophilic inflammation and an ulcer in the duodenum. When the biopsy materials of these two cases were examined, it was observed that the number of neutrophils increased in parallel with an increase in the

number of eosinophils. Neutrophil counts were found to be within normal limits in another participant with eosinophilic esophagitis and a normal FC level. In our study, it was determined that eosinophil and neutrophil counts in tissue samples were moderately, and also positively, correlated. This may explain increased FC levels in cases with severe eosinophilic inflammation. Owing to the limited number of participants with eosinophilic esophagitis and eosinophilic enteropathy in our study, it is not possible to make a definitive interpretation about the relationship between these diseases and FC levels. Another study reported that FC levels increased in parallel with disease severity in children with IBD, but FC levels were within normal limits in eosinophilic and lymphocytic colitis (23).

In one study evaluating endoscopic findings in UGIS and FC levels in children, it was stated that there was no correlation between FC values and esophageal pathologies, and there was a lack of any marker for esophageal pathologies (21). In our study, we found no significant elevation of FC levels in reflux esophagitis.

Intensive ulceration and inflammation in UGIS disease in children is a rare condition. It has also been reported that the level of neutrophils in the lumen is associated with FC levels (2,23). In two patients with critically high FC values , severe mucosal inflammation and ulceration was observed. This suggests that inflammation in the tissue may be associated with an increase in the release of neutrophils into the lumen. A significant elevation of FC levels in adults with peptic ulcer disease and erosive gastritis compared to patients with normal endoscopic findings suggests this condition (20). In the cohort study performed among pediatric patients, the sensitivity and specificity of IBD were found to be 98% and 84%, respectively. In this study, the sensitivity and specificity of FC values above the cut-off value were determined to be 75% and 35%, respectively.

Conclusion

As a result, it is important to formulate diagnostic markers for UGIS diseases without resorting to invasive procedures such as UGIS endoscopy, which requires sedation, especially for pediatric patients. However, in our study, although FC levels were found to be slightly elevated in UGIS diseases, their diagnostic value was not sufficient to establish a diagnosis. FC levels should be studied in a larger series of patients with eosinophilic esophagitis and enteropathy.

Ethics

Ethics Committee Approval: This study was approved by the local ethics committee (approval number: 77-21.4.2016).

Informed Consent: Informed consent was obtained from the family and patients.

Peer-review: Enternally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B., Y.Ç.A., G.D., Concept: M.B.,Y.Ç.A., Design: M.B., Y.Ç.A., M.K., Data Collection or Processing: M.B., Y.Ç.A., M.K., F.D.A., İ.K., D.S.K., B.E., Analysis or Interpretation: M.B., Y.Ç.A., F.D.A., İ.K., D.S.K., B.E., G.D., Literature Search: M.B., Y.Ç.A, B.E., Writing: M.B., Y.Ç.A, B.E., G.D.

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Original Article

The Role of Alvarado and Pediatric Appendicitis Score in Acute Appendicitis in Children

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ABSTRACT

Aim: Acute appendicitis (AA) is the condition that most commonly requires a surgical procedure in children presenting to the emergency department with acute abdominal pain. Alvarado and Pediatric Appendicitis scores (PAS) are the most widely used scoring systems in the diagnosis of AA in children. This study aims to evaluate the effectiveness of Alvarado and PAS scores in the diagnosis of AA in children.

Materials and Methods: One hundred and two patients aged 3-17 years, who were admitted to the pediatric emergency department with acute abdominal pain and had a clinical suspicion of AA were included in this retrospective study. The demographic characteristics, laboratory and radiological findings, and Alvarado and PAS scores were obtained from the records of the patients, retrospectively. According to the results of pathology, n=48 patients for the AA group and n=54 patients for the non-AA group were identified. The AA and non-AA groups were compared in terms of PAS and Alvarado scores.

Results: The mean age of the 102 patients was 9.38±3.90 years, and 62 (60.85%) of them were male. The median PAS score was 7.79±1.2 and 5.52±1.34, and the median Alvarado score was 7.98±1.14 and 5.89±1.53 in the AA and N-AA groups, respectively ($p<0.001$; $p<0.001$). For the PAS, the cut-off score was >7 with a sensitivity of 66.7%, a specificity of 94.4%, a positive predictive value (PPV) of 91.4%, and a negative predictive value (NPV) of 76.1% ($p<0.001$). For the Alvarado score, the cut-off was also >7, with a sensitivity of 77.1%, a specificity of 85.2%, PPV of 82.2%, and NPV of 80.7% ($p<0.001$).

Conclusion: Although both PAS and Alvarado scores provide useful diagnostic information in patients suspected of AA, neither of them is enough to diagnose AA alone.

Keywords: Appendicitis, Alvarado score, children, PAS

Introduction

Acute appendicitis (AA) is the condition that most commonly requires a surgical procedure in children presenting to the emergency department with a sudden onset of abdominal pain (1).

Although the incidence is not known precisely, it is on the scale of 5.9/10,000 in the 0-9 years old age range, and 15.3/10,000 in the 10-19 years old age range in the United States of America (2). Also, in acute abdominal pain in children, AA was diagnosed for 7.4% in Australia (3). It has been shown that children with acute abdominal pain initially received other diagnoses frequently seen in children such as acute gastroenteritis, pneumonia, urinary tract infection, or mesenteric lymphadenitis (4). Perforation and related

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complications can be seen if the diagnosis cannot be made in time (4-7). In contrast to this, the frequency of negative appendectomy is reported to be at rates of 3.7-17% (7,8). Abdominal ultrasound (USG) is the most commonly used radiological method to help in making a correct diagnosis. Its sensitivity varies from 66.2% to 85% according to the practitioner's experience (8-10). The sensitivity of abdominal computed tomography (CT) is reported to be 95-97%, but there are risks such as radiation and post-contrast reactions (11,12). Therefore, scoring systems based on symptoms, physical examination, and laboratory findings have begun to be used. The Alvarado score, which was initially applied in adults, is also used in children. Subsequently, the Pediatric Appendicitis score (PAS) was developed by Samuel (13) in 2002. The Alvarado score and PAS have been reported to reduce the use of CT in the diagnosis of AA for patients between 3 and 16 years of age (14,15). This study aims to determine the PAS and Alvarado scores in patients admitted to the pediatric emergency department and considered to have AA, and to compare them in terms of applicability, safety, and specificity in the diagnosis of AA.

Materials and Methods

The retrospective observational study was conducted between November 2014 and November 2015, in the Clinic of Pediatric Emergency of Okmeydani Training and Research Hospital. The study included children aged 3-17 years who were admitted to the pediatric emergency department. Those patients who had a pain duration of less than 96 hours and were considered to have AA (abdominal sensitivity and defense or rebound tenderness positivity) after an examination by a pediatrician were included in this study. Pregnant patients, patients with chronic inflammatory bowel disease, cystic fibrosis, or sickle cell anemia, patients who had undergone a previous abdominal operation or abdominal tomography within the last two weeks and those who had received corticosteroids for more than two weeks, and immunosuppressed patients were not included in this study. The information cards of each patient were filled out before surgical consultation. Consent forms were obtained from the parents or the children. The age, gender, duration of pain, complaint (complaints of abdominal pain and duration, fever, nausea-vomiting, diarrhea, upper respiratory tract infection) physical examination findings (right lower quadrant sensitivity, defense, rebound tenderness, percussion/cough/right lower quadrant sensitivity with jumping) leucocyte count and neutrophil percentages, biochemistry, C-reactive protein, complete urine test, and PAS and Alvarado score parameters were marked on the forms of these patients.

According to previous studies, a neutrophil count ≥75% and a body temperature over 38 °C were accepted as fever. The parameters of PAS and Alvarado scores and their scoring systems are shown in Table I (16-18). All the patients underwent USG, and USG was considered to be positive in the presence of signs such as a fixed appendix, no compression, round transverse image appendix, thickening in the intestine wall, appendix inner diameter >6 mm, decreased mobility, irregular appearance in appendix, and peri-appendicular fluid appearance (9,10). All other USG findings were accepted as negative. A pediatric surgeon consulted with all the patients. The pediatric surgeon and the radiologist were not informed about the PAS and Alvarado scores. Other laboratory data and the radiological and pathological results of the patients were obtained from the system records. Those patients who were discharged

Table I. Alvarado and pediatric appendicitis scoring system (16-18)

Alvarado score		**Pediatric Appendicitis score**	
Feature	**Point value**	**Feature**	**Point value**
Migration of pain	1	Migration of pain	1
Anorexia	1	Anorexia	1
Nausea/vomiting	1	Nausea/vomiting	1
Signs RLQ tenderness	2	Signs RLQ tenderness	2
Rebound pain	1	Cough/hopping/percussion tenderness in the RLQ	2
Elevation of temperature (38 °C)	1	Elevation of temperature (38 °C)	1
Leukocytosis ≥10^9/L	2	Leukocytosis ≥10^9/L	1
Polymorphonuclear neutrophilia ≥75%	1	Polymorphonuclear neutrophilia ≥75%	1
Total	10	Total	10

Score <4 low, 5-6 intermediate, 7-10 high risky, Score <3 low, 4-6 intermediate, 7-10 high risky, RLQ: Right lower quadrant

without being operated on were called up and asked about whether they had undergone an operation within the previous two weeks. AA was diagnosed according to the observation of the pediatric surgeon during the operation and the pathology reports. Those patients who underwent surgery and were diagnosed with AA pathologically were accepted as the AA group, and those who did not undergo surgery or who underwent surgery but pathology results did not confirm AA were accepted as the non-AA group. The demographical characteristics, clinical, radiological and laboratory results, cut-off values of PAS and Alvarado scores, sensitivity and specificity values, positive predictive values (PPV), and negative predictive values (NPV) of the AA and non-AA groups were compared. Permission from the local ethics committee for this study was received from Okmeydani Training and Research Hospital Ethics Commitee (09/02/2016-416).

Statistical Analysis

The SPSS 22.0 (IBM SPSS, Turkey) was used in the analysis of the variables in this study. The conformity of the parameters to normal distribution was evaluated by Shapiro-Wilks test. In the comparison of quantitative data, Student's t-test was used for two groups of parameters with normal distribution and Mann-Whitney U test was used for two groups of parameters without normal distribution. In the comparison of qualitative data, chi-square test and Continuity (Yates) Correction was used. In the cut-off point identification, receiver operating characteristic (ROC) analysis was used, in the sensitivity and specificity calculations, diagnostic screening tests were used. The significance level was accepted as $p<0.05$. To compare qualitative parameters, Pearson chi-square test was used. For detecting cut-off points, ROC analysis was used, and for calculating sensitivity and specificity, diagnostic and screening tests were used. The DeLong method was used to compare two areas under the receiver operating characteristics curve (AUROC). The significance level was accepted as $p<0.05$.

Results

Of the 121 patients who were included in the study with the consideration of AA, 12 were excluded from the study due to a lack of information in the records and 5 because they could not be reached by phone. Of the remaining patients, 53 underwent surgery and one was diagnosed with Meckel's diverticulum and one with ovarian cyst; these 2 patients were excluded from the study. The mean age of the final 102 patients was 9.38±3.90 years (3-15.5 years), and 62 (60.8%) of them were male. Based on the pathological diagnoses, one patient had lymphoid hyperplasia, one patient had normal tissue, and one patient had carcinoid tumor. These 3 patients were excluded from the AA group and included in the non-AA group. The number of patients was 48 (47.1%) in the AA group and 54 (52.9%) in the non-AA group. There was no statistically significant difference between the two groups in terms of age, gender, and admission times. The most common finding (97.9%) of those patients in the AA group was sensitivity in the right lower quadrant. While there was a statistically significant difference between the AA group and the non-AA group in terms of sensitivity in the right lower quadrant, pain in the right lower quadrant with cough/percussion/hopping, migration of pain to the right lower quadrant, rebound tenderness, leukocytosis, or shift to the left, there was no statistically significant difference between the two groups in terms of anorexia, nausea-vomiting, or fever (Table II).

Among the patients in the AA group, the minimum PAS and Alvarado score was 5, the maximum score was 10 and all of patients who had scores of either 9 or 10 were in the AA group, while in the non-AA group, the minimum PAS and Alvarado score was 3, and the maximum score was 8.

For the PAS score, the cut-off value was >7, [95% confidence interval (CI), area under curve (AUC)=0.88±0.3] sensitivity was 66.7%, specificity was 94.4%, PPV was 91.4%, and the NPV was 76.1% ($p<0.001$) (Figure 1). For the Alvarado score, the cut-off value was >7, (95% CI, AUC was 0.87±0.03), sensitivity was 77.1%, specificity was 85.2%, PPV was 82.2%, and NPV was 80.7% ($p<0.001$) (Figure 1). By using the DeLong method, no statistically

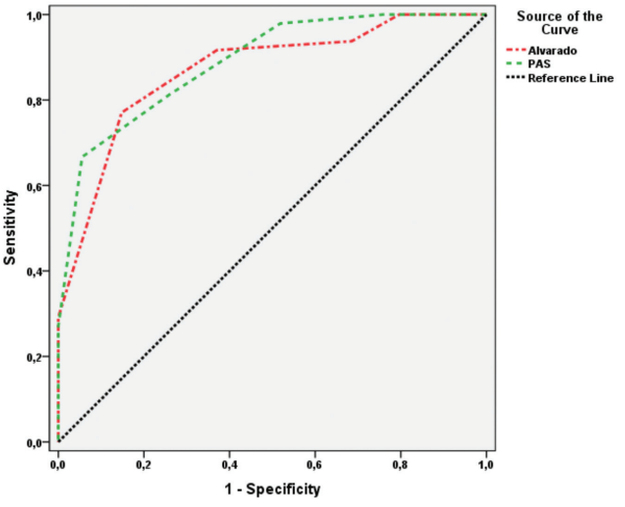

Figure 1. ROC curve of PAS and Alvarado score of patients
ROC: Receiver operating characteristic, PAS: Pediatric Appendicitis score

significant difference was found between the AUROC levels for Alvarado and PAS variables (p=0.530).

The USG findings were positive in 34 (70.8%) patients in the AA group, and 5 (9.3%) patients in the non-AA group. There was a significant difference in USG findings between the two groups (p<0.001) (Table II). For those patients in the AA group, PAS and Alvarado scores were in the intermediate or high risk group. There was statistically significant difference only for patients with Alvarado intermediate score in the comparison of USG results with the PAS and Alvarado scores of intermediate and high-risk group in patients in the AA group (p=0.047) (Table III). Abdominal CT was performed in 4 patients, and the results showed appendicitis in 2 of them, and the other patients underwent an operation after being evaluated by a surgeon.

Of the 51 patients who underwent surgery, negative appendectomy was seen in 3 (5.9%) patients. Of these patients, one had carcinoid tumor, one had lymphadenopathy and one had normal tissue (7-5); and the PAS and Alvarado scores of these patients were 8-8, 7-8, 7-5, respectively. Considering the pathological diagnoses of those patients

Table III. The comparison of ultrasound findings with the scores in acute appendicitis patients

	USG (-)	USG (+)	p
	n (%)	n (%)	
Alvarado			
5+6	20 (37.7)	7 (18.4)	**0.047***
≥7	33 (62.3)	31 (81.6)	
PAS			
5+6	25 (49.0)	11 (28.9)	**0.056**
≥7	26 (51.0)	27 (71.1)	

*: p<0.05, PAS: Pediatric appendicitis score, USG: Ultrasound

Table II. Features of group acute appendicitis and group non-acute appendicitis

	AA (n=48)	Non-AA (n=54)	Total (n=102)	p
	Med ± SD	Med ± SD	Med ± SD	
Age	10.09±3.79	8.75±3.91	9.38±3.90	[1]0.082
Duration (hour)$_{(med)}$	33.98±25.17 (24)	35.31±22.78 (24)	34.69±23.82 (24)	[2]0.386
Alvarado score$_{(med)}$	7.98±1.14 (8)	5.89±1.53 (6)	6.87±1.71 (7)	[2]<0.001*
PAS$_{(med)}$	7.79±1.2 (8)	5.52±1.34 (6)	6.59±1.71(7)	[2]<0.001*
C-reactive protein$_{(med)\ (mg/L)}$	54.51±76.98 (18.58)	31.12±62.52 (12.3)	42.13±70.33 (14.4)	[2]0.038*
Leukocytes count t (10^9/L)	16804.79±4822.02	14211.11±6825.95	15431.67±6079.71	[1]0.031*
Polymorphonuclear neutrophilia a (10^9/L)	12603.37±4220.14	10000.74±5802.04	11225.51±5258.99	[1]0.012*
	n (%)	n (%)	n (%)	
Gender				
Female	17 (35.4)	23 (42.6)	40 (39.2)	[3]0.459
Male	31 (64.6)	31 (57.4)	62 (60.8)	
Signs RLQ tenderness	47 (97.9)	42 (77.8)	89 (87.3)	[3]0.002*
Migration of pain	32 (66.7)	8 (14.8)	40 (39.2)	[3]<0.001*
Cough/hopping/percussion tenderness in the RLQ	36 (75)	18 (33.3)	54 (52.9)	[3]<0.001*
Rebound pain	35 (72.9)	13 (24.1)	48 (47.1)	[3]<0.001*
Elevation of temperature	11 (22.9)	20 (37)	31 (30.4)	[3]0.122
Anorexia	38 (79.2)	42 (77.8)	80 (78.4)	[3]0.865
Nausea/vomiting	36 (75)	35 (64.8)	71 (69.6)	[3]0.264
Leukocytosis ≥10 10^9/L	46 (95.8)	40 (74.1)	86 (84.3)	[3]0.003*
Polymorphonuclear neutrophilia ≥75%	44 (91.7)	36 (66.7)	80 (78.4)	[3]0.002*
USG	34 (70.8)	5 (9.3)	39 (38.2)	[3]<0.001*

[1]: Student t-test, [2]: Mann-Whitney U test, [3]: chi-square test ,*: p<0.05, PAS: Pediatric appendicitis score, RLQ: Right lower quadrant, AA: Acute appendicitis, Non-AA: non-acute appendicitis, SD: Standard deviation, USG: Ultrasound, Med: Median

in the AA group, 31 (64.6%) patients were diagnosed with phlegmonous appendicitis, 9 (18.7%) with gangrenous appendicitis, and 8 (16.7%) with perforated appendicitis. Of the patients in the non-AA group, 18 (35.3%) were diagnosed as acute gastroenteritis, 15 (29.4%) with constipation, 13 (25.5%) with mesenteric lymphadenomegaly, 2 (3.9%) with urinary tract infections, 1 (1.9%) with dysmenorrhea, 1 (1.9%) with Henoch-Schönlein purpura, and 1 (1.9%) with nephrolithiasis.

Discussion

Sensitivity in the right lower quadrant is the most common finding in appendicitis and is reported at a frequency of 78-100% in different series (1,15). Among other symptoms compatible with appendicitis, the shift of pain to the right lower quadrant is seen at a frequency of 33-69%, rebound pain at a frequency of 15-68%, and pain in the right lower quadrant with cough/percussion/jumping at a frequency of 64-83.2% (15,18-21). Leukocytosis frequency is 83-93% and an increase of the neutrophil ratio frequency is seen in 75-96% of cases with appendicitis (18-21). In our study, these ratios were found to be significantly higher than those of the non-AA group (p=0.003; p=0.02).

Samuel (13) determined the cut-off value to be 6 for the PAS. The cut-off value has been reported to be between 7-10, sensitivity to be between 61-86%, and specificity to be between 50-96% for the PAS in the different studies (15,19-22). The PPV values were between 50.7% and 90.1%, and NPV values were between 38% and 87.9% (15,19,20,23,24). Similarly, the cut-off value was >7, sensitivity was 66.7%, specificity was 94.4%, PPV was 91.4%, and NPV was 76.1% in our study. The cut-off values for the Alvarado score were similar to PAS. While the cut-off value was 7, the sensitivity was 68.5% to 89%, specificity was 59% to 81%, PPV was 54.9% to 93.1% and NPV was 46% to 85.3% (15,18-20,23,24). Similarly, in our study, the cut-off value was >7, while the sensitivity was 77.1%, specificity was 85.2%, PPV was 82.2%, and NPV was 80.7% for the Alvarado score. In our study, all of the patients had intermediate or high-risk scores for both PAS and Alvarado scores. On the other hand, when analyzed with ROC, there was no statistically significant difference in sensitivity, specificity, PPV and NPV between the PAS and Alvarado scores.

In the diagnosis of AA, the sensitivity of abdominal USG varies according to the experience of the practitioner, the visualization of the appendix, the gender of the patient, the patient's body weight, and the visualization of the perforated appendicitis. The abdominal USG sensitivity for AA diagnosis varies between 53% and 88.2% and specificity between 84% and 93% in different series (10,16,23,25-28). On the other hand, the false positive USG frequency is seen to be between 5% and 30% (23,29-31). It has been suggested that the USG positivity and diagnosis rate increases as the duration of pain increases in these patients (27). Although Alvarado and PAS recommends radiological methods, especially USG, for those patients with intermediate scores, some other centers also recommend using radiological methods for those patients with lower scores (28,29). In our study, abdominal USG was found to be positive in 70.8% of those patients in the AA group, and in 9.3% of those patients in the N-AA group. Sincavage et al. (25) showed intermediate risk AS (4-6), US was positive for appendicitis in 21%. Our study had similar results.

Perforation can be a fatal complication in AA in children. Perforation frequency between 7.5% and 30% has been reported in various studies (16,29,32,33). According to the results of the pathologic examinations, the simple or phlegmonous appendicitis is reported at a frequency of 25% to 57.1%, gangrenous or suppurative appendicitis at a frequency of 34% to 45%, and perforated appendicitis at a frequency of 12% to 21% (4,32,33). In our study, 31 (64.6%) patients were diagnosed with simple appendicitis, 9 (18.7%) patients with gangrenous appendicitis, and 8 (16.7%) patients with perforated appendicitis.

In children, negative appendectomy rates are reported between 3.7% and 13% in different series (7,8,16,32,33). While it is expected that the rate of negative appendectomy in high-score patients is low, Zúñiga et al. (33) found negative appendectomy to be at a rate of 4.95% in those with a PAS score above eight. In our study, all patients with a score of more than 8 were in the AA group. The pathologic diagnoses were normal tissue, fecaliths, lymphoid hyperplasia, pinworm, granuloma, Meckel's diverticulum, granuloma, fibrose obliteration, and carcinoid tumor (34,35). Three (5.9%) patients had a negative appendectomy. The scores of these 3 patients were between 5 and 8 and they were diagnosed as normal tissue, lymphoid hyperplasia and carcinoid tumor pathologically. The patient who was pathologically diagnosed with carcinoid tumor had an Alvarado score of 8, a PAS score of 8, leukocytosis, and shift to the left, but no USG findings. If positive USG findings, leukocytosis and neutrophyl count >75% are not observed together in a patient, close follow-up and radiologic re-evaluation should be considered.

Study Limitations

Firstly, this study was conducted with a limited number of patients. Secondly, the patients were not all examined

by the same physician. Finally, the fact that the patients did not apply within the same period following the onset of the complaint is thought to affect the clinical staging, laboratory findings, and radiological findings.

Conclusion

AA is a condition that is still hard to diagnose in children presenting with acute abdominal pain in the emergency department. Although both Alvarado and PAS scores provide useful information in patients suspected of having AA in the pediatric emergency department, neither of them is enough to diagnose AA alone. The possibility of AA is high in children with a PAS and Alvarado score of 5 or more in the presence of radiological findings. There is a need for new parameters in the scoring system.

Ethics

Ethics Committee Approval: Permission from the local ethics committee for this study was received from Okmeydani Training and Research Hospital Ethics Commitee (09/02/2016-416).

Informed Consent: Consent forms were obtained from the parents or the children.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.T., A.K., E.D., S.K., A.A.G., C.A., Ö.E.G.T., A.Kan., O.Ö., Design: Y.T., A.K., E.D., S.K., A.A.G., C.A., Ö.E.G.T., A.Kan., O.Ö., Data Collection or Processing: A.K., S.K., A.A.G., Ö.E.G.T., A.Kan., Analysis or Interpretation: A.K., Y.T., C.A., A.Kan., O.Ö., Literature Search: S.K., Ö.E.G.T., Writing: Y.T., E.D.

Conflict of Interest: None of the authors had conflict of interest.

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Original Article

Views of Maternity Nurses Relating to Barriers in Early Initiation of Breastfeeding: A Qualitative Study

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ABSTRACT

Aim: Although breastfeeding is common in Turkey, the proportion of breastfed infants in the first hour after birth is not at the desired level. The aim of this study was to explore the difficulties in the early initiation of breastfeeding as encountered by maternity nurses.

Materials and Methods: A phenomenology model of qualitative research design was used in this study. The data were collected by an in-depth interview method. Content analysis was used to analyze the data, and findings were categorized into themes.

Results: Barriers related to the early initiation of breastfeeding was grouped into four main themes: Delivery, culture, hospital environment and health care staff. The delivery mode, pain and the perception of insufficient milk were included in the theme of 'delivery'. The theme of 'culture' consisted of traditional practices, patriarchal family structure, and language sub-themes. The theme of 'hospital environment' involved lack of privacy and chaos. Finally understaffing, insufficient knowledge and employee turnover rates were included in the 'health care staff' theme.

Conclusion: Interviews with maternity nurses about barriers to the early initiation of breastfeeding provided deeper understanding into this critical period. These findings suggest that delivery related factors and cultural and environmental elements were obstacles in initiating early breastfeeding. Support and counseling given in the early postpartum period is important for the initiation of breastfeeding. To eliminate the barriers in this process, it is recommended to improve training and health care policy.

Keywords: Breastfeeding, breastfeeding initiation, breastfeeding barriers, nurse, qualitative research

Introduction

Breastfeeding is fundamental for an infant's survival, nutrition, growth and development, and maternal health. Early initiation of breastfeeding, defined by the World Health Organization (WHO) as giving mother's first milk, known as colostrum, to infants within the first hour of birth, provides protective factors against illnesses. The WHO recommends that children initiate breastfeeding within the first hour of birth and be exclusively breastfed for the first 6 months of life, followed by continued breastfeeding with suitable supplementary foods for up to two years or beyond. In 2012, a universal practice plan on maternal, infant and young child nutrition specifying six global nutrition targets for 2025 was endorsed. One of these targets was to increase the rate of exclusive breastfeeding in the first six months up to at least 50%. Unfortunately, breastfeeding practices are not at a desirable level throughout the world. Currently, the global average of exclusively breast-fed infants under 6 months is only 37% (1). The United Nations Children's Fund (UNICEF) indicate that most of the world's newborns are waiting too long to begin breastfeeding, and in 2017 alone, an estimated 78 million newborns had to wait more than 1 hour to begin breastfeeding (2).

The WHO and UNICEF published a joint statement in 1989 on "Protecting, promoting and supporting breastfeeding: the special role of maternity services", in

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order to support mothers and optimize the opportunity of breastfeeding in line with its recommendations. This statement listed "Ten Steps to Successful Breastfeeding". Afterwards, the WHO published the Baby-friendly Hospital Initiative in 1991 and updated it in 2009 (1,3). Since 1991, the "Promotion of Breastfeeding and Baby-friendly Health Facilities Program" which aims to protect, encourage and support breastfeeding has been carried out in Turkey. This program, which follows the recommendations of the WHO, promotes the key message that babies should start breastfeeding immediately after birth and only be breastfed for the first six months, with breastfeeding continuing until the age of two supplemented by appropriate food after the first six months (4). Breastfeeding is quite common in Turkey. According to the results of the Turkish Demographic and Health Survey 2013, 96.4% of babies were fed with breast milk for at least a while. However, only 49.9% of babies started to breastfeed within the first hour after birth. The rate of other liquids given instead of breastmilk is 25.7% three days after birth. 57.9% of babies are fed exclusively by breastfeeding until the second month. The rate of exclusively breastfed babies until the fourth month is 35.4%. This rate decreases rapidly with the child's age, and the proportion of bottle users increases with age (5). Also, in 2017 the Turkey Health Statistics Report, it was reported that 30.8% of infants between 0-6 months and 32% of infants between 7-12 months are breastfed (6). Although breastfeeding is common in Turkey, exclusively breastfed rates are not at the desired level.

Early initiation is important for the effectiveness and continuity of breastfeeding. Early breastfeeding has a positive effect on lactation, uterine involution, mother-infant interaction and the baby's development. The successful initiation of breastfeeding concerns the events and practices during labor, birth and the immediate post-birth period (7). For the initiation of breastfeeding within the first hour of life, mothers need adequate support, direction and encouragement on the positioning and breastfeeding of their newborns (8).

Causes such as the mother's knowledge of the benefits and skills of correct breastfeeding techniques, the mother's social, cultural, and economic status and support systems (particularly the family and health system personnels' skills) affect the initiation of breastfeeding (7). For example, there are some cultural practices such as feeding newborns supplemental foods or drinks by an elderly family member (8). Pre-lacteal feeding is defined as the administration of any foods or liquids other than breast milk to an infant before the initiation of breastfeeding and it can delay the initiation of breastfeeding. The practice of offering pre-lacteal feedings to a newborn is reported as an early mechanism for the disruption of exclusive breastfeeding (1). Other factors and reasons for missing out on breastfeeding in the first hour of life are lack of knowledge, caesarean delivery, and immediate separation of mothers and babies after birth (8).

Starting and maintaining a successful breastfeeding process is influenced by many factors. To ensure the success of lactation, the support of nurses in the early postpartum period is important. Resolving problems related to breastfeeding is one of the tasks of nurses. However, few qualitative studies have been conducted that specifically addressed the experiences of nurses regarding breastfeeding. Understanding the experiences of maternity nurses may help healthcare professionals to prepare education programs and care guides that enhance the quality of nursing care. In addition, these programs and guides may help healthcare professionals to recognize the early detection of breastfeeding barriers and support women to overcome these barriers in the early postpartum period. Therefore, this study was conducted to determine the difficulties in the early initiation of breastfeeding encountered by nurses working in maternity clinics.

Materials and Methods

Design

We used a phenomenological model of qualitative research design in this study.

Setting and Participants

We collected data between June and December 2018 in a university and two state hospitals. The hospitals were Baby-friendly Health Initiative accredited. The participants were nurses working in maternity clinics. The inclusion criteria were as follows: Working in a maternity clinic for a minimum of six months, voluntary participation, experience with breastfeeding counseling.

We used a purposive sampling method to select nurses in the study. There were 18 nurses working at the maternity clinics. Data collection continued until saturation had been achieved and no new themes were emerging (9). The study was completed with thirteen nurses.

Data Collection

We obtained the maternity nurses' work schedule from the chief nurse, went to the clinic diurnally and determined those nurses who met the study's sample selection criteria. We informed nurses in the study group about the

study purpose and obtained their consent. We made an appointment with the maternity nurses for the end of their shift. We collected data by the in-depth interview method in the clinic's meeting room. The interviews with nurses were conducted individually using a semi-structured interview form. The interviews were tape-recorded and additional written notes were taken.

The interview started with meeting and informing the participant. First, those questions on the participant's descriptive characteristics form were asked. Then, the interview continued with the first question on the semi-structured interview form: "What are the barriers you have to the early initiation of breastfeeding at the clinic?". During the interview, the researchers let participants speak freely and discuss opinion and thoughts. Interviews were varied in time, they lasted between 38 and 52 minutes.

We used a participant descriptive characteristics form and semi-structured interview form to collect the data. The participant descriptive characteristics form included questions on socio-demographic properties such as age, gender, education level, work experience, having children and breastfeeding status. As part of the qualitative research method, data were collected by a semi-structured interview form (Table I). It was developed based on the purposes of the study in light of the relevant literature (10-12).

Statistical Analysis

Data analysis was performed by the researchers. Sociodemographic variables were expressed as means and numbers.

We evaluated qualitative data by content analysis. According to this procedure, firstly one researcher transcribed the interviews directly into the computer without any changes. While listening to the tape recordings "who said what" was noted. Next, two researchers read the written interviews a few times individually to find out about them generally. After reading, these researchers disintegrated the text into meaningful parts, separated the content, and each significative part was attached with a code. Then, these codes were compared with regard to their similarities and differences and organized into categories. Finally, we classified and reported the data. After the classification process, themes were identified and named (9).

Table I. Semistructured interview form

Questions
1. What are the barriers you have with the early initiation of breastfeeding at the clinic?
2. What are the obstacles caused by delivery related factors in the early initiation of breastfeeding?
3. What are the obstacles caused by cultural factors in the early initiation of breastfeeding?
4. What are the obstacles caused by the hospital environment in the early initiation of breastfeeding?

We worked individually and independently from one another within these processes. Afterwards, we discussed the thematic statements and came to an agreement on the themes which described the results best.

Trustworthiness

The trustworthiness criteria in qualitative research are credibility, transferability, dependability and confirmability (9).

The first researcher conducted the interviews, and the second researcher observed and recorded the nurses' behavior in order to enhance study trustworthiness. In order to support the honesty of the participants, the nurses who volunteered to participate in the study were included in the study and it was explained that they could withdraw from the study without giving any reason. It was also stated that there were no correct answers to the questions asked to them. Furthermore, peer debriefing and member checking were used to enhance credibility. To ensure transferability of the study, purposive sampling was used. Also, during the interviews, a tape-recorder was used and additional written notes were taken. After the interviews, the recordings were copied word for word, the facial/bodily expressions of nurses were described by two of the researchers independently without additional interpretation as to their intent or meaning. For consistency, the data were analyzed independently by two researchers. Researchers resolved differences between the findings by consensus, and arranged and documented the results. For confirmability, an expert evaluated the comprehensive interview form and the final version of the thematization.

Ethical Considerations

Approval of the Ethics Committee and permission of the institution were taken (decision no: 60116787-020/29033). Verbal and written permission was obtained from the participants before the interviews commenced and were tape-recorded. In the presentation of the results, participant comments remain anonymous.

Results

Demographic Information

A total of 13 maternity nurses were included in this study. In the current study, all of the participants were female and ranged in age from 32 to 48 years. All of the participants were married, had at least one child, and all of

them gave their children breast-milk for at least 6 months. Five of the nurses had undergraduate degrees. Four had associate degrees, and four were high school graduates. The mean working years of their maternity nursing was 9.15 years. All of them were trained about breastfeeding consultancy (Table II).

The findings taken from the interviews were classified into four main themes: (1) delivery, (2) culture, (3) hospital environment, and (4) health care staff (Table III).

Delivery

This theme was investigated through three subthemes; delivery mode, pain and perception of insufficient milk.

Delivery Mode

Most of the participants expressed their belief that the delivery mode had an effect on the initiation of breastfeeding. According to the statements of the participants, cesarean delivery negatively affected the baby's first breastfeeding time, the mother's breastfeeding skill and the baby's sucking skill. In particularly, they noted: "Breastfeeding skills in cesarean birth are related to us and the sitter... Her (the mother) breastfeeding skill is passive... She is dependent on us... It is hard to get into position for breastfeeding after the mother starts to come to herself..." (N4).

Pain

The participants stated that mothers did not want to breastfeed because of pain after birth: "...she does not want to breastfeed because she is in pain..." (N8).

Perception of Insufficient Milk

All of the participants indicated that the mothers thought their breastmilk volume to be insufficient right after birth, especially in the case of a cesarean delivery. Here is a typical comment: "... In general, it is known among the people that lactation is late... In fact, the mother has milk, but the baby cannot feed, because it does not suck properly... "(N7).

Culture

Cultural barriers to the initiation of breastfeeding were traditional practices, patriarchal family structure and language.

Table III. Themes and subthemes of obstacles to breastfeeding initiation

Themes	Subthemes
1. Delivery	Delivery mode
	Pain
	Perception of insufficient milk
2. Culture	Traditional practices
	Patriarchal family structure
	Language
3. Hospital environment	Lack of privacy
	Chaos
4. Health care staff	Understaffing
	Insufficient knowledge
	Employee turn rates

Table II. Sociodemographic characteristics of participants

Participants no	Age	Gender	Marital status	Education level	Situation to have children	Breastfeeding status	Work experience in maternity clinic (year)	Breastfeeding consultancy education
N1	40	Female	Married	High school	Yes	Yes	13	Yes
N2	44	Female	Married	Undergraduate	Yes	Yes	3	Yes
N3	47	Female	Married	High school	Yes	Yes	5	Yes
N4	41	Female	Married	High school	Yes	Yes	9	Yes
N5	38	Female	Married	Associate degree	Yes	Yes	4	Yes
N6	48	Female	Married	High school	Yes	Yes	4	Yes
N7	42	Female	Married	Associate degree	Yes	Yes	16	Yes
N8	43	Female	Married	Associate degree	Yes	Yes	12	Yes
N9	39	Female	Married	Undergraduate	Yes	Yes	8	Yes
N10	35	Female	Married	Associate degree	Yes	Yes	11	Yes
N11	32	Female	Married	Undergraduate	Yes	Yes	10	Yes
N12	38	Female	Married	Undergraduate	Yes	Yes	15	Yes
N13	36	Female	Married	Undergraduate	Yes	Yes	9	Yes

Traditional Practices

The participants stated that traditional practices, such as giving the baby sherbet and putting a date in the baby's mouth, prevented the first food from being breast milk and caused problems in the initiation of breastfeeding. For example: "... The other day, I trained a mother about breastfeeding. After a while, I went back to the patient's room. There was something dark around the child's mouth. I think it was a date. I panicked thinking it was blood at first. Then I touched something sticky. Then I looked around... They had soaked the date in a cup and put napkins on top of it to make it clear" (N5).

Patriarchal Family Structure

Most of the participants indicated that they had difficulty in the initiation of breastfeeding because of elderly family members. A typical participant's statement on this concern was as follows: "... Especially if her mother-in-law or mother are with her, you need to be careful. They may want to learn everything first. They may want to do their own practice. This prevents communication with the mother..." (N6).

Language

In our study, all of the participants stated that one of the barriers to breastfeeding initiation was the language obstacle. Participants stated that immigrant/foreign mothers didn't know Turkish so they communicated with them using signs and gesture, for example: "...We often cannot communicate with immigrants. No interpreter... We are trying to communicate with them through body language. But how much can you tell with gestures! I can only show the way of breastfeeding. She showed how to hold the breast during breastfeeding on her body with her fingers. But the other parts are missing..." (N9).

Hospital Environment

The participants stated that the hospital environment was obstructive to breastfeeding because the lack of privacy and a chaotic environment creates barriers to breastfeeding.

Lack of Privacy

The participants shared their perception that they had difficulties with privacy. Here is a statement on the subject of privacy: "...Sometimes, the mother doesn't want to be talked about too much when her mother-in-law is present. For example, your nipple is too small. The woman feels disturbed when you say that. After all, she is a woman too...." (N10); "...Visitors do not respect the privacy of the patient at the time of breastfeeding. They do not want to leave the room. They say, it is my right..." (N13).

Chaos

The participants described the first moments after birth as chaotic for the mother: "A baby is crying in her lap... A continuous warning from the surroundings... The doctor says give the baby this. The nurse says do that... relatives say that we did this, we gave water... The mother is not aware of what you are saying or doing. She has just given birth. She has pain. There is a child crying. Everyone is saying something. She feels in the middle of complete confusion, chaos" (N3).

Health Care Staff

They said that initiating breastfeeding in the early period has some difficulties due to understaffing and employee turnover rates. It was also noted that some of the clinic staff were not sufficiently knowledgeable on the subject of breastfeeding.

Understaffing

The participants stated that they could not provide the necessary care and support for the initiation of breastfeeding due to understaffing. The workload was one of the obstacles to the early initiation of breastfeeding due to the multitasking structure of their work. Here is a typical comment on the subject of understaffing: "The number of patients is too many. The number of nurses is insufficient... There are both gynecology and maternity patients in our clinic. They also have treatments and follow-ups. We may have to be less concerned with breastfeeding" (N1).

Insufficient Knowledge

Some of the participants indicated that the healthcare staff did not have enough knowledge about breastfeeding: "The mother's breasts are swollen and stretched. The formula is started because there is no breast milk. Actually, there is breast milk, but the baby does not suck it properly. Unfortunately, both nurses and doctors have difficulties in evaluating breastfeeding..." (N12).

Staff Turnover Rates

Most of the participants pointed out the negative effects of staff turnover. They stated that they could not always speak a common language with other healthcare staff because of high staff turnover rates and that they had difficulties in the initiation of breastfeeding as a result. According to the statements of the participants, staff turnover leads to a lack of knowledge and the spread of some wrong practices related to breastfeeding. For example: "We may not speak a common language to discuss breastfeeding with other health personnel at the clinic. It is

caused by very rapid clinical changes of nurses and doctors. Also, different applications are emerging" (N2).

Discussion

The statistics related to breastfeeding in Turkey show that there are some problems in the initiation of breastfeeding (5). In Turkey, the healthcare staff who are seen first after the mother and baby leave the delivery room and who should support the initial breastfeeding are usually nurses in charge at the maternity clinics (13). It is important to examine in depth the practices of these nurses and the connected influencing factors. Therefore, this study was conducted to define the perceived barriers to the initiation of breastfeeding at the early postpartum period by those nurses working in maternity clinics. The identified barriers perceived as a result of the interviews, analysis and interpretation, were grouped into four themes, namely; delivery, culture, hospital environment and health care staff.

Breastfeeding problems are frequently encountered in the early postpartum period, particularly for women who have had a cesarean birth (14,15). In this study, it was determined that cesarean delivery delayed the initiation of breastfeeding. According to a qualitative study by Majra and Sılan (12), cesarean section causes about a day's delay in the initiation of breastfeeding. Adverse effects of anesthesia on the mother and infant, delayed lactation, maternal discomfort and pain are connected with the effects of cesarean deliveries (16). It has been reported in the literature that mothers who have spontaneous vaginal births have a higher rate of breastfeeding in the first hour than those who have a cesarean delivery (10,17,18). These findings are similar to our study.

Another problem is the perception of insufficient breast milk because the mother feels she does not have enough lactation to meet her baby's needs. This is all related to the mother's perceptions (19). In our study, participants expressed the notion that the perception of insufficient breast milk was an important barrier to the initiation of early breastfeeding, especially in cesarean deliveries. Similarly, it was found that mothers gave their baby supplementary formula in the first 72 hours after birth because of the perception of low milk. This was one of the results of a qualitative study examining the effects of cesarean delivery on breastfeeding (20). According to another qualitative study about barriers to exclusive breastfeeding in American Samoa conducted by Hawley et al. (21), mothers thought that they did not have enough lactation to meet their babies' needs and thus the mothers supplemented their breastfeeding with formula. Nguyen et al. (22) reported that 7.9% of the mothers included in their study were aware that newborn babies need only 5-7 mL of milk per feed in the first day of life, and half of the mothers felt confident in their ability to provide enough lactation to exclusively breastfeed for the first 24 hours.

The ten steps of successful breastfeeding recommend that newborns are to be given no nutrient or drink except for breastmilk, unless medically indicated otherwise (1,4). Cultural practices that encourage the giving of other liquids instead of breastmilk and pre-lacteal feeding caused a delay for the optimal timing of breastfeeding initiation (10,12,22-24). Hmone et al. (11) expressed that traditional practices and the intervention of family elders negatively affected breastfeeding. In a qualitative study by Lee et al. (25), they reported that the elders of the family cause a delay in the early initiation of breastfeeding. These outcomes are similar to the findings of our study.

In Turkey, the number of irregular migrants is 174,466 and the number of foreigners holding a residence permit is 461,217. By the year 2017, the count of Syrians covered by temporary protection was 3,400,195 and the number of Syrians outside temporary shelters was 3,171,721. Of these, 1,564,897 are women, and about half of these women are fertile (26). One of the obstacles preventing immigrant women from benefiting from prenatal and postpartum health services is language (27). Similarly, in our study, one of the obstacles encountered by the participants was that they could not communicate with migrant mothers because of language problems.

The other important barrier was privacy. In our study, participants expressed that they had problems with privacy due to crowded patient rooms. Postnatal wards and busy postpartum units are public places for many new mothers where privacy cannot be provided (28). Morrison et al. (29) discovered that mothers try to breastfeed but found their personal boundaries were violated in hospital. According to a qualitative study conducted in Turkey, new mothers had the perception that their privacy was severely curtailed during breastfeeding in a maternity clinic because they were uncomfortable with visitors present and thought that hospitals were not places where personal space was honored (30).

The results of our study, similar to others, confirmed that workload, understaffing and high staff turnover rates have an unfavorable impact on the early initiation of breastfeeding (12,20,25). Moreover, in our study, mothers being given conflicting information about breastfeeding

was highlighted. Studies have shown that breastfeeding training and counseling given to mothers by healthcare personnel positively affects breastfeeding (31,32). However, the information provided should be consistent. Similar to the findings of other research (12,20), it was determined that conflicting information given to mothers by healthcare personnel was a cause of confusion and led to a failure to initiate breastfeeding by mothers.

Study Limitations

Because of the qualitative nature of this study, generalizability is low. The results of this study being specific to the limited context, qualitative findings cannot be generalized to all hospitals in Turkey.

Conclusion

Interviewing nurses early about barriers to the early initiation of breastfeeding provided a deeper understanding of this critical period. As a result of our study, the barriers to the early initiation of breastfeeding were related to factors concerning delivery, culture, hospital environment and health care staff. These barriers can be overcome when pregnant women/new mothers are briefed on the procedure by healthcare staff. For this reason, prenatal education is important in overcoming obstacles related to delivery and culture. Immigrant-friendly hospitals with on-site interpreters are a solution for language-related obstacles. In overcoming the obstacles related to the hospital environment, it is suggested that a positive, calm, privacy-friendly environment be ensured. To increase the competence of health personnel, there is the need for the sufficient number of healthcare staff to be employed and regular training of staff to be given.

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Ethics

Ethics Committee Approval: Approval of the Ethics Committee and permission of the institution were taken (decision no: 60116787-020/29033).

Informed Consent: Verbal and written permission was obtained from the participants before the interviews commenced and were tape-recorded.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.S.C., B.Ç., Design: S.S.C., B.Ç., Data Collection or Processing: S.S.C., B.Ç., Analysis or Interpretation: S.S.C., B.Ç., Literature Search: S.S.C., B.Ç., Writing: S.S.C., B.Ç.

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Original Article

The Effect of Fatigue-reducing Interventions on the Fatigue Levels of Children with Cancer: A Meta-analysis Study

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ABSTRACT

Aim: This research was conducted to analyze the effect of fatigue-reducing interventions on the fatigue levels of children with cancer.

Materials and Methods: This quantitative study based on the literature relating to those interventions to reduce fatigue in children with cancer was carried out. No time limits were defined for the literature review; instead, all available studies published until February 2019 were reviewed. Data were collected from Pubmed, Cochrane, EBSCO Host, ScienceDirect, Embase/Elsevier, and Web of Science databases. The keywords "child", "cancer", and "fatigue" were used during the search process. The effect sizes and group comparisons of each study were analyzed using The Comprehensive Meta-analysis statistical software package for meta-analysis.

Results: The total sampling number of the five studies included in the study was 500. As a result of the heterogeneity test, it was determined that these studies involving interventions to reduce the fatigue of children with cancer had heterogeneous characteristics. The result of a publication bias test found no publication bias. The average effect size at 95% significance level was calculated as 1.452 for the study by Li et al. (2018), 0.560 for the study by Ramezani et al. (2018), 7.606 for the study by Kudubeş et al. (2019), -0.995 for the study by Lam et al. (2018), and 0.544 for the study by Vieira et al. (2015).

Conclusion: In this study, fatigue-reducing interventions were found to affect fatigue level.

Keywords: Child, cancer, fatigue, fatigue-reducing interventions, meta-analysis

Introduction

Children with cancer experience various symptoms due to the treatments that they receive. These symptoms include anemia, neutropenia, nausea, vomiting, alopecia, mucositis, and fatigue (1,2). Fatigue is one of the symptoms that plays a significant role in caring for pediatric oncology patients. The literature states that 51 to 86 percent of pediatric oncology patients have cancer-related fatigue (3,4).

Fatigue is a multidimensional concept which can bring about biopsychosocial and cognitive effects and change subjectively in childhood cancer. It also affects the individual's well-being, capacity to accomplish self-care, relationship with his/her environment, and state of enduring illness-related problems (5). Fatigue is a symptom that prevents the individual from working regardless of the size of the activity and constantly distresses the patient regarding their cancer. Fatigue associated with cancer in children is a deep feeling of exhaustion which is affected by various conditions, causes a lack of attention, creates negative emotions, affects play, and makes it difficult to move the limbs or even open the eyes (6).

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There are many reasons why cancer-related fatigue is often ignored by health professionals. As fatigue is an individual experience, the person tries to solve it without sharing with others. Moreover, fatigue is seen as being part of the nature of the disease process by healthcare personnel. Therefore, during the planned treatment to prolong the patient's life, this symptom can easily be ignored. However, fatigue, which is a symptom perceived by the patient, needs to be correctly identified. Fatigue should be identified in detail in all stages of the disease and its treatment and it should be controlled and intervened with using a multidisciplinary approach (7,8).

The role and autonomy of nurses in symptom management of pediatric oncology patients is quite significant. The nurse is responsible for assessing the findings of fatigue symptoms that have an important effect on the child's quality of life (QoL) and the factors that affect them. In addition, it is emphasized that the nurse should plan appropriate fatigue-reducing interventions and provide relevant training to the children and their families (9-12). In the literature, it has been reported that fatigue training given to children suffering from cancer and their families reduces the fatigue levels of these patients compared to the pre-treatment period. In addition, training for symptom control relieves children, improves the life quality of these children, and provides trust in healthcare personnel (10-12). There are many intervention options to reduce the fatigue of those children with cancer. These options include managing the fatigue symptoms, regulating activity, ensuring adequate and balanced nutrition, regulating the sleep schedule, and coping with stress (9-12).

A review of studies on reducing the fatigue levels of pediatric oncology patients has revealed that there are limited quantities of meta-analysis studies available. It has been found in these studies that exercise interventions, nutritional support, and training plans are available to reduce fatigue in pediatric oncology patients (12-14). However, the number of studies is limited and the majority of them relate to the adult population. This variation in study findings makes it difficult to come up with a straightforward conclusion on the matter. This situation requires the subject to be studied using sophisticated statistical methods. The meta-analysis approach is one of those statistical approaches (15,16). Meta-analysis is defined as the grouping of similar studies on a topic, theme or field of study under certain criteria and integrating and interpreting the quantitative findings of these studies (17). Meta-analysis differs from traditional statistical methods. While a significant difference is sought in traditional methods, in meta-analysis, the effect size of this difference and the direction of this effect make up the focal point of the analysis (18,19). However, there are very few studies investigating the effect of fatigue-reducing interventions on the fatigue level of pediatric oncology patients using the meta-analysis method. In our country, no studies investigating this topic using the meta-analysis method have been found. That constituted the key starting point for this study's planning. The purpose of this study was to synthesize the results of studies investigating the effect of fatigue-reducing interventions on the fatigue levels of pediatric oncology patients using the meta-analysis method. The results of this study are thought to contribute to the planning of care for nurses, who play a major role in managing the symptoms and improving the QoL of pediatric oncology patients. It is also expected that this study will provide researchers with a new vision for future studies.

Materials and Methods

This study aimed to analyze the effect of fatigue-reducing interventions on the fatigue levels of children with cancer.

Research Question

What is the size of effect of fatigue-reducing interventions on the fatigue levels of children with cancer?

Literature Review

Within the framework of this study, observational research on fatigue-reducing interventions in children with cancer were examined in order to evaluate the impact of these fatigue-reducing interventions on the fatigue rates of children with cancer. Since there was a limited number of meta-analysis studies on fatigue in pediatric oncology patients, no time limits were established; instead, all available studies published until February 2019, when the literature review was performed, were reviewed. Pubmed, Cochrane, EBSCO Host, ScienceDirect, Embase/Elsevier and Web of Science databases were searched. Printed publications accessed in this way were researched, but congress papers were not researched. During this search, the keywords "cancer", "child" and "fatigue" were used in Turkish and English. During the data collection phase, full-text research papers published on this subject were utilized.

Inclusion Criteria

The following criteria were used for the inclusion of the accessed papers in the meta-analysis: (a) having a sampling of children with cancer, (b) having quantitative analysis data, (c) analyzing fatigue variables in children with cancer, (d) sufficient statistical data for calculating the size of the

effect, (e) analyzing at least one of the fatigue-reducing interventions, and (f) access to the full-text version of the study.

Figure 1 presents the flow diagram which summarizes the process of including studies in the meta-analysis. A total of 2,370 studies were obtained through the database search. Hundred and ten duplicate studies were removed. Two thousand two hundred and sixty studies were reviewed and 2,236 of them were removed based on their titles. Two researchers reviewed the abstracts of the remaining 24 studies according to inclusion and exclusion criteria, and their full-text copies were analyzed where appropriate. Nineteen studies which did not meet the requirements for inclusion were excluded. The lack of the fatigue variable or differences in the study population were among the reasons for removal. As a result, this study was based on a total of 5 previously published studies. Two researchers independently carried out the collection of these studies for meta-analysis. When the selection process for the two researchers was later measured, it was found that there was a 100 percent agreement on the researchers' paper selection. The studies included in the review have been planned according to the "PRISMA Flow Diagram Directive". There is a representation of this in Figure 1 (20).

Coding of the Studies

A form of data coding has been used to gather data from the 5 studies included. The researchers developed the form using the related literature (21,22). The data coding form was used to obtain the statistical data and the characteristics of the sample method, measurement, measuring devices, type of publication, etc. needed to measure the effect size from each sample. The title of the report, author, year of publication, form of the report, design, sample size, findings and conclusion were coded for each study using "Cohen's d effect size". Comparing the coding of the first researcher with second researcher ensured reliability of the coded results. A value of positive effect size would indicate that fatigue-reducing interventions affect fatigue in children with cancer positively, whereas a negative value would indicate that fatigue-reducing interventions affect fatigue in children with cancer negatively. It can therefore be concluded that if the effect size is zero (0) or close to zero, the fatigue-reducing interventions do not have any effect on fatigue.

Statistical Analysis

The study employed the Group Difference method, one of the group comparison meta-analysis types. The comparisons of the effect sizes and the groups of each study were performed using the CMA software package (The Comprehensive Meta-analysis software). Hedges' g was used to measure the effect size because of variations in sampling and measuring instruments (15,23). Hedges' g is determined by dividing the product of standardized mean difference between groups by means of a combined standard deviation of the two groups (23). Cohen (24) says that the effect size is small if it is less than 0.20 and large if it is greater than 0.80. According to this classification, an effect size of d <0.20 is small, 0.20< d <0.80 is medium, and d ≥0.80 is large. In meta-analysis studies, a fixed-effect or a random-effect model is used according to heterogeneity (15). If the universe effect sizes of the studies in the meta-analysis do not change, the fixed-effect model is employed, whereas the random-effect model is used if the universe effect sizes vary from study to study. In this study, a random-effect model was employed because of the heterogeneity of the studies, which was determined as a result of homogeneity tests (15). Cochran's Q statistics, p-value, and I^2 tests were employed to test the heterogeneity

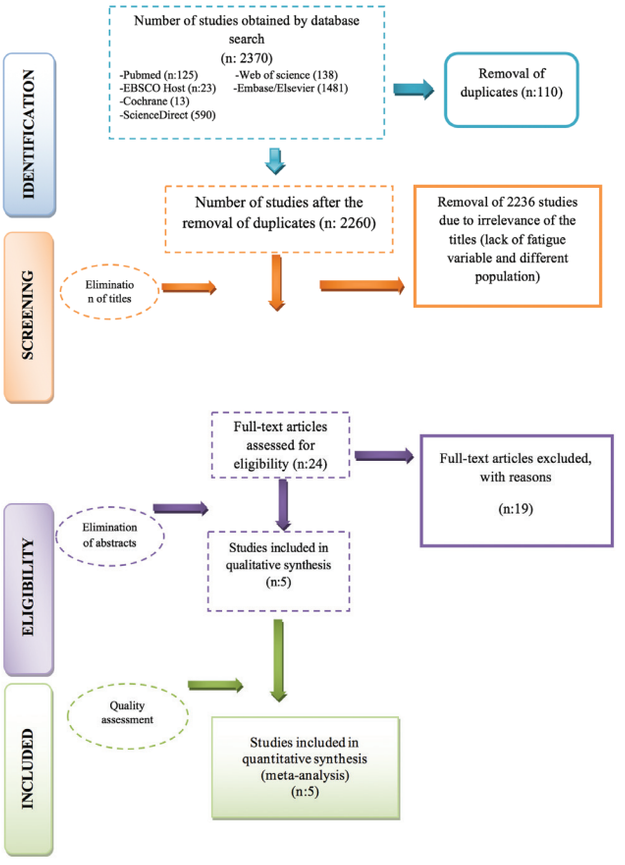

Figure 1. Flow diagram for the selection of studies

of the effect sizes. In determining the significance of the Q test, it is recommended that the limit value for p should be taken as 0.1. In the heterogeneity assessment, if the heterogeneity ratio (I2) is below 25%, it does not exist; it is low if the ratio is between 25-50%; it is moderate if the ratio is between 51-75%; and high if the ratio is greater than 75% (25). To test publication bias, Rosenthal and Orwin fail-safe N, Funnel plot chart, Duval and Tweedie's trim and fill test, rank correlation, Egger regression, and Begg and Mazumdar rank correlations tests were employed (15). As the significance levels of the papers included in the study ranged between 0.01 and 0.05, the significance level of the statistical analyzes in this study was taken as 0.05.

Ethical Consideration

This study was approved by the Institutional Review Board of the University (IRB approval number: 4753-GOA-2019/12-04). Since the literature review model is used in the study, it does not directly affect humans or animals. Therefore, patient consent is not required.

Results

All of the five studies included in the meta-analysis were research papers. Theses were not included in this study. The research included in the meta-analysis included research dating from 2015 to 2019 (Table I).

Table I. Articles included in meta-analysis

Article information	Aim	Methods	Statistical Analysis	Conclusion
Kudubes AA, Bektas M, Mutafoğlu K. (12). The effect of fatigue-related education on pediatric oncology patients' fatigue and quality of life. Journal of Cancer Education, 1-12.	This study aims to analyze the effect of fatigue-related education for pediatric oncology patients aged 7-12 and their parents on their fatigue and quality of life.	This study was conducted with 80 children with cancer and their parents who were assigned to either the control group (n=40) or the experimental group (n=40). The experimental group received a fatigue-related educational program. The data were collected three times: prior to the program, 3 months later, and 6 months afterwards.	Multidimensional variance analysis, the Bonferroni adjusted t-test and regression analysis were used to analyze the data.	A significant difference was found among the experimental and the control group for total mean scores and the mean scores of subdimensions of the scale for the Assessment of Fatigue-child form in terms of the interactions of group, time, and group*time ($p<0.05$). Significant differences were found among the experimental and control groups' mean scores on the scale for the Quality of Life-child and Parents form in terms of the interactions of group, time, and group*time ($p<0.05$). Fatigue-related education is an effective education model as a way to reduce fatigue and increase the quality of life of children with cancer. The use of fatigue-related education by nurses in pediatric oncology clinics will have positive effects on children and their parents.
Lam KK, Li WH, Chung OK, Ho KY, Chiu SY, Lam HS, Chan GC (28). An integrated experiential training programme with coaching to promote physical activity, and reduce fatigue among children with cancer: A randomised controlled trial. Patient education and counseling, 101 (11), 1947-1956.	This study examined the effectiveness of an integrated programme in promoting physical activity, reducing fatigue, enhancing physical activity self-efficacy, muscle strength and quality of life among Chinese children with cancer.	A randomised controlled trial was conducted in a Hong Kong public hospital. Seventy eligible children were randomly assigned to an experimental group (n=37) or a control group (n=33). The experimental group received an integrated programme with 28 home visits from coaches over a 6-month period. The control group received a placebo intervention. The primary outcome was fatigue at 9 months (3 months after intervention completion). Secondary outcomes were physical activity levels, physical activity self efficacy, muscle strength and quality of life at 9 months, assessed at baseline, and 6 and 9 months after starting the intervention.	To minimise attrition bias, the intention-to-treat principle was applied, and participants were analysed according to their initial group assignment. Inferential statistics (e.g. independent sample t-tests, chi-square and Fisher's exact tests) were performed to compare age, gender, parents' educational attainment, diagnosis and treatment received between those who were willing to participate and those who were not.	The experimental group reported significantly lower levels of cancer-related fatigue, higher levels of physical activity and physical activity self-efficacy, greater right- and left-hand grip strength and better quality of life than the control group at 9 months. The programme is effective and feasible to implement among children with cancer and offers an alternative means of ameliorating the healthcare burden.

Vieira MLDS, Fonseca FLA, Costa LG, Beltrame RL, Chaves CMDS, Cartum J, Rocha KC. (29). Supplementation with selenium can influence nausea, fatigue, physical, renal, and liver function of children and adolescents with cancer. Journal of medicinal food, 18 (1), 109-117.	The present study evaluated the health-related quality of life of patients undergoing chemotherapy for the treatment of leukemias and lymphomas (LL) and solid tumors (ST) while receiving Selenium (Se) supplementation.	This is a randomized, double-blind, crossover study that evaluated the quality of life (EORTC-QLQC30 questionnaire), renal and liver functions of patients supplemented with Se.	The results analyses were performed by adopting a significance level of 5% in compliance with the following models: Descriptive statistics; measure of central tendency; equality of means test (Student's t-test); equality of medians test (Mann-Whitney U).	There was no statistically significant alteration in LL patients. However, the fatigue and nausea scores after 30 days did decrease in this group as well as in the ST group. After 1 year supplementation with Selenium, a more noticeable decrease in the scores concerning fatigue and nausea could be observed in the ST group, when compared with the beginning of the study. The LL patients also presented a decrease in the fatigue scores and physical functions. The kidney function as well as liver function has improved after Se supplementation when compared with the placebo intake in LL and ST patients, more remarkably in the LL group. Supplementation with Selenium promotes the reduction of chemotherapy side effects in cancer patients, especially by improving the conditions of patients with fatigue, nausea, and impaired physical function. Renal and liver functions have also improved.
Li WH, Ho KY, Lam KKW, Lam HS, Chui SY, Chan GC, Chung OK. (26). Adventure-based training to promote physical activity and reduce fatigue among childhood cancer survivors: A randomized controlled trial. International journal of nursing studies, 83, 65-74.	This study examined the effectiveness of an adventure-based training programme in promoting physical activity, reducing fatigue, and enhancing self-efficacy and quality of life among Hong Kong Chinese childhood cancer survivors.	A prospective randomised controlled trial. Hong Kong Chinese childhood cancer survivors aged 9-16 years who reported symptoms of fatigue and had not engaged in regular physical exercise in the past 6 months. The experimental group underwent a 4-day adventure-based training programme. The control group received a placebo intervention. The primary outcome was fatigue at 12 months. Secondary outcomes were physical activity levels, self-efficacy and quality of life at 12 months.	We performed intention-to-treat analyses. Descriptive statistics, independent sample t-tests, chi-square, ANOVA test	From 6 January, 2014 to 8 June, 2015, we randomly assigned 222 eligible childhood cancer survivors to either an experimental (n=117) or a control group (n=105). The experimental group showed statistically significantly lower levels of cancer-related fatigue ($p<0.001$), higher levels of self-efficacy ($p<0.001$) and physical activity ($p<0.001$), and better quality of life ($p<0.01$) than the control group at 12 months.
Ramezani N, Moafi A, Nadjarzadeh A, Yousefian S, Reisi N, Salehi-Abargouei A. (27). The Effect of Soy Nut Compared to Cowpea Nut on Body Weight, Blood Cells, Inflammatory Markers and Chemotherapy Complications in Children with Acute Lymphoblastic Leukemia: A Randomized Controlled Clinical Trial. Nutrition and cancer, 70 (7), 1017-1025.	The present randomized controlled clinical trial studied the effect of soy nut on children with B-cell acute lymphoblastic leukemia (ALL) who were in the maintenance phase of chemotherapy.	The eligible patients were randomized to receive 30 g/day soy or cowpea nut powder for 12 weeks.	Wilcoxon Mann-Whitney U Anova Kolmogrov-Smirnov test T-test	Dietary intake, physical activity, anthropometric measurements, complete blood count, serum albumin, serum highly sensitive C-reactive protein (hs-CRP), and Tumor necrosis factor alpha (TNF-α) as well as chemotherapy side effects were assessed at the start and the end of the study. In total 29 and 27 children completed the study (aged 6.34±2.44 and 5.85±2.35 years) in soy and cowpea nut groups, respectively. The total energy and protein intake, and physical activity as well as body weight, body mass index, number of red blood cells, hemoglobin and hematocrit levels, and fatigue were significantly improved in the soy nut group compared to patients who consumed cowpea nut ($p<0.05$). Soy nut intake might improve the nutritional status, anemia, and fatigue in children with ALL.

Table II displays the check results for the homogeneity of the studies included in the meta-analysis. Q and I2 values were calculated as 237,165 and 98,313 for fatigue, respectively.

Table III presents the effect sizes of the studies investigating the effect of fatigue-reducing interventions on the fatigue levels of children with cancer. The mean effect sizes at 95% significance level were determined as follows: -1.452 for Li et al. (26); 0.560 for Ramezani et al. (27); 7.606 for Kudubeş et al. (12); -0.995 for Lam et al. (28); and 0.544 for Vieira et al. (29) (Table II, Figure 2). As a result, it was determined that the effect of Li et al. (26) on fatigue had a negative large effect size, the effect of Ramezani et al. (27) on fatigue had a positive medium effect size, the effect of Kudubeş et al. (12) on fatigue had a positive large effect size, the effect of Lam et al. (28) on fatigue had a negative large effect size, and the effect of Vieira et al. (29) on fatigue had a positive medium effect size (12,26-29).

To check the publishing bias, Rosenthal and Orwin fail-safe N, Funnel plot map, Duval and Tweedie's trim and fill check, rank correlation, Egger regression, and Begg and Mazumdar rank correlation test were used. It was determined that for an effect size of 0, five studies were needed when the Rosenthal fail-safe N according to the fatigue level of children with cancer was examined, one study was needed when the Orwin fail-safe N was examined, and two studies were needed when the Duval and Tweedie's trim and fill test was considered. No publication bias was found

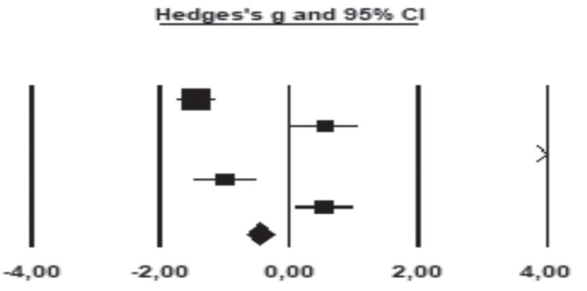

Figure 2. The effect sizes of the studies investigating the effect of fatigue-reducing interventions on the fatigue levels of children with cancer according to hedges' g test
CI: Confidence interval

Table II. Homogeneity test results

	Q	df	Table X² value	p	I²
Fatigue	237.165	4	9.488	0.000	98.313

Table III. The effect sizes of the studies investigating the effect of fatigue-reducing interventions on the fatigue level of children with cancer

Fatigue	n	Mean Effect Size	SE	Variance	95% CI		Z	p
Li et al. (26)	222	-1.452	0.151	0.023	-1.748	-1.156	-9.614	0.000
Ramezani et al. (27)	56	0.560	0.273	0.074	0.026	1.094	2.054	0.040
Kudubes et al. (12)	80	7.606	0.642	0.412	6.349	8.863	11.856	0.000
Lam et al. (28)	70	-0.995	0.254	0.064	-1.493	-0.498	-3.921	0.000
Vieira et al. (29)	72	0.544	0.240	0.058	0.074	1.015	2.267	0.023
		-0.471	0.104	0.011	-0.675	-0.268	-4.534	0.000

SE: Side effect, CI: Confidence interval, n: Number

Table IV. Cochrane collaboration's tool for assessing risk of bias

	Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
	Random sequence generation	Allocation concealment					
Li et al. (26)	-	-	+	+	-	-	-
Ramezani et al. (27)	-	-	-	+	-	-	-
Kudubes et al. (12)	+	+	+	+	-	-	-
Lam et al. (28)	-	-	+	-	-	-	-
Vieira et al. (29)	-	-	-	-	-	-	-

according to Begg and Mazumdar rank correlations tests, Funnel plot graph, or Egger regression analysis (p>0.05).

Four studies describe the sequence of allocations, and two studies describe a blinded study design for participants and personnel. Two studies describe a blinded study design for outcome assessment. All of the studies describe attrition bias, reporting bias and other bias (Table IV).

Discussion

Due to the rising occurrence of cancer cases and survival rates in children, management of symptoms has become more important. The definition of fatigue, which is one of the most important symptoms impacting the QoL of pediatric oncology patients, has been on the agenda in recent years. Especially, the high rates of fatigue in pediatric oncology patients have attracted attention to this issue and revealed the need for determining fatigue-reducing interventions (8). Therefore, the aim of this meta-analysis study was to determine the effect of fatigue-reducing interventions on the fatigue levels of pediatric oncology patients.

In this meta-analysis study, the total sampling size in the five studies included in the study was 500. The detailed sampling sizes in the five studies analyzed in this study were determined as follows: 222 in Li et al. (26); 56 in Ramezani et al. (27); 80 in Kudubes et al. (12); 70 in Lam et al. (28); and72 in Vieira et al. (29) (12,26-29).

Q, p and I^2 values were used in the Heterogeneity test regarding the studies included in the meta-analysis. Q values were found to range between 26.916 and 1,033.459, while I^2 values ranged between 81.424% and 99.419%. In the literature, while the statistical significance limit value for p is accepted as 0.10 for the significance of Q test in heterogeneity evaluation, if the heterogeneity ratio (I^2) is below 25%, heterogeneity is considered non-existent; heterogeneity is low if the ratio is between 25-50%; heterogeneity is moderate if the ratio is between 51-75%; and high if the ratio is greater than 75% (25). According to the heterogeneity test in this study, the effect of fatigue-reducing interventions on the fatigue level of children with cancer yielded a heterogeneous distribution (Q=237.165, I^2=98.313%, p<0.001). In line with this finding, the studies included showed a heterogeneous characteristic and the average effect sizes were calculated according to the random effects model.

The effect sizes of studies investigating the effect of fatigue-reducing interventions on the fatigue levels of pediatric oncology patients were found as follows: the effect determined by Li et al. (26) on fatigue was a negative large effect size (-1.452), the effect determined by Ramezani et al. (27) on fatigue was a positive medium effect size (0.560), the effect determined by Kudubeş et al. (12) on fatigue was a positive large effect size (7.606), the effect determined by Lam et al. (28) on fatigue was a negative large effect size (-0.995), and the effect determined by Vieira et al. (29) on fatigue was a positive medium effect size (0.544) (12,26-29). In the literature, a significant negative correlation was found between fatigue-reducing interventions and fatigue (9-12). It was observed that as the interventions both in this meta-analysis study and in the above discussed studies from the literature increased, the levels of fatigue decreased. Fatigue symptoms are very common in children with cancer because of both the cancer itself and the method of treatment (2,5). Although the symptoms of fatigue are often ignored by health professionals, studies emphasize that diagnosing fatigue and administering fatigue-reducing interventions are important in managing fatigue (8). Activity and exercise regulation, providing appropriate nutritional support, stress management, and regulation of sleep in children with cancer significantly reduce fatigue levels. It is also emphasized that regular training activities on these issues are also effective (12). This is why fatigue-reducing interventions are thought to be effective in reducing fatigue experienced by pediatric oncology patients.

To test the existence of publication bias in this meta-analysis, Rosenthal and Orwin fail-safe N, Funnel plot chart, Duval and Tweedie's trim and fill test, rank correlation, Egger regression, and Begg and Mazumdar rank correlations tests were employed. These analyses calculate the number of studies that may be missing in a meta-analysis (15,25). It is recommended that using a single method should be avoided and other methods should also be used to determine publication bias. When the Rosenthal error protection coefficient, one of the technical methods, was considered, the number of studies required to bring the effect size to zero was large, which indicated that no publication bias existed in this study. However, when other methods were examined in this study, it was found that this study might include publication bias in the majority of the methods. When analyzing the results of this meta-analysis study, it is recommended that this fact be considered.

Conclusion

It was determined that fatigue-reducing interventions administered to children with cancer affected the status of fatigue in these children. Due to the small number of studies included in this meta-analysis review and the

likelihood of publishing bias based on this small number, new studies with a high level of evidence concerning the delivery of fatigue-reducing treatments in children with cancer are required to explain the findings. In particular, it is recommended that randomized-controlled experimental studies should be planned and effect sizes and power analysis should be carried out in these studies.

Implications for Nursing Practice

Fatigue management is very important in children with cancer. Reducing their tiredness helps to boost the child's QoL. Therefore, studies in this area are valuable. Studies investigating efforts to reduce fatigue should be increased. This meta-analysis examines studies that reduce fatigue and shows the power effects of interventions applied to children with cancer.

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Ethics

Ethics Committee Approval: This study was approved by the Institutional Review Board of the University (IRB approval number: 4753-GOA-2019/12-04).

Informed Consent: Patient consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.A.K., Design: A.A.K., Data Collection or Processing: A.A.K., Analysis or Interpretation: M.B., A.A.K., Literature Search: M.B., Writing: A.A.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Iron and Ferritin Levels of Children and Adolescents with Attention Deficit Hyperactivity Disorder and Attention Deficit Hyperactivity Disorder-Not Otherwise Specified

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ABSTRACT

Aim: The study aimed to compare the levels of iron and ferritin in children with Attention Deficit Hyperactivity Disorder (ADHD) and Attention-Deficit Hyperactivity Disorder-Not Otherwise Specified (ADHD-NOS) and to assess the relationship between ADHD symptom severity and anxiety symptom severity with iron and ferritin levels.

Materials and Methods: This study was planned as a cross-sectional, retrospective study. The study was performed by scanning the records of patients who applied to our clinic between January 2012 and January 2013. Accordingly, 205 ADHD and ADHD-NOS case records were evaluated. Patients were diagnosed clinically according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria. ADHD symptom severity was assessed by the Turgay DSM-IV-TR-Based Child and Adolescent Behavior Disorders Screening and Rating scale. Anxiety symptom severity was assessed by The Screen for Anxiety Related Emotional Disorders.

Results: Among the whole sample, 99 (48.3%) patients had ADHD and 106 (51.7%) had ADHD-NOS. In the ADHD group, the average age of the children was 10.88±3.02 years, while that of the children in the ADHD-NOS group was 9.93±2.49 years. Iron and ferritin were measured in 81 of the 205 patients participating in the study. There was no statistically significant difference between the two groups in terms of iron or ferritin levels ($p>0.05$). Statistically significant negative correlations between ADHD hyperactivity symptom severity and iron levels, and ADHD attention deficit symptom severity and ferritin levels were found. Ferritin levels correlated statistically with the total number of psychiatric diagnoses in the children.

Conclusion: Iron and ferritin levels may be differentially affected in children with ADHD. The results we obtained from our study should be supported by studies with larger samples.

Keywords: ADHD, iron, ferritin

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is defined as an important childhood neuropsychiatric disorder with different clinical features, such as inattention, hyperactivity, and impulsivity. It is also often related to cognitive deficit (1). It is reported that the prevalence of ADHD ranges from 8.0% to 12.0% worldwide (2). The prevalence of ADHD in school-age children was determined to be 8.1% in Turkey (3). Attention-Deficit Hyperactivity Disorder Not Otherwise Specified (ADHD-NOS) is a diagnosis in Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR)

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for disorders with pronounced symptoms of inattention/hyperactivity-impulsivity that do not meet the ADHD criteria (4). Faraone et al. (5) showed that individuals with ADHD and ADHD-NOS had similar patterns of functional impairment, comorbid psychiatric disorders, and familial inheritance. ADHD-NOS, which has been replaced by the term ADHD- Unspecified in DSM-5, is a diagnosis that can be made for situations where children may experience moderate attention problems that may affect learning or executive function in school (4). In addition, rather than being categorized as ADHD Other Specified and Unspecified types, many young people are defined as having borderline or subclinical levels of ADHD instead. Pharmacotherapy including stimulants and atomoxetine is the first choice in the treatment of both these disorders (2).

There are many theories regarding the etiology of ADHD, but it is not yet fully understood. A clear identifiable factor has not been demonstrated, but ADHD is known to be multifactorial (6). Studies have reported that the hereditary ratio of ADHD varies between 76.0% and 80.0% (7,8). The exact cause is still unknown, but various prenatal and perinatal factors such as socio-psychological stress, exposure to toxins and heavy metals, diet, deficiency of neurotransmitters, gene variants and structural/functional abnormalities of the brain have been reported to contribute to the etiology (2,7). ADHD is also frequently accompanied by comorbid conditions including iron deficiency (ID), electroencephalographic abnormalities, epilepsy, learning disabilities and depressive disorders (9).

Iron has an important role in systems that have vital features for the organism, such as the immune system, oxygen transport to cells, deoxyribonucleic acid synthesis and nitric oxide metabolism (10). In addition, iron is required in neurochemistry, proper brain morphology, and bioenergy processes (11). It has been shown that poor brain myelination associated with ID has a long-term effect on behavior in the early development period (12,13). The regions where brain iron concentrations are highest are the Globus pallidus, substantia nigra, red nucleus, nucleus caudate and putamen (14). Fast iron accumulation in these areas is essential for the development of the brain and can contribute significantly to behavioral organization (14). Iron is a co-factor of tyrosine hydroxylase, an enzyme important in dopamine synthesis. Dopamine plays a significant role in ADHD pathophysiology (15). Various studies trying to elucidate ADHD neurobiology have shown that nutritional factors can affect brain function and play a role in the pathogenesis of ADHD (16-18). ID, which is one of these nutritional factors, is important because it plays a crucial role in the organizing of dopaminergic activity associated with the pathogenesis and symptoms of ADHD (19). Sever et al. (20) showed that children who were given iron supplements had increased ferritin levels and had decreased ADHD symptoms. These results suggest that iron supplements may be beneficial in children with non-anemic ADHD. In a systematic review examining the relationship between iron and ADHD, statistical analysis on iron status indices has not been shown (21).

Studies have shown strong evidence that ID in young children gives rise to developmental delays (22). ID has also been found to be related to cognitive changes in adolescents (23). In one study on rats, behavioral changes were shown to continue despite the ID being treated (24). Children with ID have more anxiety and/or depression with attentional and social problems (25).

We aimed to compare the levels of iron, ferritin and hemoglobin in children with ADHD and ADHD-NOS in this study. We also aimed to assess the relationships of ADHD symptom severity and anxiety symptom severity with respect to iron, ferritin and hemoglobin levels.

Materials and Methods

Study Center and Time-frame

The study was planned as a cross-sectional, retrospective study. It was conducted at the outpatient department of the Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Child and Adolescent Psychiatry. The study was performed by scanning the records of patients who applied to our clinic between January 2012 and January 2013. Accordingly, 205 ADHD and ADHD-NOS case records were evaluated. The diagnosis of 205 patients' records and their comorbid psychiatric disorders was made clinically by the consensus of child psychiatry residents and the clinical supervisor according to DSM-IV-TR criteria (4). The diagnosis of ADHD was based on 6 of the 9 attention deficit criteria, 6 of the 9 hyperactivity criteria, onset of symptoms before the age of 6 years, the presence of symptoms in at least 2 different settings, symptoms having continued for at least 6 months and an impairment of functionality. The ADHD-NOS was diagnosed for those patients who did not meet the criteria for ADHD (e.g. age, number of symptoms), but who had a behavioral pattern marked by sluggishness, daydreaming, and hypoactivity.

Inclusion criteria were a primary diagnosis of ADHD or ADHD-NOS according to DSM-IV-TR criteria, adequate information on laboratory values and psychometric measures in the patient records and application to the

outpatient department during the specified time-frame. Patients with comorbid medical and psychiatric disorders were included. Both ADHD and ADHD-NOS groups received methylphenidate treatment at 1 mg/kg/day. Those patients with inadequate records were excluded. Ethics committee approval of the study was obtained from Bolu Abant İzzet Baysal University Clinical Trials Ethics Committee (date: 16.05.2018, number: 164).

Measures

Turgay DSM-IV-Based Child and Adolescent Behavior Disorders Screening and Rating scale (T-DSM-IV-S): This parent and Teacher-reported scale was developed by Turgay by transforming the DSM-IV criteria into questions without changing their meanings and includes 9 items for attention deficit, 6 items for hyperactivity, 3 items for impulsivity, 8 items for oppositional defiant disorder, and 15 items for conduct disorder. Each item is rated on a scale of 0= none, 1= occasional, 2= much, and 3= very much. When subscales are evaluated, 2 to 3 points per item are assessed as symptomatic (1), while 0 to 1 are assessed as non-symptomatic (0) (26). The validity and reliability study of this scale was established previously (27).

The Screen for Anxiety Related Emotional Disorders (SCARED): There are 41 questions on this scale. It asks parents to show how often an explanatory sentence about how their children felt correct during the previous three months. Participants can choose from 0 to 2 points (28). When subscales are evaluated, 2 points for an item is assessed as symptomatic (1), while 0 to 1 are assessed as not symptomatic (0). Both the child's and parent's reports were used. The scale also includes generalized anxiety, separation anxiety, somatic/panic, social anxiety and school fear subscales. The SCARED Turkish form's validity and reliability were established by Cakmakci (29).

The Clinical Global Impression-Severity scale (CGI-S): CGI-S is a scale that measures the functionality that varies with treatment evaluated by clinicians (30). The CGI-S score is a 7-point scale of disease severity. CGI-S is often used in Turkish Child and Adolescent psychiatry clinics. There are many Turkish studies using CGI-S. In this study, CGI-S was used to demonstrate symptom severity.

Statistical Analysis

For the data in this study, the Statistical Package for Social Sciences (SPSS) version 22.0 was used. Summary statistics are given for continuous variables. Unless otherwise stated, this refers to the number of patients (n), mean and standard deviation (SD). Categorical data are presented as absolute or relative frequencies. Demographic data were compared using the chi-square tests by applying the corrections of Yates and Fisher when necessary. The normal distribution of the data was examined with the Kolmogorov-Smirnov method. Since the distribution of the data was normal, group comparisons were assessed by Student t-test or One-Way ANOVA according to the group numbers. The relationship between continuous variables was made using pearson correlation analysis. All tests were considered statistically significant for $p<0.05$.

Results

The records of 205 patients were analyzed. Of these, 99 (48.3%) patients were ADHD and 106 (51.7%) patients were ADHD-NOS. In the ADHD group, the average age of the children was 10.88±3.02 years, while that of the children in the ADHD-NOS group was 9.93±2.49 years. A significant difference was determined between the average ages of the groups (p=0.015). Thirty-four children in the ADHD group were female, and 34 children in the ADHD-NOS group were female. There was no statistically significant difference between the groups in terms of gender (p=0.73). There were 45 cases with a positive family history and 51 cases with a medical disease history in the ADHD group. In the ADHD-NOS group, there were 54 cases with a positive family history and 48 cases with a medical disease history. Both family history and medical disease history between the two groups did not display statistically significant differences (p=0.095, p=0.958 respectively) (Table I). T-DSM-IV-

Table I. Comparison of sociodemographic data of the attention-deficit hyperactivity disorder not otherwise specified and attention deficit hyperactivity disorder groups

	ADHD-NOS group (n=99)	ADHD group (n=106)	p
Age	10.88±3.02	9.93±2.49	0.015
Gender			
Male	65	72	0.73
Female	34	34	
Family history			
Positive	54	45	0.095
Negative	45	61	
Medical disease history			
Positive	48	51	0.958
Negative	51	55	

ADHD: Attention deficit hyperactivity disorder, ADHD-NOS: Attention-deficit hyperactivity disorder not otherwise specified, n: Number of patients

S-parent subscales, SCARED subscales and CGI-S scores between the two groups are presented in Table II.

Comorbid psychiatric disorders were detected in 144 cases (70.24%) of the 205 cases included in the study. It was found that conduct disorder (28.29%) and specific learning difficulties (21.95%) were the most common comorbid psychiatric disorders. While 71 of these 144 cases had only one comorbid psychiatric disorder, the other 73 cases had multiple comorbid psychiatric disorders. It was found that 76 cases in the ADHD group and 68 cases in the ADHD-NOS group had comorbid psychiatric disorders. There was no statistically significant difference between the groups in terms of comorbid psychiatric disorders (p=0.531).

Iron and ferritin levels were measured in 81 of the 205 patients participating in the study. Hemoglobin levels were measured in 61 of the 205 patients participating in the study. While the averages of iron and ferritin levels were 65.53±33.21 and 38.77±44.66 respectively in the ADHD group (n=47), they were 73.04±27.08 and 32.83±17.11 respectively in the ADHD-NOS group (n=34). No statistically significant difference was determined between the groups in terms of iron and ferritin levels (p>0.05). While the average of the hemoglobin level was 13.66±4.24 in the ADHD group (n=39), it was 12.77±1.06 in the ADHD-NOS group (n=22). There was no statistically significant difference between the groups in terms of hemoglobin levels (p=0.339) (Table III).

When the relationship between iron and ferritin levels and comorbidity was evaluated, iron and ferritin levels were 75.18±33.51 and 33.56±16.39 respectively in the non-comorbid group (n=27), and they were 63.89±29.70 and 33.91±20.82 respectively in the single comorbid group (n=24), and they were 65.81±29.48 and 40.61±53.90 respectively in the multiple comorbid group (n=30). There was no statistically significant difference between the three groups in terms of iron and ferritin levels (p=0.453, p=0.709 respectively). When the relationship between hemoglobin level and comorbidity was evaluated, hemoglobin levels were 13.91±5.31 in the non-comorbid group (n=25), they were 12.90±1.04 in the single comorbid group (n=20), and they were 13.02±0.73 in the multiple comorbid group (n=16). There was no statistically significant difference between the three groups in terms of hemoglobin levels (p=0.217). When the relationship between the total number of psychiatric diagnosis and iron, ferritin and hemoglobin levels was assessed, a statistically significant negative correlation was found between the total number of diagnosis and ferritin levels (r=-351, p=0.001), but the same relationship with iron and hemoglobin levels was not observed (p>0.05).

When the relationship between the symptoms of ADHD and iron, ferritin and hemoglobin was evaluated, while there was a significant negative correlation between ADHD Hemolytic Anemia (HA) sub-scores and iron levels

Table II. Comparison of disorders screening and rating scale -parent subscales, the screen for anxiety related emotional disorders subscales and the clinical global impression-severity scale scores of the attention-deficit hyperactivity disorder not otherwise specified and attention deficit hyperactivity disorder groups

	ADHD-NOS group (n=99)	ADHD group (n=106)	p
T-DSM-IV-S-parent			
Attention	2.41±1.72	6.28±2.15	<0.001
Hyperactivity	1.40±1.85	4.52±3.18	<0.001
Opposition-defiance	1.45±2.02	3.56±2.99	<0.001
CD	0.11±0.46	0.56±1.22	0.001
Total score	21.34±11.41	42.37±17.02	<0.001
SCARED			
Somatic/panic	1.85±2.31	2.29±2.47	0.441
Generalized anxiety	1.32±1.98	3.11±2.64	0.001
Separation anxiety	1.43±1.68	2.68±1.92	0.003
Social anxiety	2.09±1.95	2.85±2.22	0.106
School phobia	0.53±1.31	1.15±0.142	0.050
Total score	25.89±12.86	34.00±12.89	0.007
CGI-S	3.39±0.74	4.55±0.64	<0.001

ADHD: Attention deficit hyperactivity disorder, ADHD-NOS: Attention-deficit hyperactivity disorder not otherwise specified, SCARED: The screen for anxiety related emotional disorders, CGI: The clinical global impression-severity scale, T-DSM-IV-S: Disorders screening and rating scale, n: Number of patients

(p=0.027), and between ADHD AD sub-scores and ferritin (p=0.011), no correlation was found between the symptoms of ADHD and hemoglobin (p>0.05) (Table IV). When the relationship between SCARED scores and iron, ferritin and hemoglobin levels was evaluated, no relationship was found between iron, ferritin and hemoglobin levels and the SCARED subscales and total scores (p>0.05).

Table III. Comparison of iron, ferritin and hemoglobin levels of the attention-deficit hyperactivity disorder not otherwise specified and attention deficit hyperactivity disorder groups

	ADHD-NOS group	ADHD group	p
Iron µg/dL (rr: 60-180 µg/dL)	73.04±27.08	65.53±33.21	0.349
Ferritin, ng/mL (rr: 10-204 ng/mL)	32.83±17.11	38.77±44.66	0.464
Hemoglobin g/dL (rr: 11.5-17.5 g/dL)	12.77±1.06	13.66±4.24	0.339

ADHD: Attention deficit hyperactivity disorder, ADHD-NOS: Attention-deficit hyperactivity disorder not otherwise specified, rr: Reference range

Table IV. Examination of the relationship between attention deficit hyperactivity disorder symptoms and iron, ferritin and hemoglobin levels (Pearson correlation analysis)

		Iron level	Ferritin level	Hemoglobin level
T-DSM-IV-S-parent				
Attention	r p	-0.118 0.398	-0.303 0.011	0.136 0.350
Hyperactivity	r p	-0.303 0.027	-0.077 0.527	0.197 0.175
Total score	r p	-0.231 0.095	-0.127 0.294	0.186 0.202

T-DSM-IV-S: Disorders screening and rating scale

Discussion

In this retrospective study, we aimed to compare the levels of iron, ferritin and hemoglobin in children with ADHD and ADHD-NOS, and to assess the relationship between ADHD symptom severity, anxiety symptom severity and iron, ferritin and hemoglobin levels. While we did not find significant differences between the ADHD and ADHD-NOS groups in terms iron, ferritin and hemoglobin levels, we found a statistically significant negative correlation between ADHD HA symptom severity and iron levels, and ADHD AD symptom severity and ferritin levels. We also found a statistically significant negative correlation between the total number of psychiatric diagnoses and ferritin levels.

The main outcome of this study is that we have observed a significant negative correlation between ADHD HA symptom severity and iron levels, and ADHD AD symptom severity and ferritin levels. This means that as the iron level decreases, ADHD HA symptom severity increases, and as the ferritin level decreases, ADHD AD symptom severity increases. In studies evaluating iron, ferritin and ADHD relations, ADHD and healthy control groups were compared for iron and ferritin levels (19,31-37). The data comparing ADHD and healthy control in terms of iron, ferritin was inconsistent. Some studies have shown no significant difference in ferritin levels between ADHD and healthy groups (31,33), while others found significantly lower ferritin levels in children with ADHD (19,34-37). In a meta-analysis study, it was shown that serum ferritin levels were lower in ADHD patients than healthy controls. In the same meta-analysis, no relation was found between serum iron levels and ADHD (38). In studies evaluating the correlation between ADHD symptoms and ferritin levels, the data were inconsistent. While some studies had different findings from our study in that ADHD hyperactivity scores were negatively associated with serum ferritin level in ADHD (39-41), Menegassi et al. (32) found no relationship between ADHD symptoms and ferritin level.

Iron is a crucial element which plays a central role in brain processes (42). Therefore, ID causes structural and functional brain abnormalities such as dopamine metabolism alterations, energy metabolism and myelination (43). ID is associated with ADHD etiopathophysiology with several mechanisms (21). While there was a direct relationship between ADHD symptom and ferritin, the same direct relationship for ADHD and iron level could not be found in the literature. By finding a negative correlation between ADHD HA symptom severity and iron level; we believe our study can contribute to the ADHD literature.

We also observed a significant negative correlation between the total number of psychiatric diagnosis and ferritin levels. This means that as the number of psychiatric diagnosis increases, the ferritin level decreases. Comorbidities are common in ADHD (9). In one study, serum ferritin levels were found to be higher in psychiatric disorders other than ADHD. (19). In a meta-analysis study, it was found that patients with ADHD as well as serum ferritin levels in other psychiatric comorbidity are higher than those without psychiatric comorbidity, which did not align with our findings. This difference in the literature and our study can be explained by different diet and pathophysiologic changes related to other psychiatric disorders.

Another finding we have in our study is that there is no significant difference between the ADHD and ADHD-NOS groups with regard to iron, ferritin and hemoglobin levels. Our study may be a contribution to the literature on this subject. There is no study in the literature assessing the association between ADHD and ADHD-NOS in terms of iron, ferritin and hemoglobin levels.

Study Limitations

Our findings should be evaluated within the context of certain limitations. Firstly, this study did not have a healthy control group. Secondly, the study was retrospective and depended on information recorded routinely in clinical records. This dependence resulted in some missing data which may have affected the results. Thirdly, the study was conducted on a clinical sample evaluated at a single center and may not reflect patient populations in other centers or in the community.

Fourthly, the laboratory evaluations were conducted as part of the baseline examination prior to commencing pharmacotherapy at the study center but due to dependence on patient charts, we could not ascertain whether the patients were drug naive or were receiving treatment at the time of evaluations. Despite these limitations, this is the only study that we are aware of which evaluates psychometric features and laboratory values of children diagnosed with ADHD and ADHD-NOS.

Conclusion

In conclusion, a significant negative correlation between ADHD HA symptom severity and iron levels, and ADHD AD symptom severity and ferritin levels and an inverse relationship between the number of psychiatric diagnoses and ferritin with ADHD has been shown by our study. These findings suggest that iron and ferritin levels are related to ADHD symptoms and assessing ferritin levels may be beneficial if the number of psychiatric diagnoses increases in children with ADHD. However, there is a need for further study in this area because our findings and the literature available to date are both inadequate and inconsistent to generalize.

Ethics

Ethics Committee Approval: Ethics committee approval of the study was obtained from Bolu Abant İzzet Baysal University Clinical Trials Ethics Committee (date: 16.05.2018, number: 164).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.Ö., Z.T., Design: Y.Ö., N.D., Data Collection or Processing: Y.Ö., Z.T., N.D., Analysis or Interpretation: A.E.T., Literature Search: Z.T., N.D., Writing: Y.Ö., A.E.T.

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Original Article

Infantile Colic in Infants Aged One-Six Months and the Practices of Mothers for Colic

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ABSTRACT

Aim: The aims of this study were to explore the infantile colic in infants from 1-6 months of age, and the mother's practices to eliminate colic and the relationships between infantile colic scores and sample characteristics.

Materials and Methods: This descriptive study included 232 mothers of infants who were admitted to the hospital for child monitoring at two university hospitals in the Aegean region. Information on demographics, colic characteristics, and practices of mothers to eliminate colic was collected.

Results: According to reports of the mothers, 67.2% of the infants had colic, and 28.5% of the infants met all of the Wessel criteria. The average scale score of infantile colic was 65.2±12.6. To eliminate the colic, 67.6% of the mothers held their infants on their lap, 68.7% rocked them, 63.2% changed the position of their infants, 57.4% massaged the infants' bellies, and 37.1% stated that they used a warm application.

Conclusion: One out of every three-four babies in the study met the diagnosis of infantile colic according to Wessel criteria's. The average score obtained from the infantile colic scale is quite high. Mothers usually use behavioral methods to prevent and reduce colic symptoms, they also use pharmacological and traditional methods in this study.

Keywords: Infantile colic, mother, practices

Introduction

Infantile colic is observed in 10-30% of infants in the first 3 months of life (1-3). Wessel et al. (4) stated that "paroxysmal fussing/infantile colic" is a somatic response to tension in the environment. Infantile colic is characterized by uncontrollable crying episodes in healthy infants, especially in those less than 3 months of age (5). Infantile colic is related to many conditions, its pathogenesis is not completely known and has different definitions (2-6,7). There are many methods for preventing infantile colic. In addition to behavioral methods such as rocking and massage, pharmacological and traditional approaches can be used (2-9). It is a very important topic that affects the family deeply and especially makes them feel insufficient (10-12).

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The crying episodes can last more than 3 hours per day, and 3 days per week, for at least 3 weeks (5). This "rule of 3" description is the most widely accepted description, and was introduced by Wessel et al. (4) We also used this "rule of 3" description to diagnose colic in this study. There is no single definition of colic. Wessel's criteria can be used, and some researchers adopt behavioral signs of infants and parental reports to determine infantile colic (1-13). The study by Helseth and Begnum (13) found that infants identified by parents and nurses cried more than 3 hours a day and crying occurred on more than 3 days in any 1 week and lasted for more than 3 weeks similar to Wessel's criteria.

In addition to these descriptions, many studies indicate that infantile colic is seen during the first 3 months of life - when they are between 3 weeks and 3 months old especially in the afternoons (after 3 o'clock) or at night (1-7). The diagnostic criteria for infantile colic were also determined by Benninga et al. (6). There are some differences in this definition such as colic symptoms' start/stop time (<5 months), not being preventable or resolvable by caregivers, and caregiver reports about infants crying for 3 or more hours per day on 3 or more days within a 7-day period (6). Beside these definitions, the Infant Colic scale can be used to diagnose colic and it includes five possible explanations related to colic such as cow's milk/soy protein allergy or intolerance, immature gastrointestinal system, immature central nervous system, difficult infant temperament, and parent-infant interaction problems (14).

The aims of this study were:

• to diagnose infantile colic in infants aged 1-6 months.

• to examine mothers' practices in the elimination of colic.

Material and Methods

Setting and Study Design

In this descriptive and cross-sectional study, 232 mothers of infants between 1 and 6 months of age who requested their children to be monitored at two university hospitals between August 2015 and October 2015 were enrolled in the study. The inclusion criteria were as follows: mothers who volunteered to participate in the study, babies aged 1-6 months, babies who did not have a chronic disease, and mothers who were primarily responsible for their baby's care.

Instruments

Sociodemographic and Colic Characteristics Form

This form includes items questioning the babies' sociodemographic characteristics such as age, gender, whether the baby is breastfed or not, if it is breastfed exclusively or breastfed and supplemented with formula, the use of a pacifier, and the type of baby bottle. The form also includes items questioning the mothers' sociodemographic characteristics such as age, educational status, working status, income, and their number of children. This form also includes a place for the mothers' statements about whether their infants suffer from colic, the nature of their colic, crying spells, the nutrients that cause colic according to the mothers' opinion, whether the mothers eat these foods or not, and the practices used by the mothers to eliminate colic.

The nature of colic included in the Wessel (4) criteria are as follows: the crying episodes last more than 3 hours per day, more than 3 days per week, for at least 3 weeks. It also states that the crying episodes take place during the first 3 months of life - when they are between 3 weeks and 3 months old especially in the afternoons (after 3 o'clock) or at nights.

The Infant Colic Scale

This scale was developed by Cirgin Ellett et al. (14) to diagnose colic in infants. This scale includes 22 items in 5 subscales: (1) Cow's Milk/Soy Protein Allergy/Intolerance (2 items), (2) Immature Gastrointestinal System (4 items), (3) Immature Central Nervous System (8 items), (4) Difficult Infant Temperament (4 items), and (5) Parent-Infant Interaction and Problematic Infant (4 items). It was reported that in the Turkish validity and reliability study of this scale, item 5 (Baby vomits milk that looks like it did before it was drunk) and item 6 (Baby has no difficulty passing stool) of the immature digestive system subscale and item 11 (Baby eats at the same time every day) of the immature central nervous system subscale were removed. Consequently, the Turkish form of this scale only consists of a total of 19 items. The items are evaluated on a 6-point Likert-type scale, ranging from 1 (strongly disagree) to 6 (strongly agree). A low total score is positive for showing colic, and a high score indicates negative (14). The reliability and validity study of the Turkish version of the scale was conducted by Çetinkaya and Başbakkal (15). While the Cronbach's alpha coefficient ranged from 0.55 to 0.89 for the subscales, it was 0.73 for the total scale. The 19-item and 5 subscale Infant Colic Scale was determined to be valid and reliable for the Turkish population (15).

Data Collection

The researchers reached out to the mothers in outpatient waiting areas. The researchers asked the mothers of infants who came to the hospital for child monitoring if their

babies had a chronic disease, and were between 1 and 6 months of age. If the baby did not have a chronic disease and was 1-6 months of age, the researchers explained the study aims to the mother, and obtained written informed consent. After written consent was obtained, socio-demographic information, data relating to the infantile colic of the baby and the mother's practices to eliminate the colic were collected using the "Sociodemographic and Colic Characteristics form", and "The Infant Colic scale". The data were collected from the mothers in outpatient waiting areas in the hospital setting via face to face interviews.

Statistical Analysis

The data were analyzed using the SPSS 23.0 (SPSS, Inc., Chicago, IL) for Windows. Sample characteristics were summarized using means and standard deviations for continuous variables and proportions for categoric variables. The independent sample t-test and ANOVA test were used to investigate statistical difference between infantile colic scores and other variables. An alpha level of $p<0.05$ was considered statistically significant.

Ethics

The study was approved by the Ethics Committee of Faculty (IRB: 2015-82). Written informed consent was obtained from the participants before enrollment.

Results

The study included 232 infants who met the inclusion criteria. The mean age of the infants was 3.6±1.8 months. Of the infants, 54.3% were male, 56.9% were breastfed exclusively, 58.2% did not use a feeding bottle, and 46.1% did not use a pacifier. The mean age of the mothers was 28.6±5.3 years. Of the mothers, 42.2% were primary school graduates, and 57% had 1 child (Table I).

According to the mothers' statements, 67.2% of the infants suffered from colic, 41.3% continuously cried for more than 3 hours per day, 70.5% had crying spells 3 days per week, 50.6% cried for at least 3 weeks. Of them, 28.5% met all of the Wessel criteria. Of the infants, 67.6% started to cry when they were between 3 weeks and 3 months old (during the first 3 months of life), and 70.1% generally cried in the afternoons or at nights.

The breastfeeding mothers reported that dry legumes (64.7%), milk (12.9%), and carbonated beverages (23.7%) caused colic in their infants. Of the mothers, 70.7% stated that in their opinion, they ate certain foods that caused the colic. To prevent colic, of them, 67.6% held the infants on their laps, 68.7% rocked the infants, 53.4% gave the infants abdominal massage, 43.1% took the infant to a calm and dimly lit environment, and 40.1% had their infants drink olive oil (Table II).

The mean score obtained from the overall scale was 65.2±12.6 (minimum: 35, maximum: 98) (Table III). The total mean score had statistically significant differences for colic in terms of the mothers' statements ($t=3.064$, $p=0.002$), the infant's age ($p=0.000$), and the mother's education level ($F=3.338$, $p=0.020$); however, the total mean score was found to have no statistically significant differences in terms of the infant's gender ($t=-0.242$, $p=0.809$), the way of feeding ($F=1.323$, $p=0.268$), the use of a feeding bottle ($t=-0.685$, $p=0.494$), the use of a pacifier ($t=0.452$, $p=0.652$), the mother's working status ($t=-0.680$, $p=0.497$), the number of children ($F=2.423$, $p=0.091$), and the consumption of foods that cause flatulence ($t=0.509$, $p=0.611$).

Table I. Descriptive characteristics

	n	**%**
Infant's gender		
Female	106	45.7
Male	126	54.3
Infant's age (month)	**M ± SD (min-max)** 3.6±1.8 (1-6)	
Breastfed	n	%
Yes	219	94.4
No	13	5.6
Way of feeding the infant		
Breast milk only	132	56.9
Breast milk and formula	87	37.5
Formula	13	5.6
Using a feeding bottle		
Yes	97	41.8
No	135	58.2
Using a pacifier		
Yes	125	53.9
No	107	46.1
Mothers age	**M ± SD (min-max)** 28.6±5.3 (18-43)	
Education level	**n**	**%**
Literate	16	6.9
Primary school	98	42.2
High school	49	21.1
University/Graduate school	69	29.8
Working status		
Yes	62	26.7
No	170	73.3
Number of children	**M ± SD (min-max)** 1.5±0.7 (1-3)	

M: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum

Table II. Infantile colic in the infants and mothers' practices regarding infantile colic

	n	%
Mothers' statements about whether their infants suffer from colic		
Yes	156	67.2
No	76	32.8
Nature of the colic		
The infant was continuously crying more than 3 hours per day*	74	41.3
The infants had crying spells 3 days per week*	124	70.5
The infants had been crying for at least 3 weeks*	89	50.6
The infants generally started to cry when they were 3 weeks or 3 months old/ during the first 3 months	121	67.6
The infants generally cry in the afternoon (after 3 o'clock) or at night	124	70.1
Infants' status of having colic according to all of the Wessel criteria		
Yes	66	28.5
No	166	71.5
The nutrients that cause colic according to the mothers' opinion		
Legumes (chickpea, bean, lentil)	150	64.7
Carbonated beverages	55	23.7
Milk	30	12.9
Broccoli, cabbage, cauliflower	33	14.2
Spicy and hot spicy foods	13	5.6
Do the breastfeeding mothers eat the foods which they consider a cause of colic?		
Yes	164	70.7
No	68	29.3
Practices used by the mothers to eliminate colic Behavioral		
Taking the infant to a calm and dimly lit environment	100	43.1
Taking the infant on lap	144	67.6
Rocking the infant	145	68.7
Positioning the infant	84	36.2
Massaging the infant's abdomen	124	53.4
Using warm application	76	37.1
Listening to music	83	35.8
Swaddling the infant	47	20.3
Pharmacological		
Simethicone	46	19.8
Fennel tea	67	28.9
Anise tea	50	21.6
Using suppository	85	36.6
Traditional		
Giving the infants sherbet	63	27.2
Giving the infants water with lemon juice	61	26.3
Giving the infants olive oil	93	40.1
Quitting breastfeeding	4	1.7
Referring to the hospital due to colic		
Yes	136	58.6
No	96	41.4

*Wessel Criteria's; 3 hours per day, and 3 days per week, for at least 3 weeks

Table III. Infant colic scale scores

Subscales	M ± SD (Min-Max)
Cow's Milk/Soy Protein Allergy/Intolerance	9.1±2.8 (2-12)
Immature Gastrointestinal System	6.6±2.5 (2-12)
Immature Central Nervous System	25.1±6.0 (10-40)
Difficult Infant Temperament	13.8±4.4 (4-24)
Parent-Infant Interaction +Problem Infant	10.4±3.2 (4-21)
Total scale	65.2±12.6 (35-98)

M: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum

The total mean score on the scale was found to have statistically significant differences with the Wessel criteria; "the infants cried persistently for more than 3 hours per day" (t=1.765, p=0.049), "the infants had crying spells 3 days per week" (t=4.589, p=0.000), and "the infants cried for at least 3 weeks" (t=2.278, p=0.024). No statistically significant difference was found between the mean score on the scale and meeting all of the Wessel criteria (t=1.663, p=0.098). In addition, the total mean score on the scale was found to have statistically significant differences with "the infants generally started to cry when they were between 3 weeks and 3 months old" (t=2.898, p=0.004), and "the infants generally cry during the afternoon (after 3 p.m.) or at night" (t=2.396, p=0.017).

Discussion

Infantile colic can be a cause of maternal distress, the effect of which remains unclear due to the self-limiting nature of the illness. Of the mothers in this study, 67.2% answered "yes" to the question, "Do you think your baby is suffering from colic?". Of the infants, 28.5% met all of the Wessel criteria. In another study, according to the mothers' statements, the infantile colic incidence was 80.1%, but the total incidence assessed using the Wessel's criteria was 16.3% (16). This may be due to the definition of the illness, the educational status of the mother, and the subjective assessment of the mother caused by the psychological changes she experiences.

Karabel et al. (17) identified a history of colic in 75% of 170 infants aged between 6 and 9 months, and found no difference between the infants who did or did not meet the definition of colic according to the Wessel criteria in terms of birth weight, gender, type of delivery, accompanying diseases, maternal care and food supplementation in the first 3 months, food allergy, exposure to smoking, or mothers' age. The present study also did not reveal a significant difference between the mean score on the colic

scale and the infants' gender, way of feeding, use of a feeding bottle, use of a pacifier, mother's working status, number of children, or eating foods that cause flatulence.

There is no consensus on whether the manner of feeding and the development of colic are related to each other or not. However, it is known that breastfeeding for the first six months is a preventive factor against infantile colic. Karabel et al. (17) revealed that the incidence of colic was 74% among breastfed infants and 26% among the infants who were not breastfed or fed with formula along with breast milk, and found no significant differences between the incidence of colic and the manner of providing nutrition. In the present study, almost all of the infants were breastfed, and no significant difference was found between the manner of providing nutrition and mean scores on The Colic scale. This result is similar to the results of other studies and provides a significant contribution to the literature. Lucassen et al. (18) stated that gender, socioeconomic level, type of feeding, family history of atopy, and maternal smoking were not associated with colic. Exclusive breastfeeding is associated with reduced colic, and nocturnal-breast milk contains substantial levels of melatonin (19).

The mothers in the present study mostly held their infants on their laps (67.6%), used pacifiers (53.9%), took their infants to a calm and dimly lit environment (43.1%), rocked them (68.7%), gave the infants abdominal massage (53.4%), played music for their infants (35.8%), and swaddled their infants (20.3%) to eliminate colic. Çiftçi and Arıkan (20) stated in their studies that mothers held their infant in their arms (87.9%), used massage (80.9%), repositioned and rocked the infant (79.4%), brought the infant to a dark quiet room (48.2%), and warmed the infant (41.1%). Behavioral methods noted in this study were similar to those seen in other studies. The detection of these methods is also a guide for nurses and provides important results in directing mothers to manage colic symptoms. Swaddling is a traditional practice, and it is used to reduce infantile colic. However, swaddled infants are under an increased risk of sudden infant death syndrome, and late-term dysplasia of the hip. Therefore, mothers should be advised to avoid placing their swaddled infants in straight, lateral positions while they are sleeping (20,21).

The most commonly known pharmacological approach to reducing infantile colic is the use of simethicone, which increases intestinal movements and reduces the development of gas, thereby reducing colic attacks. In the present study, the rate of simethicone use was 19.8% (22). Karabel et al. (17) reported that the pharmacological treatments most frequently recommended by doctors were copper natural, simethicone drops, Nurse Harvey's herbal oil, fennel oil, and apple oil, respectively. However, they found no difference between the groups who did or did not receive pharmacological treatment in terms of the healing time of colic. The present study showed that mothers used fennel-anise tea and suppositories as well as simethicone. It has been reported that a herbal tea prepared with chamomile, verbena, licorice, fennel and lemon balm reduces crying spells in infants with colic when it is given to the infant 3 times a day (23). Biagioli et al. (24) stated that herbal agents, sugar, dicyclomine and cimetropium bromide should not be recommended for colicky infants. The most frequently used drug therapy reported in the present study was the use of suppositories. In other studies, suppository use is rare (25,26).

The mothers in the present study also gave olive oil (40.1%), sherbet (27.2%), and water with lemon juice (26.3%) to their babies with infantile colic. In another study, 44.7% of the mothers gave sherbet, 2.1% gave water with lemon juice, and 1.4% gave olive oil to their infants (25). Akçam and Yılmaz (27) compared oral hypertonic glucose and sterile water, and found that the group receiving oral hypertonic glucose had significantly reduced colic symptoms than did the other group.

The rate of mothers presenting their infants to a hospital due to colic was 58.6% in the present study. Another study reported this rate to be 50.4% (25). Infantile colic can easily be managed by healthcare professionals, and the symptoms can be reduced with behavioral treatment without the need for drug therapy. Families should be informed about infantile colic, and assured that there is no underlying disease. They should also be told that infantile colic is a temporary condition that will spontaneously improve after approximately three months. The present study did not analyze the families' behavioral changes. This can be regarded as a limitation of this study.

Keefe et al. (28) conducted an intervention program (REST routine- weekly home visits by nurses) for healthy infants with colic. This program decreased the length of crying over the 8-week study period (0.9 hours) and infant irritability was resolved (<1 hour of unexplained crying) in 62% of the treatment group. It is necessary to observe existing supervision practices and warn them about the colicky infants. A care strategy for reducing colic symptoms and maternal fatigue has the potential to improve family well-being (12).

More than half of the mothers reported that they believed their infants suffered from colic. However, only 28.5% of infants met the definition of colic according to the Wessel criteria. The mean score obtained from the scale was found to be high. Most of the mothers used behavioral treatment to reduce the symptoms of colic. Drug therapies such as herbal tea or simethicone were also frequently used. In addition to these methods, the mothers also used cultural methods. More than half of the study sample had sought help from a hospital due to colic. Healthcare professionals play an important role in the management of colic symptoms.

Studies on the effectiveness of the most used behavioral methods can be planned. Healthcare professionals can be specialized to manage colic symptoms and provide support to mothers about the correct methods to be used. Rocking, massaging, swaddling, playing music and providing a quite environment are behavioral methods that every mother can easily apply. Healthcare professionals should support mothers using these methods and encourage them either by visiting the mother at home or by telephone counseling to create a healthy relationship between mother and baby.

Study Limitations

The lack of data on the discharge diagnosis of infants and mothers psychological state is one of the limitations of this study.

Conclusions

One out of every three or four babies in the study met the diagnosis of infantile colic according to Wessel's criteria. The average score obtained from The Infantile Colic scale is quite high. Mothers usually use behavioral methods to prevent and reduce colic symptoms, they also used pharmacological and traditional methods in this study.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Faculty (IRB: 2015-82).

Informed Consent: Written informed consent was obtained from the participants before enrollment.

Peer-review: Enternally and internally peer-reviewed.

Authorship Contributions

Concept: N.A.D., Z.B., G.Ö.G., Design: N.A.D., Z.B., G.Ö.G., Data Collection or Processing: B.Y., T.A., M.A., Analysis or Interpretation: N.A.D., G.Ö.G., Literature Search: N.A.D., Z.B., G.Ö.G., B.Y., Writing: N.A.D., G.Ö.G.

Conflict of Interest: None of the authors had conflict of interest.

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Original Article

The Use of Artificial Neural Networks for Differential Diagnosis between Vesicoureteral Reflux and Urinary Tract Infection in Children

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ABSTRACT

Aim: Vesicoureteral reflux (VUR) and urinary tract infection (UTI) are common problems in children. Our goal is to use different models for the clinical decision of differential diagnosis of VUR and UTI in children.

Materials and Methods: This was a retrospective cross-sectional study with 611 pediatric patients enrolled. Detailed information for the patients was obtained from hospital records and patient files. Three models including different variables were evaluated via an artificial neural network for the differential diagnosis of VUR and recurrent UTI. Clinical findings were included in Model 1, clinical and laboratory findings were included in Model 2, and clinical, laboratory and detailed urinary ultrasonography (USG) findings were included in Model 3. A cross-validation technique was used to evaluate predictive models by partitioning the original sample into a training set to train the model, and a test set to evaluate it.

Results: Of the 611 children, 425 (69.6%) had VUR and 186 (30.4%) had UTI. The sensitivity of Model 1 and Model 2 were 0.682 and 0.856, respectively. Also, Model 3 showed the best performance and highest sensitivity with 0.939 for differential diagnosis.

Conclusion: Differential diagnosis between VUR and UTI in children can be predicted by using clinical, laboratory and USG variables via an Artificial Neural Network. Model 3, which included clinical, laboratory and USG variables together, showed the best performance and highest sensitivity.

Keywords: Artificial neural network, differential diagnosis, urinary tract infection, urinary ultrasonography, vesicoureteral reflux

Introduction

Vesicoureteral reflux (VUR) should be considered in children who have urinary tract anomalies diagnosed by fetal ultrasonography (USG) and who have recurrent urinary tract infection (UTI). For child patients presenting with primary or recurrent UTI, 25-40% are found to have VUR. However, the actual cause-and-effect relationship between VUR and UTI is controversial (1-3). In a meta-analytic study, it was reported that children with VUR had a higher risk of pyelonephritis and renal scarring than those without VUR (Odds Ratio: 2.8 and 3.8, respectively) (4). The prevalence of VUR in children with end-stage renal failure is 3-25% (5). Some authors have suggested that renal scarring is not caused by VUR alone, but by concomitant congenital renal hypoplasia or dysplasia (6).

Around one-third of children who were diagnosed with a UTI have VUR. Imaging tests are used for diagnosis in cases where VUR is suspected. Voiding cystourethrogram (VCUG),

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the gold standard radiographic test used to diagnose VUR, is a widely accepted test (7). Screening of VCUG for VUR in siblings and neonates with prenatal hydronephrosis is recommended (7,8).

However, renal/bladder ultrasound (RBUS), which is a noninvasive procedure, is commonly used as an initial screening test to determine VUR. The diagnostic accuracy of RBUS is controversial. Several studies report that the RBUS has low sensitivity and specificity for detecting VUR in children with UTI, and sometimes it may be not possible to detect high-grade VUR. In one study, RBUS sensitivity for high-grade (i.e., Grades IV-V) VUR was found to be 86%, while in another study, RBUS sensitivity and specificity was found to be 40% and 76%, respectively. At the same time, the diagnostic value of ultrasound with clinical and laboratory findings is not discussed in the mentioned studies above (9-12). Children with low-grade reflux should be followed under observation. Prophylactic antibiotic therapy should be suggested for patients with bladder and bowel dysfunction and for patients who are not toilet trained. Surgical correction is performed in those patients with persistent grade IV or grade V after they have reached two or three years of age. Conversely, the treatment method in patients with low-grade VUR and renal scarring is controversial.

The presence of renal scarring changes the management of VUR. Early detection of VUR should be performed to prevent the development of renal scarring, and prognosis of the patient should be closely monitored. In addition, the accuracy of early detection and prognostic monitoring methods should be known. In recent studies, some different analytical methods, such as artificial neural network (ANN), have been used to determine the accuracy of diagnostic tests and differential diagnosis with classical approaches. Classical approaches lead to misinterpretations when complex data structures are encountered. Therefore, analytic methods such as ANN, in which multiple data are evaluated together, are important for management (13-15).

In the neural network model, the activation function is defined as the following linear function f(x)= x, where *x* represents a parameter of the activation function. b is called the bias term and it is associated with each inter connection to introduce a supplementary degree of freedom. The weighted sum S to the i_{th} neuron in the k_{th} layer (k ≥2) is

$$S_{k.I} = \sum_{f=1}^{N_{k=I}} [(w_{\kappa-1} x_{\kappa-1+f}) + b_{\kappa-1}]$$

where *w* is the weight parameter between each neuron-neuron inter connection (8).

Multiple data structures should be evaluated together in children with VUR for an early differential diagnosis and for the prediction of prognosis (16). In the literature, classical statistical methods such as multivariate regression analysis are frequently used in the prediction of differential diagnosis and prognosis of VUR (17). In these analytic methods where there are multiple linear connections, the co-evaluation of multiple variables has some drawbacks. Strong predictions by ANNs can be made in situations where data structures need to be evaluated together (18). The structure of these networks supports capturing very complex relationships between predictor variables and dependent variables. This study has features that require analysis with ANN. The high number of variables and the possibility of multiple correlations form the basis of different statistical approaches.

In this study, three different ANN models were created in which different clinical, laboratory and imaging variables were included. Thus, the contribution of different findings in these models to the differential diagnosis was evaluated.

Materials and Methods

This study was approved by the Ethics Committee of the Faculty of Medicine of Ege University (The protocol number: 13-6.1/56). Patients gave their informed consent for inclusion in the study.

In this retrospective cross-sectional study, 611 pediatric patients who had been admitted to Ege University Faculty of Medicine Pediatric Nephrology Outpatient Clinic and Tepecik Training and Research Hospital were included. Informative data about the patients were obtained from hospital records and patient files. The conversion of records into data was carried out by pediatric nephrologists in the study team and a database was created. Four basic variables of the patients (gender, age group, history of UTI, and urine culture positive UTI) were examined descriptively. 39 characteristics including these four variables were evaluated by ANN algorithms (Figure 1).

In our study, 39 features (physical findings, laboratory and imaging findings) were presented (Table I). VUR or UTI, which is classified by pediatric nephrologists, constitutes the outcome variable to be estimated. History of UTI and culture positive UTI used to estimate the outcome variable are two independent variables. Since recurrent UTI which occurs commonly in children with VUR is an important variable, history of UTI was included in the

model. Three different models were created by an ANN method for the differential diagnosis of VUR/UTI. ANN analyses of 14 clinical and 16 laboratory variables without any ultrasonographic variables were included in Model 1. In Model 2, 14 clinical, 16 laboratory and 2 ultrasonographic variables (hydronephrosis and dilatation) were included in the ANN analysis. In Model 3, 14 clinical, 16 laboratory, and 10 ultrasonographic variables (9 hydronephrosis and 1 dilatation) were included in the ANN analysis.

All variables were used in the analysis as shown in Table I. Variability of the differences of AP (anteroposterior diameter) was used by re-coding in Model 3 (19).

Eleven children had bilateral VUR. The anteroposterior (AP) renal pelvis diameter of the right and left kidneys were not included separately in the model as kidney diameters were determined in only 3 children. Values of AP right and left renal diameter difference are defined categorically according to the values of either ≤5 mm or ≥5 mm in analytic methods.

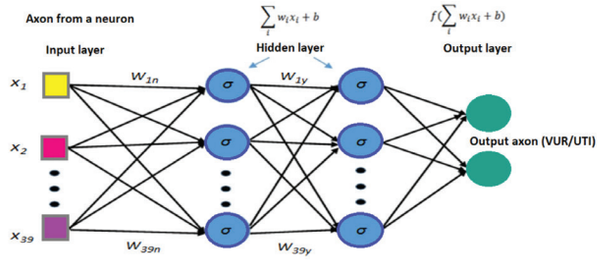

Figure 1. Perceptron network structure with 39-input and 2-output multilayer forward feed
VUR: Vesicoureteral reflux, UTI: Urinary tract infection

As a result, an axon leading from an ANN, reaching the synapse dendrite (studied with 39 variables) was studied by the linear modeling system and the variable output was predicted as VUR/UTI.

Statistical Analysis

Statistical analyses were performed using Waikato Environment for Knowledge Analysis (WEKA 3.8).

K-fold cross-validation technique was used to evaluate predictive models by partitioning the original sample into a training set to train the model, and a test set to evaluate it. In 10-fold cross-validation, the original sample was randomly partitioned into 10 equal-sized subsamples. Of the 10 subsamples, a single subsample was retained as the validation data for testing the model, and the remaining 9 subsamples were used as training data. The cross-validation process was then repeated 10 times, with each of the 10 subsamples used exactly once as the validation data. The 10 results were then averaged to produce a single estimation.

The significance of the area under the curve (AUC)

Table I. The variables used in the artificial neural network analysis

Clinical variables	Laboratory variables	USG variables
Diagnosis(V/I) Model 1,2,3	u-culture(c) Model 1,2,3	USG-R-grade (ordinal:0,1,2) Model 3
Gender (cat: Male/Female) Model 1,2,3	ud-density(c) Model 1,2,3	USG-L-grade (ordinal: 0,1,2) Model 3
Age(c) Model 1,2,3	ud-nitrite(cat:Y/N) Model 1,2,3	USG-AP diameter differences (cat:≤5, 5<) Model3
Fever(cat:Y/N) Model 1,2,3	ud-l.esterase(cat:Y/N) Model 1,2,3	USG-R-Hydronephrosis (cat:Y/N) Model 2, 3
Emesis(catty/N) Model 1,2,3	ud-erythrocyte(cat:Y/N) Model 1,2,3	USG-L-Hydronephrosis (cat:Y/N) Model 2, 3
Incontinence(cat:Y/N) Model 1,2,3	ud-protein(cat:Y/N) Model 1,2,3	USG-R-L Hydronephrosis (cat:Y/N) Model 2, 3
Collywobbles(cat:Y/N) Model 1,2,3	us-erythrocyte(cat:Y/N) Model 1,2,3	USG-Bladder wall thickening(cat:Y/N) Model 3
Urgency(cat:Y/N) Model 1,2,3	us-leukocyte(cat:Y/N) Model 1,2,3	USG-Bladder diverticulum(cat:Y/N) Model 3
Frequent urination(cat:Y/N) Model 1,2,3	ud-leukocyte(cat:Y/N) Model 1,2,3	USG-Ureter dilatation (cat:Y/N) Model 3
Dysuria(cat:Y/N) Model 1,2,3	us-bacteria (cat: Y/N) Model 1,2,3	
Restless(cat:Y/N) Model 1,2,3	us-leukocyte cylinder(c) Model 1,2,3	
Anorexia(cat:Y/N) Model 1,2,3	b-leukocyte (c) Model 1,2,3	
UTI on story(cat:Y/N) Model 1,2,3	b-thrombocyte(c) Model 1,2,3	
Prolonged neonatal jaundice(cat:Y/N) Model 1,2,3	b-urea(c) Model 1,2,3	
	b-creatinine(c) Model 1,2,3	
	b-uric acid(c) Model 1,2,3	

All categorical variables are defined as binary (cat: Y/N: Yes/No and Gender: cat: Male/Female), c: Continuous variable, cat: Categorical variable, UTI: Urinary tract infection, ud: Urine dipstick, us: Urine sediment, USG: Ultrasonography, b: Blood, R: Right, L: Left, AP: Anterior-Posterior, u-le: Urine-leukocyte esterase

statistics was assessed by receiver operating characteristic (ROC) analysis. The statistics (sensitivity, specificity and precision value) obtained from the ROC analysis were also evaluated.

Results

Of the 611 children, 425 (69.6%) had VUR and 186 (30.4%) had UTI. Some descriptive characteristics of the children with VUR and UTI are presented in Table II.

41.2% (175) of the children with VUR were boys and 58.8% (250) were girls. 41.9% (78) of the children with UTI were boys and 58.1% (108) were girls. 55.1% of those children with VUR and 58.1% of those children with recurrent UTI were in the age group of 0-24 months. During medical examination, 76.5% of children with VUR and 78.9% with UTI presented their urinary cultures. The respective numbers of children with urinary culture were similar for both VUR and recurrent UTI. Since VUR data was related with UTI variable, it is hard to differentiate the diagnosis of VUR and UTI. The results of ANN for VUR and UTI are shown in Table III.

Table II. Characteristics of children with vesicoureteral reflux and urinary tract infection

Variables	VUR		UTI	
	n (425)	%	n (186)	%
Gender				
Boy	175	41.2	78	41.9
Girl	250	58.8	108	58.1
Age groups				
0-24 months	234	55.1	108	58.1
25-60 months	83	19.5	34	18.3
61 months and over	108	25.4	44	23.7
Presence of urinary culture in previous UTI				
Present	315	76.5	146	78.9
Not present	97	23.5	39	21.1
UTI based on notification				
Not present	101	23.8	31	16.7
1-4	259	60.9	118	63.4
5 and over	65	15.3	37	19.9

VUR: Vesicoureteral reflux, UTI: Urinary tract infection

Table III. Results of model 1, 2 and 3

Model	Sensitivity	Specificity	Precision	AUC
Model 3	0.939	0.418	0.779	0.809
Model 2	0.856	0.425	0.744	0.747
Model 1	0.682	0.216	0.527	0.601

AUC: Area under the curve

By the cross-validation method, the sensitivity to differentiate VUR in Model 3 was found to be 0.939 whereas the specificity was 0.418. Also, AUC was found to be a significantly high value at 0.809. Model 2 included 2 ultrasonographic variables (hydronephrosis and dilatation). The sensitivity of model 2 was found to be 0.856 and AUC was found to be 0.747. In Model 1, in which there was no ultrasonographic variable included, the sensitivity was found to be 0.682 and AUC was found to be 0.601. The ROC which was obtained from ANN analysis is presented Figure 2. When the AUC's were evaluated for ANN, it was found that the RBUS variable is very important in differentiating VUR and UTI.

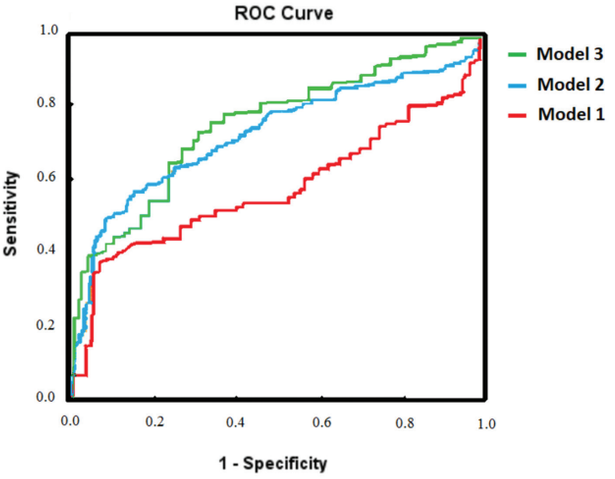

Figure 2. Comparisons of the model 1, model 2, model 3
ROC: Receiver operating characteristic

Discussion

In this study, the performances of 3 different models were evaluated via ANN analysis for the differential diagnosis of VUR and recurrent UTI, which is an important urinary problem in children. Different sensitivity, specificity and predictive values were obtained by using different models that incorporate different findings. When detailed RBUS findings are added to the models, the sensitivity value increased from 0.682 to 0.939, while specificities were similar. However, the predictive value of Model 3 was higher than the other models because the differential diagnosis of VUR was the main objective. In the model that includes most variables, clinical, laboratory and detailed urinal tract findings (10 clinical variables, 14 laboratory variables, and

10 ultrasonographic variables) were used together and the predictive value and sensitivity were determined to be 0.779 and 0.939, respectively. In the literature, lower sensitivity and lower specificity estimates of RBUS in the detection of VUR have been reported. However, the findings of RBUS have not been detailed in these studies.

In one study, the sensitivity of colored doppler RBUS in the evaluation of ureteral jet opening in children with VUR was determined to be 85% for grade III-IV and 94% for grade IV-V (20).

The sensitivity of RBUS was found to be 0.939 in our study. We think that the diagnostic sensitivity of VUR will increase when RBUS with a discriminative capability such as color doppler RBUS is used together with conventional RBUS.

Some studies reported that ANN and other data mining methods supported medical decisions regarding VUR and some nephrological problems (21-27).

In one study, data was evaluated with ANN, and the results were compared with logistic regression analysis results for the surgical treatment decision of VUR (24). Better performance has been achieved with ANN.

Study Limitations

In our study, the performance of ANN was tested and found to be distinctive in a more chaotic decision, such as the differential diagnosis between VUR and UTI. We found that the model with the highest distinguishing characteristics was the model including the detailed RBUS variables.

The algorithms were trained on the training set via a multilayer perceptron before the estimates were made on the test set (28). The iterations were continued until the error values between the input and output parts of the algorithm were minimized. Algorithms which were obtained via k-fold cross validation were applied on the validation set.

It is important to select the ANN function according to the suitability of the data structure. A linear ANN algorithm was used in our study. Since this approach involves the training stages of the algorithms, more successful estimates can be obtained than with classical linear approaches (29). When the outputs of our study are evaluated together with the literature, detailed clinical findings and detailed ultrasonographic evaluation were observed to be important for the differential diagnosis between VUR and UTI. For this reason, the importance of RBUS, which is frequently performed in renal disease, can be seen and so negating the need for invasive methods. Results with high sensitivity were obtained by evaluating multiple data using different analysis techniques such as ANN. Some studies also have reported that better estimates were obtained by the ANN method.

Conclusions

VUR is a urinary system anomaly that causes adverse outcomes in children such as renal scarring when late diagnosis or uncontrolled prognosis occurs. Early diagnosis and differential diagnosis from UTI are important. ANNs can build computer models that are useful for medical decision-making. When the data from detailed clinical findings and noninvasive imaging techniques such as USG are evaluated together via ANN analysis, better estimates and higher sensitivity can be obtained for the differential diagnosis of VUR and UTI.

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Ethics

Ethics Committee Approval: The study was approved by the ethics committee of the Ege University Faculty of Medicine the protocol number: 13-6.1/56 the date of approval: 29.07.2013).

Informed Consent: Patients have given informed consent for participation in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.K., S.Ö., Design: A.K., S.Ö., Data Collection or Processing: A.K., S.Ö., Analysis or Interpretation: A.K., S.Ö., Writing: A.K., S.Ö.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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Original Article

Association of Parents' Body Esteem and Body Mass Index with Children's Body Esteem and BMI: A Study from Turkey

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ABSTRACT

Aim: Childhood obesity is associated with psychosocial and medical comorbidities. Children affected by obesity have significantly lower self-esteem than children with normal weight, and families have an important role in the development of body image and body dissatisfaction. We aimed to examine the relationship between the body esteem of obese children and their parents' body esteem and weight status.

Materials and Methods: This study was carried out in Ankara, the capital city of Turkey, and was rolled out to 9 to 11-year-old children and their parents in 46 schools (2,066 parent-child dyads). The data were collected via parent and child questionnaires. Anthropometric measurements were conducted by the project team. Four different logistic regression models were performed separately; body esteem and Body Mass Index (BMI) for fathers and sons, fathers and daughters, mothers and sons, and mothers and daughters.

Results: Mothers obesity was associated with their daughters' and their sons' obesity status. Fathers' obesity status appears to be positively associated with their sons' status only but not their daughters'. The body esteem of the obese boys was adversely affected only by their own BMI in the models with their fathers and mothers. Compared to boys, girls were affected adversely by their own BMI, their body esteem, and their mothers' and fathers' body esteem.

Conclusion: A strong association between both the mothers' and fathers' role in the body esteem of daughters was found. Further research should be conducted to better understand the socio-cultural dynamics that may be unique to the development of childhood obesity.

Keywords: BMI, body esteem, childhood obesity, parental relations, daughter, sons, Turkey

Introduction

The prevalence of childhood obesity has increased in the last three decades worldwide. This poses a serious public health problem in both developing and developed countries (1). The prevalence of obesity also increased from 8.3 to 9.9% from 2013 to 2016 among 7-8-year-old children, and it is considered as an emergent public health problem in Turkey (2).

Obesity in childhood increases the risk of obesity in adulthood, and is associated with psychosocial and medical comorbidities (3). Children affected by obesity have significantly lower self-esteem than children with

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normal weight. (4,5). Body esteem is a measure of body image that includes at least three aspects: feelings about one's general appearance, feelings about one's weight, and evaluations attributed to others about one's body (6). Children with low body esteem consider themselves "stupid", "ugly", "unhappy", "less competent", "sloppy", "lazy" and/or "socially isolated", and they usually lack self-discipline, motivation and personal control (7).

Families play an important role in the development of body image and body dissatisfaction among children. In particular, past research has shown that girls' identification with their mothers is a strong factor in their development, including the formation of gender-role identity, body image, and self-esteem, as mothers serve as both role models and sources of information and guidance (8). Perez et al. (9) found that daughters' thin body ideal was significantly affected by their mothers' body dissatisfaction. By the same token, mothers' self-objectification can also influence their daughters' self-objectification. On the other hand, research on the role of fathers into this issue is limited (10). In contrast to general expectation, Johannsen et al. (11) showed that fathers can have higher concerns about their daughters' weight in the future, more so than their sons'.

The roles of the mothers' and fathers' body esteem and weight status on their children's body esteem have not been studied previously in Turkey. In the present study, we examined these relationships in a relatively large population-based sample of parent-child dyads in Ankara, Turkey.

Materials and Methods

This research is a part of Childhood Obesity Study in the metropolitan area of Ankara (COSA) (12). The surveys were rolled out to grade 4 children (aged 9-11 years) and their parents in 46 schools [15 schools were from low socio-economic status (SES), 17 schools from medium SES, and 14 schools from high SES]. The schools were selected from all SES by using probability proportional-to-size methodology in order to match the general population. The detailed study protocol can be found in the article by Yardım et al. (12). Parental questionnaires were sent to parents by their corresponding schools, and they were returned with an informed consent form to the schools. Children consented to the study and completed the questionnaires under the supervision of the research team and teachers in the schools. Anthropometric measurements were conducted with personal privacy by the research team. The study was approved by the Ministry of National Education and the Non-interventional Clinical Research Ethics Board Hacettepe University, Ankara, Turkey (GO 14-429-07).

Measurements

The body esteem scale was developed for adolescents and adults by Mendelson et al. (6) and includes 23 items. It consists of three dimensions to gauge a respondent's feelings about his/her body: Appearance (Cronbach's alpha= 0.92), Attribution (Cronbach's alpha= 0.81) and Weight (Cronbach's alpha= 0.94). Tests of validity and reliability of the scale were performed in children and adults in COSA. The results were found for Appearance (Cronbach's alpha= 0.76) for Attribution (Cronbach's alpha= 0.69) and for Weight (Cronbach's alpha= 0.85). According to confirmatory factor analysis (CFA), the results showed a root mean square error of approximation (RMSEA)= 0.039 (Pclose <1.00), chi-square to df ratio (CMIN/df)= 3.406 ($p<0.001$), SRMR= 0.040, and goodness-of-fit index (GFI)= 0.979. Test-retest analysis presented the following statistics: Appearance (Spearman's r=0.68), Attribution (Spearman's r=0.57) and Weight (Spearman's r=0.68) for children. CFA was conducted with the 12-item three-factor version for adults. Results of CFA were obtained as RMSEA=0.067 (Pclose <1.00), and GFI=0.959. The test-retest reliability of the BE scale has Appearance (Spearman's r=0.59), Attribution (Spearman's r= 0.72) and Weight (Spearman's r=0.68), for adults, respectively. We presented the total scores of the body esteem scale in the analyses section.

Height and weight measurements of the children were conducted to assess the children's Body Mass index (BMI). BMI-for-age was calculated using the WHO ANTHRO Plus program and classified as underweight, normal, overweight or obese. The BMI of the mothers and fathers were calculated based on their self-reported weight and height and they were classified based on standard adult cut-offs (13). Only one parent (either the mother or father) served as the primary respondent to the questionnaire for each student.

Statistical Analysis

The Kruskal-Wallis test was used to compare scaled variables across more than two independent groups. Pairwise comparisons were performed using Dunn's test. Pearson's chi-square test was used to compare proportions. We combined underweight and normal weight into one group and compared it to the overweight and obese groups. The total body esteem score in children was the outcome measure. Receiver operating characteristic (ROC) curve analyses were performed to evaluate the cut-off point for the total body esteem score (14). The best cut-off point of total score for boys and girls was obtained as 36. Individual scores lower than the cut-off point were classified as low

body esteem; and scores higher than the cut-off point were classified as high body esteem (15). The parents' body esteem scores were treated as a continuous variable in our analyses. Analyses were performed using SPSS version 23.0 (Chicago, IL) with alpha set as $p<0.05$.

Logistic regression models were used to examine the risk of low body esteem among children in relation to their own weight status, their parents' weight status and their parents' body esteem. Four separate logistic regression models were performed by using the father-son, father-daughter, mother-son, and mother-daughter data sets. SES status (reference is high SES), child obesity status (reference is normal BMI), the relevant parent's obesity status (reference is normal BMI), the relevant parent's body esteem (corresponding dimension or total score) were included as the main effects in relation to the low body esteem of children. In the regression model, those children having higher scores than the cut-off point were coded as "0" which means "having high body esteem" and the ones having lower scores than the cut-off point were coded as "1" which represents "having low body esteem".

Results

The study sample included 2,066 parent-child dyads. The sample included 53.1% girls and 46.9% boys. 53.2% of children were from low SES, 34.6% from middle SES and 12.1% from high SES. The education level of the fathers was higher than the mothers; 60.3% of fathers vs. 47.9% of mothers had high school or higher degree education levels ($p<0.001$). Most of the fathers were employed (95.5%) while the percentage of employment among the mothers (33.0%) was lower ($p<0.001$) The rates of being overweight or obese among mothers were 34.8% and 16.1%, respectively; these percentages in fathers were 50.5% and 19.6%, respectively. The rates of the child being overweight or obese were 21.2% and 14.6% respectively (12).

Table I shows the comparison of children's weight status with their mothers and fathers. Results showed that the mothers' obesity was associated with their daughters' and their sons' obesity status ($p<0.001$, $p<0.001$). However, the fathers' obesity status appears to be positively associated with their sons' status only ($p<0.001$), as it was not found to be statistically associated with their daughters'.

Figure 1 shows that there is a statistically significant negative correlation between BMI and body esteem among girls, boys, mothers and fathers, suggesting that self-esteem increases as BMI decreases ($p<0.001$ for all four group).

The body esteem of boys was significantly negatively associated with their own BMI ($p<0.001$) when a logistic regression model was used to examine the association between mothers and their boys. Body esteem of the obese boys was 9.01 times lower, compared to normal weight boys. For girls, they were affected by their mothers' body esteem score ($p=0.001$) in addition to their own obesity status ($p<0.001$) in the model that examined the association between mothers and their daughters. Overweight girls had 5.32 times and obese girls had 16.88 times lower body esteem than girls who had normal BMI, and the body esteem of girls increased as the body esteem of their mothers increased (Table II).

The body esteem of boys was adversely associated with their own BMI ($p=0.006$) in the model examining the association between fathers and their boys. The body esteem of the overweight and obese boys were 3.08 and 4.55 times lower than the body esteem score of normal weight boys respectively. In this model, there was a dose-response relationship observed between girls' total body esteem and their BMI status ($p<0.001$) for fathers and their daughters. Overweight and obese girls have lower body esteem (4.96 and 9.92, respectively) compared to normal weight girls. The other result we observed was that the body esteem of girls increased as the body esteem of their fathers increased (Table III).

Discussion

To the best of our knowledge, this is the first study in Turkey to explore the association between mothers' and fathers' body esteem with their daughters and sons. We found a significant relationship between mothers' and fathers' body esteem with the body esteem of their daughters. For both boys and girls, their body esteem was significantly associated with their own BMI, suggesting that among Turkish children, high BMI may have deleterious psychological effects.

With the increasing epidemic of obesity in Turkey (2), there is a great need for public health policies to address the psychosocial sequelae of obesity among children. In the COSA study, we have previously reported that the prevalence of obesity among 10-year-old children was 14.6%, which is much higher than prior studies and the most recent estimates of national prevalence in Turkey (12). This suggests that children in the larger urban areas in the country may be at higher risk for obesity-related comorbidities (1,3). Çolpan et al. (16) found that children affected by obesity had lower self-esteem than children who were not affected by obesity. They also found a

relationship between self-esteem and other emotional and behavioral problems. Our results corroborate the findings of their study which also used a population-based sample from Ankara. We also found a statistically significant negative correlation between BMI and body esteem for girls and boys; and showed their self-esteem increased as BMI decreased. This negative correlation is consistent with prior research in the United States and Europe (3-5,17).

Obesity in children can be affected by parental obesity status, suggesting that family dynamics, including family lifestyle, attitudes and perceptions on obesity are important determinants for obesity in childhood. Eating habits, family and environmental factors serve as targets of prevention and treatment interventions in many studies (9).

In our study, fathers' weight status and their sons' weight status were correlated, similar to prior research (9,18). However, the same was not true between fathers and daughters, in contrast with the study by Power et al. (19). It is possible that the effect of the same-gender parents (i.e. father/son or mother/daughter) may be stronger in the cultural context (9). In Turkey, the main responsibilities of the mothers are "cooking" and "taking care of children" and fathers have the main responsibility for providing the income in the family (20). It is likely that mothers have more influence on their children than fathers in Turkish communities in terms of weight-related outcomes.

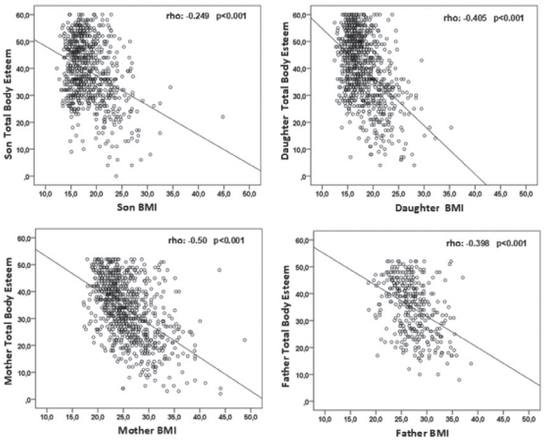

Figure 1. Correlation diagram BMI by self-esteem in children and parents
BMI: Body mass index

Table I. The percentage distribution of children' body mass index by families' body mass index

		BMI				
	BMI	**Underweight and normal**	**Overweight**	**Obese**	**Total**	**p**
		Daughters				
Mother	Underweight and normal	67.7	25.1	7.2	279	**<0.001**
	Overweight	59.2	26.7	14.1	206	
	Obese	47.1	27.1	25.9	85	
	Total	61.6	26.0	12.5	570	
Father	Underweight and normal	77.3	18.2	4.5	66	0.335
	Overweight	70.2	18.2	11.6	121	
	Obese	75.0	21.2	3.8	52	
	Total	73.2	18.8	7.9	239	
		Sons				
Mother	Underweight and normal	62.0	18.8	19.2	250	**<0.001**
	Overweight	62.1	23.7	14.1	177	
	Obese	51.2	11.6	37.2	86	
	Total	309	99	105	513	
Father	Underweight and normal	77.5	12.5	9.9	71	**<0.001**
	Overweight	47.8	30.4	21.7	115	
	Obese	55.3	10.5	34.2	38	
	Total	58.5	21.4	20.1	224	

BMI: Body mass index

Unfortunately, we could not ask about gender roles and parenting behaviors in terms of mothers vs fathers in our study, but this issue warrants further research. More research is needed to understand the underlying cultural mechanisms of the relations between paternal and childhood obesity in different countries.

The strong relationship between mothers' body esteem and their daughters also corroborates with previous studies (9,21). Cooley et al. (22) found that mothers have a strong influence on their daughters' lifestyle and behaviors such as eating and physical activity, and also their feelings and attitudes towards themselves. It is also known that the mother-daughter relationship affects the daughter's body perception. Since parents are role-models for their children, mothers worrying about their weight and their body image could also become negative role-models for their daughters (23). In light of our findings, psychological support targeting mothers may be particularly important in the design of obesity interventions in Turkey.

We also found a significant association between fathers' and daughters' body esteem. The father's role in childhood obesity has only begun to be discussed in recent years, but the literature does not specifically include relationship between the body esteem of obese fathers and their obese daughters (24). Sagkal et al. (25) showed that the father-daughter relationship is related to the well-being of the daughters in the Turkish population. Our findings suggest that family interventions in Turkey could potentially benefit from the inclusion of fathers as well, at least with regard to their daughters' weight-related outcomes.

Results showed that the body esteem of sons was not affected by their mothers' or fathers' body esteem (the sons' own weight status was the significant correlate of their body esteem). Studies show that girls are more affected by parental body esteem and body image than boys (8-10).

Weinberger et al. (26) showed that, in general, women reported more dissatisfaction with their bodies than men, but some studies also show that men affected by obesity have lower body esteem than those who are not affected by obesity. In our study, we found a statistically significant negative correlation between BMI and body esteem among mothers, but this relationship was not statistically significant among fathers. Lengerke et al. (27) discussed that the lack of awareness on overweight and obesity status among low socioeconomic status individuals can also cause lower body dissatisfaction; Bibiloni et al. (28) mentioned that cultural factors, such as acceptance of body image,

Table II. Impact of socio-economic status, body mass index, and mothers' body esteem on their children' body esteem

	Sons' body esteem				**Daughters' body esteem**			
			95% CI				**95% CI**	
	p	**Exp (B)**	**Lower**	**Upper**	**p**	**Exp (B)**	**Lower**	**Upper**
School SES	0.38				0.16			
High (reference)								
Middle	0.19	1.71	0.76	3.86	0.06	2.04	0.95	4.37
Low	0.49	1.33	0.58	3.05	0.30	1.49	0.69	3.19
Children BMI	**<0.001**				**<0.001**			
Normal (reference)								
Overweight	0.09	1.66	0.91	3.04	**<0.001**	5.32	3.11	9.08
Obese	**<0.001**	9.01	4.49	18.07	**<0.001**	16.88	6.66	42.79
Mother BMI	0.66				0.85			
Normal (reference)								
Overweight	0.99	1.00	0.54	1.84	0.95	0.98	0.55	1.74
Obese	0.41	0.69	0.29	1.66	0.60	0.79	0.34	1.86
Mother body esteem score	0.75	0.99	0.96	1.02	**0.001**	0.95	0.93	0.98
Constant	0.053	0.29			0.66	0.77		

Note. Parental body esteem variable corresponds to the specific dimension or total score among children, BMI: Body Mass index, CI: Confidence interval, SES: Socio-economic status

Table III. Impact of socio-economic status, body mass index, and fathers' body esteem on their children' body esteem

	Sons' body esteem				Daughters' body esteem			
			95% CI				95% CI	
	p	Exp (B)	Lower	Upper	p	Exp (B)	Lower	Upper
School SES	0.74				0.11			
High (reference)								
Middle	0.99	1.00	0.28	3.55	0.24	0.52	0.17	1.55
Low	0.64	0.73	0.19	2.70	0.040	0.26	0.07	0.94
Children BMI	**0.006**				**<0.001**			
Normal weight (reference)								
Overweight	**0.030**	3.08	1.11	8.55	**0.001**	4.96	1.92	12.80
Obese	**0.003**	4.55	1.67	12.34	**0.008**	9.26	1.80	47.46
Father BMI	0.30				0.20			
Normal (reference)								
Overweight	0.13	0.48	0.19	1.24	0.43	0.71	0.30	1.67
Obese	0.29	0.50	0.13	1.83	0.07	0.35	0.11	1.11
Father body esteem score	0.38	0.98	0.94	1.02	**0.024**	0.95	0.91	0.99
Constant	0.86	1.19			0.07	5.78		

Note: Parental body esteem variable corresponds to the specific dimension or total score among children, BMI: Body Mass index, CI: Confidence interval, SES: Socio-economic status

can also vary among countries. We could not explain the differences between men and women in this study, however research on adults can help to answer the body esteem differences between men and women by investigating social and cultural factors.

Study Limitations

This study also has some limitations. There can be other possible explanations to describe the relationship between parental BMI, parental body esteem, and children's body esteem. More contextually rich research including the use of qualitative or mixed methods will be helpful to investigate the underlying mechanisms. Our study may not be generalizable to other populations in Turkey.

Conclusion

In conclusion, the family has a crucial role in the physical, mental and social development of children. Our study highlights the need for more evidence-based approaches to help families improve their children's health and quality of life. We found strong evidence that both mothers and fathers have a role to play in the body esteem of daughters, suggesting a whole family approach may be essential so as not to lose sight of the importance of fathers. Our research adds another dimension to the national strategy for obesity by shedding light on potential family levers in the design of prevention and treatment interventions. As obesity continues to rise in emerging economies worldwide, countries such as Turkey are in critical need of culturally tailored intervention strategies. Our research adds to the emerging literature and should inform obesity prevention and control programs and policies in Turkey.

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Ethics

Ethics Committee Approval: The study was approved by the Ministry of National Education and the non-

interventional Clinical Research Ethics Board Hacettepe University, Ankara, Turkey (GO 14-429-07).

Informed Consent: They were returned with an informed consent form to the schools.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.Ö., U.E.A., S.Ü., Ö.A., H.K.Ü., M.Y., N.B., T.T.H., Design: H.Ö., U.E.A., S.Ü., Ö.A., H.K.Ü., M.Y., N.B., T.T.H., Data Collection or Processing: H.Ö., U.E.A., S.Ü., Ö.A., H.K.Ü., M.Y., N.B., T.T.H, Analysis or Interpretation: H.Ö., U.E.A., H.K.Ü., T.T.H., Literature Search: H.Ö., U.E.A., S.Ü., Ö.A., H.K.Ü., M.Y., N.B., T.T.H., Writing: H.Ö., U.E.A., Ö.A.

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Original Article

Examining the Effect of a Program Developed to Address Bullying in Primary Schools

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ABSTRACT

Aim: The purpose of this study is to evaluate effectiveness of a bullying prevention program developed using an integrated approach, in the short-term and long-term, in primary schools.

Materials and Methods: This study was made using a quasi-experimental control group pretest-posttest design; one of the experimental research methods. This study comprised 113 students receiving 6th grade education at 2 randomly selected schools in the districts of Konak and Karabağlar, in Izmir, Turkey. The data were gathered using the Demographic Data Questionnaire and The Peer Bully Adolescent Form. In the experimental group, students attended a bullying prevention program and their parents and teachers attended seminars for 5 weeks. The data were collected before the training, 2 weeks after the training, at the 6th month, and at 1 year after the training. In the analysis of the data, ANOVA was used in repetitive measurements and t-tests were used in dependent and independent groups with Bonferroni correction.

Results: A statistically significant difference was found among the control and experimental group victim subdimension point averages in the group interaction (F=68.28, p=0.001), time interaction (F=7.39, p=0.001), and group-time interaction (F=14.04, p=0.001). A statistically significant difference was found between the control and experimental group bully subdimension point averages in the group interaction (F=7.63, p=0.007) and time interaction (F=20.21, p=0.001). No significant difference was determined in the group-time interaction (F=1.10, p=0.349).

Conclusion: It was found that the bullying prevention program based on the Social Cognitive Theory is effective in decreasing the rates of students who are bullies or are the victims of bullying, and this effect continues in the victims through to the end of the 1st year; however, it becomes nonsignificant in bullies by the end of the 1st year.

Keywords: Bullying, bullying prevention program, victim, bully, nursing

Introduction

Bullying is one of the negative relationships that students experience in schools and it has important negative effects that threaten a child's health and development. Olweus (1) defined bullying as when a student is exposed to the negative behaviors of one or more students in a repeated way, more than one time, meaning this individual is then exposed to bullying or has become a victim. The characteristics of bullying behavior are a real or perceived strength imbalance, an intentional deed with the purpose of harming, and being repetitive (1).

Studies made on bullying in Norway (1), Spain (2), Ireland (3), Finland (4), Australia (5), Greece (6), Malaysia (7), Canada (8), Italy (9), Belgium (10), America (11), Holland (12), Japan (13), and Germany (14) show that bullying is a problem experienced all over the world.

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In the studies made in Turkey (15-18), it was stated that there is a significant increase in the cases of violence and bullying in schools and immediate prevention is required. In the study "Health Behavior in School-aged Children" which was arranged by the World Health Organization, and in which 41 countries took part, the situation of students' being victim and bully over a 12-month period were investigated. Out of 41 countries, Turkey ranked 1st in the victim age group of 11-year-olds and 10th in the age group of 13-year-olds. Moreover, Turkey was 3rd in the bully age group of 11-year-olds and 10th in the age group of 13-year-olds. These results show that bullying is an important problem in Turkey as well (19).

In many countries around the world, bullying prevention programs have been applied for years and nation-wide studies have been made on this subject. There are a limited number of studies about bullying prevention in Turkey (20-25). Most of the studies are just programs oriented towards students (21-23,25-27). It is stated in the literature that integrated programs including the student, teacher, and parents altogether are more effective in preventing bullying (28). It is also stated that conducting a prevention program in the early adolescent period will be a protective factor in the later life and adulthood of the student (29).

There is a necessity for programs that will provide a healthy learning environment for students, with awareness training that addresses bullying and will include the student, family, and teacher in an integrated way, as well as a necessity for studies that evaluate the long-term effects of these programs. These programs will enable enterprises to work towards a solution by raising awareness concerning the problem of bullying as well as by decreasing the rates of bullying. The purpose of this study is to evaluate the long-term and short-term effectuality of the programs developed to address bullying in primary schools.

Materials and Methods

Design

This study was carried out using the "quasi-experimental control group pretest-posttest design" which is one of the experimental methods to evaluate the effectiveness of bullying prevention programs (Figure 1).

Sample

This study was carried out in 2 primary schools dependent on the Ministry of National Education in the districts of Konak and Karabağlar in Izmir, Turkey. The schools to be included in the study were selected from the half-day public schools that are under the supervision of Konak and Karabağlar District National Education Directorate. Data from the Izmir National Education Directorate were used to select districts and schools; districts and schools that show similar characteristics were selected. The primary schools in the districts of Konak and Karabağlar were chosen as they serve students from low, middle and high income backgrounds and have mixed student groups. There are a total of 50 primary schools, 47 of which are public and 3 of which are private schools, under the Karabağlar District Directorate of National Education, and 38 of these schools are half-day. There are 70 primary schools, 65 of which are public and 5 of which are private schools under the Konak District Directorate of National Education, and 46 of these schools are half-day.

In Izmir, from the schools that serve under the Konak and Karabağlar District National Education Directorate, two schools were selected among those half-day public schools by a simple random method. As it is stated in the literature that early intervention is important, 6th grade students (approx. 12-year-olds) were taken as samples. The schools to be included in the experimental and control groups and the classes to be selected from the schools were determined by random method.

Two classes were selected from both schools in order to cover the number of samples. An experiment and a control group were generated from both schools. For the determination of the classes, the experimental group was selected from the morning classes and the control group was selected from the afternoon classes. D and B classes were included in the sample under the experimental group and D and F classes were included in the sample under the control group. Before starting the research, the class lists of the sample classes were requested and the number of classes was determined according to the number of students.

The study was carried out between February 2007 and June 2011. Schools and branches that would be in the experimental and control group were randomly selected and 6th grade students were taken as the sample. The demographic data of the students are given in Table I.

In order to determine the sample size, an analysis was made by taking the Type II error 0.20 (80% power), Type I error 0.05 significance level, and the data in the study by Ayas (20), and it was found that the sample size should be at least 32 for each group. Thus, 2 classes from each school were included in the study.

According to the power analysis, it was calculated that there should be at least 32 students in a group. As the

number of students in the classes in the experimental group was 29 and 27, no students were excluded from the class and the training for the prevention of bullying was conducted with the whole class. The posttests were taken from 97 students, however 77 students, whose names/nicknames match with the first measurement, were included in the posttests (1 year after the interference), because of reasons such as some of the students had left the school or some new students had arrived.

The t-test was used in order to determine the difference between the averages of the students' ages in the experimental and control groups; x^2 analysis was made in order to determine the difference between the groups in terms of gender, and it was found that there was no significant difference between the groups in terms of age and gender (Table I, p>0.05).

The t-test was used in order to determine the difference between the victim and bully pretest point averages of the students in the experimental and control groups, and it was found that there was no significant difference (Table II, p>0.05). The fact that there was no change between the groups in terms of the age, gender, victim, and bully subdimensions shows that the groups were homogenous.

Table I. Demographic data of the students

Group	**Age**		**t**	**p**
	n	**X ± SD**		
Experimental	56	11.5±0.6	0.587	0.558
Control	57	11.6±0.5		

Group	Gender				**X²**	**p**
	Female		Male			
	n	**%**	**n**	**%**		
Experimental	30	53.7	26	46.3	0.435	0.510
Control	27	47.4	30	52.6		

N: Number of patients, SD: Standard deviation

Table II. Comparison of the bully and victim pretest point averages of the students

Group	**Victim Subdimension Points**		**t**	**p**
	n	**X ± SD**		
Experimental	56	76,21±22.23	0.577	0.565
Control	57	78.49±19.62		
	Bully Subdimension Points		**t**	**p**
	n	**X ± SD**		
Experimental	56	58.17±13.24	1.572	0.119
Control	57	62.03±12.83		

n: Number of patient, SD: Standard deviation

Research Instruments

The data were collected using the Demographic Data Collection Form and The Peer Bully scale-adolescent form (30). The demographic data collection form consists of questions such as nickname, name, and age.

The Peer Bully scale-adolescent Form

The Peer Bully scale-adolescent form developed by Pişkin and Ayas (30) consists of 2 scales called the "Bully scale" and the "Victim scale". This scale consists of 53 items covering physical bullying, verbal bullying, bullying by isolation, bullying by spreading rumor, bullying with threatening objects, sexual bullying, and victim subdimensions. The scale is graded as "never" (1) and "almost every day" (5), and as the number increases, the cases of being a bully or victim increase. In this study, the sexual bully and victim subdimensions could not be used due to the fact that it was not permitted by the Directorate of National Education. For this reason, the lowest point score possible was 43 and the highest point possible was 215, taken from the bully and victim subdimensions. Pişkin and Ayas (30) stated the Cronbach alpha internal consistency coefficient to be 0.93 for the total Victim scale and 0.92 for the total Bully scale. In this study, the Cronbach α coefficient was computed as 0.88 for the total Victim scale and 0.91 for the total bully scale.

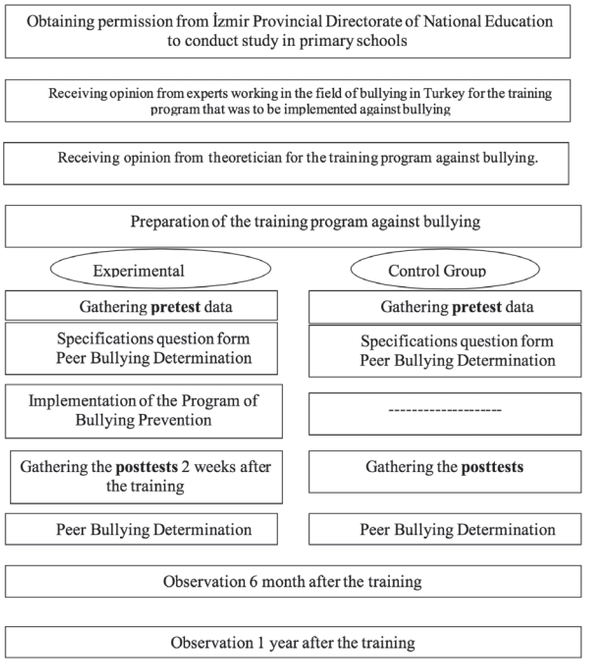

Figure 1. Study plan

Application of the Training Program

In this study, a bullying prevention program was prepared in 2 steps. In the first step, the conceptual framework of the bullying prevention program was prepared according to Bandura's Social Cognitive Theory. In this study, a training program was prepared in accordance with reciprocal determinism, self-sufficiency, self-regulation, and the indirect learning principles of Social Cognitive Theory (31).

The second step was to take expert opinions. In this study, a training program for bullying was prepared by taking opinions from different disciplines. A Bullying Training Content Questionnaire was prepared by the researchers in accordance with the literature and sent to the experts via e-mail. Experts were also requested to state training subjects that they thought should be in the student, teacher, and parent training program, and those subjects that were not in the questionnaire, but they thought should be. A scanning method was chosen to reach these experts in Turkey. Consequently, the authors of 50 studies made in Turkey before and during 2009 were sent e-mails. In order to reach all of these experts, reminder e-mails were also sent; however, just 15 experts replied back. All of these responders stated that there should be "awareness training" and "problem solving skills" at the highest level in the student, teacher, and parent training programs. The training contents were formed in line with these expert opinions.

Program Carried Out with the Students

The training program for students was conducted as weekly 40-minute sessions over a 5-week period. The program lasted for 7 weeks with sessions in which pretest and posttest data were collected. The program was held in the seminar hall of the school on the same day, at the same hour, every week, due to the fact that only counseling courses were allowed for the application of the program by the school administrators.

Within the program content carried out with the students, there were awareness training sessions that included information about bullying, sessions in which scenarios about the direct and indirect aspects of bullying were solved with problem solving techniques, an intervention techniques session in which what to do in a cases of being exposed to bullying was discussed, and a session oriented towards antibullying activities. In the student training, the methods of slides, videos, pictures, scenarios, mnemonic games about the subject, acting roles, question-answer activities, and the preparation and presentation of posters were used.

The study data were collected 4 times: before the training (pretest: with 113 students), 2 weeks after the training (posttest 1: with 112 students), 6 months after (posttest 2: with 87 students), and 1 year after (posttest 3: with 97 students). The attendance rate was 86%. In total,

Table III. The effect of the program addressed to the bullying on the victim subdimension points

Time Groups	Pretest X ± SD	Posttest 1 X ± SD	Posttest 2 X ± SD	Posttest 3 X ± SD	F	p
Experimental	73.5±18.3	54.4±11.5	52.7±9.8	55.0±10.8		
Control	78.4±19.2	76.8±23.3	81.9±26.5	90.5±22.4		
				Group	68.28	0.001
				Time	7.39	0.001
				Group*Time	14.04	0.001

SD: Standard deviation

Table IV. The effect of the program addressed to bullying on the bully subdimension points

Time Groups	Pretest X ± SD	Posttest 1 X ± SD	Posttest 2 X ± SD	Posttest 3 X ± SD	F	p
Experimental	58.5±13.5	46.9±4.9	47.5±5.8	50.3±9.4		
Control	62.1±14.1	55.2±16.1	52.1±10.6	54.2±11.7		
				Group	7.63	0.007
				Time	20.21	0.001
				Group*Time	1.10	0.349

SD: Standard deviation

the analyses of only 77 students could be made as the passwords of some of the students did not match or they did not have the 6-month or 1-year follow-up data, or they had changed schools.

Parental Participation

In studies carried out with parents, the inadequate attendance of parents is a common problem. In order to mitigate this problem, a letter was prepared about the importance of the training program and the attendance to the program for the parents of the students in the experimental group, and these were sent to the families by the school administration. Moreover, information was given about the study and the parents' questions were answered at parent training sessions. These parent training sessions took place twice a day, each session lasted for 60 minutes and 26 parents attended the training. In the parent training session content, there was awareness training for bullying, the negative results of bullying, adolescence, and subject of communication with an adolescent.

Program Carried Out with the Teachers

In the 5th training session of the program, training was given for the teachers. In line with the suggestions of the experts, school administrators were also included in this training. In the training session content for teachers, there was awareness training for bullying, the negative results of bullying, activities that could be done to prevent bullying school-wide, and subjects that dealt with the relationship between bullying and teacher attitudes. As just 6th grade classroom teachers were to take part in the study, training materials were shared with the school administrators attending the study and it was aimed to inform all of the teachers.

Statistical Analysis

The study data were assessed using SPSS Software in a computer environment, ANOVA for repetitive measurements, and t-test for dependent and independent groups with Bonferroni correction (32).

Ethical Dimensions of the Study

In order to conduct this study, written permission was taken from the Dokuz Eylül University Faculty of Nursing Ethics Committee (18.10.2007/1169), the Izmir Provincial Directorate for National Education, and from the parents of those students involved, and verbal permission was taken from the students. All of the parents allowed their children to take part in the study.

Results

In this category, the findings of this study are given under 2 titles, namely, the effect of the program on the victim and on the bully subdimension points of students.

In Table III, a comparison of the averages of the Peer Bully scale victim subdimensions are given. A significant difference was found between the experimental and control group victim subdimension point averages according to the group (F=68.28, p=0.001), time (F=7.39, p=0.001), and group*time (F=14.04, p=0.001).

In this study, it was found that there was a significant difference between the measurements before the training, the 6-month, and 1-year measurements in the experimental group in which the bullying prevention program was applied. It was also found that the program carried out with the students in the experimental group caused more of a decrease in the victim levels than for those students in the control group, and this was a long-term situation.

In Table IV, the averages of The Peer Bully scale bully subdimensions are given. In the results of the analysis, a statistically significant difference was found between the bully subdimension point averages according to the group (F=7.63, p=0.007) and time (F=20.21, p=0.001). No significance difference was confirmed for the group and time interaction (F=1.10, p=0.349).

Discussion

In this category, findings obtained from the data of the study are discussed with the titles of victim and bully subdimensions and, additionally, suggestions are offered.

The Effect of the Program for Bullying on the Victim Subdimension Points of the Students

In this study, it was determined that the bullying prevention program is effective in decreasing the victim subdimension points of the students in the experimental group and that this effect continues in the measurements of the 6-month and 1-year follow-up (Table III). This finding is similar to some studies in the literature that evaluate to effectiveness of programs developed to prevent bullying (1-4,6,8,11,20,22-25,27). This differs from the findings of some studies stating that bullying prevention programs are not effective (5,7,9,21,26). In the systematic collected work, Vreeman and Carrol (28) analyzed the effectuality of bullying prevention programs and they determined that integrated programs were more effective compared to other programs. In this study, the effectiveness of the

program can be assessed as a result of its being integrated and the attendance of the students, teachers, and parents.

There are a limited number of studies in which the long-term effectiveness of programs developed for bullying prevention are analyzed in the literature (1,5,6,12) and the results also differ. In the review study made by Mytton et al. (29), which evaluates the effectiveness of programs developed to decrease violence and bullying in schools, it was stated that a 12-month follow-up was conducted in only 7 studies.

In this study, it was found that while the experimental group victim points decreased, the control group victim points increased. It is thought that in a branch in the control group, being exposed to bullying with the change in class and peer relationships and victim points increased in the 1-year follow-up. It was stated in the study by Totan and Yöndem (33) that as peer relationships increase, cases of being the bully increase but cases of being the victim decrease.

The Effect of the Program for Bullying on the Bully Subdimension Points of the Students

In this study, it was determined that the bullying prevention program was effective in decreasing the bully subdimension points of those students in the experimental group; however, this effect did not continue in the measurements of the 6-month and 1-year follow-up (Table IV). This finding is similar to the results of other studies (3,4,12,23). The fact that the effectiveness of the program continued into the 6-month follow-up showed that it was effective in the long-term. In a study in which follow-ups were made for less than 6 months (20), it was found that the program was not effective for the rates of being bullied. This result is different from the findings of the present study. In the 1-year follow-up of this study, the fact that there was not a significant difference in the bully rates between the groups made us think that in order to make effectiveness continue, further training was necessary 1 year after the initial training. Fekkes et al. (12) stated that the program should be repeated every year for greater effectiveness.

Study Limitations

The inadequate attendance of the parents, the inclusion of only 6th grade classroom teachers in the study, and students dropping out or changing classes while the follow-up data during the 1st year were being collected were the restrictions of this study.

Conclusion

That the program is effective in decreasing the victim points 2 weeks after and at the 6-month and 1-year follow-up and is effective in decreasing the bully points 2 weeks after and at the 6-month follow-up; however, it is not effective at the 1-year follow-up.

In line with the results obtained in this study, it is suggested that:

That training should be repeated every year in programs applied for bullying prevention, mnemonic training should be made, and activities about bullying should be planned.

It is recommended to conduct studies in which the negative consequences of bullying are defined and the effectiveness of the programs developed for these negative consequences are evaluated.

Ethics

Ethics Committee Approval: Written permission was taken from the Dokuz Eylül University Faculty of Nursing Ethics Committee (18.10.2007/1169).

Informed Consent: Consent forms were filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.K., C.Ö., Design: H.K., C.Ö., Data Collection or Processing: H.K., Analysis or Interpretation: C.Ö., Literature Search: C.Ö., Writing: H.K., C.Ö.

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Original Article

Evaluation of Patients Diagnosed with Brain Death in Paediatric Critical Care

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ABSTRACT

Aim: We aimed to investigate the rate of brain death (BD) determinations and organ donations in our tertiary pediatric intensive care unit (PICU), and to report the data on demographic pattern and supplementary descriptive data on the BD declarations.

Materials and Methods: Age and gender of the cases were recorded, and also clinical conditions causing BD were grouped and recorded under six headings including; traumatic brain injury (TBI), brain neoplasm, cardiac arrest, intracranial haemorrhage, central nervous system infection and other causes. The time from the PICU admission to the diagnosis of BD, the time from suspicion of BD to final diagnosis, duration of survival after diagnosis of BD, confirmatory tests used, whether apnea test is performed, and inherent alterations (diabetes insipidus, hyperglycaemia, hypothermia) that occurred following diagnosis of BD were recorded. Organ donation rate, the name and number of organs harvested were determined.

Results: Twenty-three patients were diagnosed with BD. The mean age of the patients diagnosed with BD was 5.5±4.9 years. The causative mechanism leading to BD was classified into six groups, out of which the most common diagnosis was TBI with a rate of 39.3%. The meantime from PICU admission to BD diagnosis was 5.7±5.5 days. The time period from suspicion of BD to the final diagnosis was 1.6±0.7 days and the meantime to develop cardiac arrest after diagnosis of BD was 13.1±21.6 days in non-donor cases. The most commonly used confirmation test was brain computed tomography angiography with 82.6%. The most common alteration that follow BD was diabetes insipidus (56.5%). Four patients became organ donors (17.4%).

Conclusion: Patients with severe TBI are the most likely candidates for BD declaration and suitable for organ donation for children in need. Early diagnosis and good donor care are of great importance, especially for paediatric patients waiting for organ transplants.

Keywords: Brain death, paediatric intensive care, organ donation

Introduction

Brain death (BD) is a clinical condition characterized by the irreversible loss of all brain functions, including the brain stem (1). Patients diagnosed with BD can be accepted as organ donors by obtaining family consent for the transplantation (1,2). In Turkey, the diagnosis of BD is made in accordance with the Turkish Law numbered 2,238 called "Harvesting, Storage, Grafting, and Transplantation of Organs and Tissues" (passed on June 3rd, 1979) with article 11 establishing the cause of death and saying 'In connection with the enforcement of this law, the case of medical death is established unanimously by a committee of 4 physicians consisting of one cardiologist, one neurologist, one neurosurgeon, and one anesthesiologist, according to the rules, methods, and practices equivalent to the level of science reached in the country'. Also, a subsequent

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amendment numbered 6,514 made according to the law which was adopted on the 2nd of January 2014 established that BD must be diagnosed by a committee of two physicians; one neurologist or neurosurgeon and one anesthesiology and reanimation specialist or intensive care specialist (3,4). Despite its acceptance as a legal mode of death in Turkey since 1979, progress in the awareness and declaration of BD has been slow and difficult in the pediatric population.

The number of patients waiting for organ transplantation due to organ failure is increasing in Turkey as in many other countries. The one and only treatment for these patients is organ transplantation (5). Therefore, the diagnosis of BD and the provision of good care for the donor have become more important for patients waiting for life saving organ transplantation. Approximately 25,000 patients are on a waiting list for organ transplantation in Turkey according to the current data (6).

The incidence of BD in the pediatric intensive care unit (PICU) is between 16% and 20% (7,8). In developed countries, acute brain injuries are the most common cause of BD in pediatric patients with traumatic brain injuries (TBI). TBI is the leading cause of death in children over 1 year of age (9-11). Diagnosis criteria for BD vary between countries due to some variables such as the requirement of certain tests and the number of physicians needed to establish the diagnosis. There have been a limited number of studies conducted on children regarding the diagnosis of BD and the subsequent process.

In this study; the demographic characteristics of pediatric patients diagnosed with BD in the last 4 years at the PICU of our hospital were examined retrospectively along with the confirmatory tests used in the diagnosis, the time to the occurrence of cardiac arrest after the diagnosis and the acceptance or rejection rates concerning organ donations of the families.

Materials and Methods

Twenty-three pediatric cases with a diagnosis of BD were retrospectively analyzed at Goztepe Training and Research Hospital in the tertiary level of PICU, between March 2015 and September 2019. This study was granted permission by the Ethical Board of Medeniyet University, Goztepe Training and Research Hospital (approval number: 2019/0328).

The BD diagnosis was made in accordance with the conditions outlined in the applicable law and the criteria specified in the guidelines (3,12). An apnea test was performed after the establishment of normothermia, normotension, normovolemia and the value of arterial blood gas partial carbon dioxide pressure ($PaCO_2$) was between 35-45 mmHg and partial oxygen pressure (PaO_2) was above 200 mmHg. Patients who did not meet these criteria or those who were hemodynamically unstable were not subjected to the apnea test. The result of the apnea test was considered positive in the absence of spontaneous breathing, despite the establishment of $PaCO_2$ ≥60 mmHg and/or an increase by 20 mmHg or more from the baseline. Brain computed tomography angiography (CTA) or transcranial doppler ultrasonography (dUSG) was performed as a confirmatory test in cases where an apnea test could not be performed or completed and in patients for whom it was not desirable to wait for a second neurological examination.

The age and gender of the patients were recorded, and also the clinical conditions leading to BD were grouped and recorded under six headings as follows; TBI, brain neoplasm, cardiac arrest, intracranial hemorrhage (non-traumatic), central nervous system infection and other causes. In addition; archive files, computer records, and BD declaration forms were evaluated. The time from the PICU admission to the diagnosis of BD (admission - BD diagnosis time), the time from suspicion of BD to final diagnosis (duration of diagnosis of BD), duration of survival after diagnosis of BD, confirmatory tests used, whether or not an apnea test was performed, and inherent alterations [such as diabetes insipidus (polyuria, urine density <1.005, and serum Na level >145 mEq/dL), hyperglycemia (blood glucose >180 mg/dL), hypothermia (<35 °C)] that occurred following the diagnosis of BD were recorded. The organ donation rate and the type and number of organs harvested were determined using data obtained from the department of organ transplantation coordination.

Statistical Analyses

The data were evaluated using SPSS version 18.0 (SPSS, Inc., Chicago, IL). Categorical variables were presented as frequency and percentage. Continuous variables were presented as mean ± standard deviation [(minimum (min), maximum (max)].

Results

In the study period, a total of 1,738 admissions were recorded at the PICU with an overall mortality rate of 6.9% (n=121). Within this period, there were 23 BD declarations. The frequency of BD was 1.3% in our patient population. The mean age of those children diagnosed with BD was 5.5±4.9 (min: 0.4, max: 15 years) years. From these, 65.2% of patients (n=15) were male and 34.8% (n=8) were female. The causative mechanism leading to BD was classified into six groups, out of which the most common diagnosis was TBI with a rate of 39.3% (n=9) (Table I).

Among those patients with certified BD, the meantime from PICU admission to BD diagnosis was 5.7±5.5 days (min: 1, max: 24 days). The time period from suspicion of BD to the final diagnosis was 1.6±0.7 days (min: 1, max: 3 days) and the meantime to the occurrence of cardiac arrest after the diagnosis of BD was 13.1±21.6 days in non-donor cases (min: 1, max: 95 days). An apnea test was applied in all cases for the proper diagnosis of BD. The test could not be completed due to hemodynamic and respiratory instabilities in four cases. Regardless of the outcome of apnea test, all patients underwent confirmatory testing (radiological imaging) to avoid unnecessary waiting for a second neurological examination. As a confirmation test, brain CTA was performed in 19 (82.6%) of the patients, transcranial dUSG was performed in one patient (4.4%) and both brain CTA and transcranial dUSG were performed in 3 (13.0%) of the patients. Regarding certain alterations that follow BD, 34 alterations were observed in 23 patients. Among these alterations, 56.5% (n=13) developed DI, 52.2% (n=12) developed hypothermia, and 39.1% (n=9) developed hyperglycemia. Four patients became organ donors (17.4%). From these four donors, 8 kidneys, 4 livers and 2 hearts were transplanted successfully. The detailed analyses of the 23 children with BD are shown in Table II.

Discussion

We assume that our findings may be considered acceptable as a representation of tertiary level PICUs in Turkey. We reported the incidence rate of BD to be 1.3% in our PICU. In the adult ICU settings, the reported incidence was 2.7% (13). Our observed BD rate seems to be similar to previous reports in the literature (14,15).

In our study, it is reported that BD was more frequent in patients with severe TBI in the PICU. According to our study, 39.3% of BD cases occurred after trauma. However, in other PICUs, trauma was responsible for 8.6-73% of all BD cases in Turkey (15-17).

There are several past studies which evaluated the following; the duration of the diagnosis of BD, the duration of survival after its diagnosis and the time from ICU admission to BD diagnosis. Sucu et al. (18) performed a study in pediatric patients and found that the mean duration of the diagnosis of BD was 2.1±1.1 days. In another study, this period was found to be 5.9±1.3 days (17). The present study revealed that the duration of the diagnosis of BD was 1.6±0.7 days. Meanwhile, Ozmert et al. (17) conducted a study and found that the mean duration of survival after diagnosis of BD was 6.9±7.4 days with the longest surviving case reported to be 25 days. In another study, these periods were 6.8 days for patients less than18 years old and 2.5 days for those greater than 18 years old (19). In our study, the duration of survival after the diagnosis of BD was found to be 13.1±21.6 days, which is longer than

Table I. Demographic and clinical characteristics of the patients

Variables	Values
Age (years), mean ± SD (min-max)	5.5±4.9 (0.4-15)
Gender, n (%)	
Male	15 (65.2)
Female	8 (34.8)
Causative Mechanisms, n (%)	
Traumatic brain injury	9 (39.3)
Falls	5 (21.8)
Motor vehicle accident	4 (17.5)
Brain neoplasm	3 (13.0)
Cardiac Arrest	3 (13.0)
Asphyxia	2 (8.7)
Trauma	1 (4.3)
Intracranial haemorrhage	3 (13.0)
Arteriovenous malformation	2 (8.7)
Subarachnoid haemorrhage	1 (4.3)
Central nervous system infection	2 (8.7)
Other	3 (13.0)
Hydrocephaly	1 (4.3)
Shock and Multiple organ failure	2 (8.7)
Confirmatory tests	
Brain CTA	19 (82.6)
Transcranial dUSG	1 (4.4)
Brain CT angiography + Transcranial dUSG	3 (13.0)
Alterations that follow brain death, n (%)	
Diabetes insipidus	13 (56.5)
Hypothermia	12 (52.2)
Hyperglycaemia	9 (39.1)
[a]PICU admission - BD diagnosis time, mean ± SD (min-max)	5.6±5.5 (1-24)
[b]Duration of diagnosis of BD (days), mean ± SD (min-max)	1.6±0.7 (1-3)
Duration of survival after diagnosis of BD (days), mean ± SD (min-max)	13.1±21.6 (1-95)
Organ donation, n (%)	4 (17.4)

SD: Standard deviation, CTA: Computed tomography angiography, dUSG: Doppler ultrasonoghraphy, PICU: Paediatric intensive care unit, BD: Brain death, [a]: The time from the PICU admission to the diagnosis of BD, [b]: The time from suspicion of BD to final diagnosis, Min: Minimum, Max: Maximum

Table II. Detailed analysis of brain death patients

Patient no	Age (years)	Gender	Diagnosis	Confirmatory tests	PICU admission-BD diagnosis time (days)	Duration of diagnosis of BD (days)	Duration of survival after diagnosis of BD (days)	Complications	Apnoea test
1	4.6	Male	Tuberculous meningoencephalitis	CTA	11	2	5	DI, hypothermia, hyperglycaemia	Yes
2	7.8	Male	Diffuse intrinsic pontine glioma	Transcranial dUSG	1	1	1	ϕ	Yes
3	14.9	Female	Arteriovenous malformation	CTA	2	1	9	DI, hypothermia	Yes
4	9.2	Male	Diffuse intrinsic pontine glioma	CTA	10	3	Donor	Hypothermia	Yes
5	9.3	Male	Non-traumatic subarachnoid haemorrhage	CTA	11	2	2	ϕ	No
6	4.9	Male	Cardiac arrest (trauma)	CTA	1	1	14	DI, hypothermia	Yes
7	3	Male	Fall	CTA	2	1	2	DI, hyperglycaemia	Yes
8	3	Female	Meningoencephalitis (unidentified)	Transcranial dUSG CTA	6	2	34	DI, hypothermia, hyperglycaemia	Yes
9	0.8	Female	Fall	Transcranial dUSG CTA	9	3	17	DI, hypothermia	Yes
10	0.6	Male	Motor vehicle accident	Transcranial dUSG CTA	3	1	1	Hypothermia	Yes
11	12	Female	Motor vehicle accident	CTA	8	2	Donor	ϕ	Yes
12	6.3	Male	Motor vehicle accident	CTA	14	2	22	DI, Hypothermia	Yes
13	2.2	Male	Fall	CTA	1	1	1	Hyperglycaemia	No
14	2.2	Female	Shock	CTA	4	2	95	DI, hyperglycaemia	Yes
15	1.8	Male	Glioblastoma multiforme	CTA	4	2	3	DI	Yes
16	1.5	Female	Fall	CTA	1	1	1	ϕ	Yes
17	0.4	Female	Shock	CTA	5	2	3	Hypothermia	No
18	14.4	Male	Ventricular shunt malfunction	CTA	24	3	11	Hypothermia	Yes
19	1	Female	Asphyxia	CTA	3	1	4	DI	No
20	15	Male	Arteriovenous malformation	CTA	3	1	Donor	DI, hyperglycaemia	Yes
21	3	Male	Asphyxia	CTA	3	1	Donor	DI, Hyperglycaemia	Yes
22	1.8	Male	Fall	CTA	2	1	9	Hypothermia	Yes
23	6.8	Male	Motor vehicle accident	CTA	3	1	15	DI, hypothermia, hyperglycaemia	Yes

PICU: Paediatric intensive care unit, BD: Brain death, CTA: Computed tomography angiography, dUSG: Doppler ultrasonography, DI: Diabetes insipidus

what is seen in the literature. The reason for this longer period might be due to the fact that the longest surviving BD case in our study had 95 days of life after her diagnosis of BD and she is the longest surviving patient in the literature as far as we are aware.

In the physiopathology of BD, the irreversible loss of brain functions leads to the disruption of the central regulatory mechanisms. Hypothalamic pituitary adrenocortical regulation ceases. Antidiuretic hormone deficiency occurs in 65-90% of those patients with BD due to neurohypophyseal damage (20). Hypothermia is inevitable as the primary thermoregulation center is affected by hypothalamic injury, and vasoplegia-induced body heat loss occurs (21). In our study, DI was observed in 56.5% of BD cases, and hypothermia was observed in 52.2% of cases. In parallel, Bonetto et al. (8) found similar complication rates in their study.

Out of the 23 cases in our study, only 4 (17.4%) families agreed to organ donation. Previous studies on pediatric BD patients from Turkey determined different donation rates varying from 0% to 27% (15-18). The organ donation rate was found to be 47% in 135 BD cases in a study from Canada which was based in four different PICU centers (22). In a multicenter study from Argentina, 147 cases of BD were evaluated and the organ donation rate was found to be 25% (8).

Golchet et al. (23) evaluated the factors affecting organ and tissue donation and reported that religious beliefs and to a lesser extent personal fears and concerns about the integrity of body parts have reduced organ donation rates. Similarly, we found that most of the families did not accept organ donation either because of religious beliefs or an unwillingness to disrupt body integrity. However, factors affecting organ donation rates are not limited to beliefs. In one study, the authors reported that the coordinator of organ transplantation who communicated with the families about organ donation had a significant effect (24). Another study which included 268 patients with a family approval rate of 78.4% for organ donation revealed that the organ donation approval rate increased with the increased frequency of meetings held by an organ transplantation coordinator with the family after the declaration of BD (25). As can be seen, family approval rates for organ donation vary greatly in different health centers. We believe that regular and effective interviews conducted by an experienced and trained organ transplantation coordinator from the beginning of the process will increase donation rates.

The final decision of BD diagnosis and management in the subsequent period vary according to the medical and legal regulations in different countries. The clinical evaluation must be verified by more than one physician in many countries as is also the case in Turkey. Until recently, approval of a neurologist, neurosurgeon, cardiologist and anesthesiologist were required for the declaration of BD as enforced by the law which was in force at that time. With an amendment in 2014, the approval of two physicians, one neurologist or neurosurgeon and one anesthesiologist and reanimation specialist or intensive care specialist is now sufficient for a diagnosis of BD (3). According to the law; in the presence of the clinical findings of BD and apnea test positivity, a second neurological examination should be performed at intervals varying according to age groups. The diagnosis of BD is established when clinical findings of BD persist in the second neurological examination which is conducted after a 48 hour period in infants less than 2 months old, after 24 hours in infants aged between 2 months to 1 year, after 12 hours in children above 1 year old and in adults, and after 24 hours in the presence of cardiopulmonary arrest or acute hypoxic-ischemic brain injury (3). If an apnea test cannot be performed for the diagnosis of BD or the established time for a second neurological examination is not appropriate, then confirmatory tests for cerebral blood flow (brain CTA, transcranial dUSG, magnetic resonance imaging angiography and brain scintigraphy) or Electrophysiological tests [electroencephalogram (EEG) and evoked potentials] should be performed (3). In our study, the apnea test was performed in all suspected BD cases. However, the test could not be completed in four patients due to hemodynamic and respiratory instabilities. In our study, confirmation tests (brain CTA, transcranial dUSG) were performed in all cases because of the requirement for patients who could not undergo the apnea test and also an unwillingness to wait for the second neurological examination in some cases. In our study, brain CTA was the most preferred confirmatory test (82.6%). There were two main reasons to prefer this method; first of all, it was easy to transport patients to the imaging service/center which is located near to the PICU, secondly, it was difficult to find a neuroradiologist with sufficient experience in transcranial dUSG. In addition to brain CTA as a confirmatory test, there are some centers which prefer transcranial dUSG or EEG as well (8,16-19).

Livers, kidneys and hearts were the solid organs which were donated and successfully transplanted in our unit. Although in Turkey, the transplantation of livers and kidneys

are seen frequently, cadaveric transplant rates are not as frequent as kidney and liver transplantations (26). A total of 27,146 organ transplantation procedures were carried out between January 2008 and December 2016, and only 27% of them were harvested from cadavers. (Data acquired from the Turkish Ministry of Health, Directorate for General Health Service, Organ, Tissue Transplantation and Dialysis Services Department). Raising awareness of BD with its early diagnosis, good donor care and awareness of the importance of organ donation in the society may increase organ transplantation rates from cadavers.

Conclusion

We concluded that patients with severe TBI are the most likely candidates for BD declaration and are suitable for organ donation to those children in need. Early diagnosis and good donor care are of great importance, especially for pediatric patients waiting for organ transplants. The organ transplantation rates from a cadaver are still far below the targeted rates in Turkey. Careful and planned public educational broadcasting campaigns with spiritual and religious leaders' speeches addressing the concerns of the relatives of patients may increase donation rates. In addition, we believe that donation rates may increase if family meetings are held by a trained and experienced coordinator.

Ethics

Ethics Committee Approval: This study was granted permission by the Ethical Board of Medeniyet University, Goztepe Training and Research Hospital (Approval number: 2019/0328).

Informed Consent: Patients diagnosed with BD can be accepted as organ donors by obtaining family consent for the transplantation

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.D., Z.K., Design: M.D., Z.K., Data Collection or Processing: M.D., Z.K., Analysis or Interpretation: M.D., Z.K., Literature Search: M.D., Writing: M.D., Z.K.

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Original Article

Relationships between Vitamin B12, Folate Levels and Clinical Features in Attention Deficit Hyperactivity Disorder and Attention Deficit Hyperactivity Disorder-Not Otherwise Specified

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ABSTRACT

Aim: In this study, we aimed to compare the levels of vitamin B12 and folate in children with Attention Deficit and Hyperactivity Disorder (ADHD) and Attention Deficit and Hyperactivity Disorder-Not Otherwise Specified (ADHD-NOS).

Materials and Methods: This study was planned as a cross-sectional, retrospective study. Patients were recruited between January 2012 and January 2013 and 205 case records were evaluated. The ADHD and ADHA-NOS groups were compared according to vitamin B12 and folate levels. Symptom severity was evaluated by the Turgay DSM-IV-Based Child and Adolescent Behavior Disorders Screening and Rating scale. Anxiety symptom severity was assessed by The Screen for Anxiety Related Emotional Disorders.

Results: The average age of the children in the ADHD group was 10.88±3.02 (n=99) years, and the average age of the children in the ADHD-NOS group was 9.93±2.49 (n=106) years. There was no statistically significant difference between two groups in terms of Vitamin B12 level and folate level ($p>0.05$). A statistically significant negative correlation between the total number of diagnoses of a child and vitamin B12 levels was found. Folate levels correlated significantly with anxiety total scores generalized anxiety subscale.

Conclusion: Vitamin B12 levels may be affected in children with impairing ADHD symptoms and increased comorbidities. The results of the study should be supported by future studies.

Keywords: ADHD, vitamin B12, folate

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder having different clinical symptoms including inattention, hyperactivity, and impulsivity. It is also frequently related to cognitive deficit (1). It has been reported that the prevalence of ADHD ranges between 8.0-12.0% worldwide (2). The prevalence of ADHD was found to be 8.1% in school-age children in Turkey (3). Attention Deficit Hyperactivity Disorder-Not Otherwise Specified (ADHD-NOS) was defined for disorders with inattention/ hyperactivity-impulsivity that do not fully meet the criteria for ADHD in Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) (4). It was replaced with ADHD- Unspecified in DSM-5 (1) and seems to be used as a diagnosis for conditions where children have moderate attention problems in school, which can be better characterized as difficulties in children's learning or executive function (4). In addition, rather than being categorized as ADHD Other Specified and Unspecified

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types, many young people are defined as having borderline or subclinical levels of ADHD instead. Pharmacotherapy including stimulants and atomoxetine is the first choice in the treatment of both disorders (2).

The etiology of ADHD is complex and has not yet been identified. There is no specific definable factor, ADHD is multi-component (5). Its heritability was found to differ between 76.0% and 80.0%. Thus, it is one of the highest hereditary neuropsychiatric disorders (6,7). Although the exact reason is not known, exposure to heavy metals and toxins in the prenatal and perinatal period, socio-psychological stress, diet, gene variants and abnormalities of the brain, neurotransmitter deficiency and dysfunction in the frontostriatal, as well as fronto-cerebellar catecholaminergic circuits, were reported to contribute to the etiology (2,6,8).

Psychiatric symptoms related to vitamin B12 and folate deficiencies may consist of irritability, agitation, negativism, disorientation, confusion, amnesia, problems of concentration and attention and insomnia. It is considered that vitamin B12 and folate in the carbon transfer metabolism (i.e. methylation) play a significant role in psychiatric symptoms. They are also essential in the production of dopamine, serotonin, other monoamine neurotransmitters and catecholamines (9). Basal ganglia, which is considered to play a significant role in ADHD, may be especially vulnerable to deficiencies of vitamin B12 and folate. Studies on the relationship of ADHD with folate and related metabolic pathways have focused on the consequences of folate deficiency in the prenatal period (10). A recent study from Turkey also reported that ADHD may be related to methylene tetrahydrofolate reductase polymorphisms (11).

The lack of vitamin B12 and folate levels in the diet were associated with ADHD (12). It was reported that there is a relationship between maternal folate deficiency during early pregnancy and childhood hyperactivity (10). The serum levels of vitamin B12 were conversely related to depression in some studies (13-15) but not in others (16,17). Although major depressive disorder and anxiety disorder are common comorbid diseases, the effect of serum vitamin B12 and folate levels in anxiety has been investigated in few studies.

We aimed to compare the levels of vitamin B12 and folate in children with ADHD and ADHD-NOS in this study. We also aimed to assess the relationship between ADHD symptom severity, and anxiety symptom severity with vitamin B12 and folate levels.

Materials and Methods

Study Center and Time-frame

This study was planned as a cross-sectional, retrospective study. It was conducted at the outpatient department of Child and Adolescent Psychiatry in Abant İzzet Baysal University Faculty of Medicine. Patients were recruited between January 2012 and January 2013 and 205 case records were evaluated. The diagnoses of 205 patients' records and their comorbid psychiatric disorders were made clinically by the consensus of child psychiatry residents and the clinical supervisor according to DSM-IV-TR criteria (4).

Inclusion criteria were a primary diagnosis of ADHD or ADHD-NOS according to DSM-IV-TR criteria, adequate information on laboratory values and psychometric measures in patient records, and application to the outpatient department between the specified time-frame. Patients with comorbid medical and psychiatric disorders were included. Both ADHD and ADHD-NOS groups received methylphenidate treatment at 1 mg/kg/day. Patients with inadequate records were excluded.

The Ethics Committee approval of the study was obtained from Bolu Abant İzzet Baysal University Clinical Trials Ethics Committee (date: 16.05.2018, number: 164).

Measures

Turgay DSM-IV-Based Child and Adolescent Behavior Disorders Screening and Rating scale: This scale was developed by Turgay by transforming the DSM-IV criteria (T-DSM-IV-S) into questions without changing their meanings. It includes 41items (attention deficit = 9 items, hyperactivity = 6 items, impulsivity = 3 items, oppositional defiant disorder = 8 items, and conduct disorder = 15 items). Each item is rated on a scale of 0 = none, 1 = occasional, 2 = much, and 3 = very much. When subscales are evaluated, 2 to 3 points per item are assessed as symptomatic (1), while 0 to 1 are assessed as not symptomatic (0) (18). The validity and reliability study of this scale was established previously (19).

The Screen for Anxiety Related Emotional Disorders (SCARED): There are 41 items to determine anxiety symptom severity in the previous three months in this scale. Participants may choose a score between 0 (not true or hardly ever true) and 2 (very true or often true) (20). When the subscales are evaluated, 2 points per item is assessed as symptomatic (1), while 0 to 1 are assessed as not symptomatic (0). It was used according to both the child and their parent's report. The scale also includes generalized anxiety, somatic/panic, social anxiety, separation anxiety

and school fear subscales. The validity and reliability for the SCARED Turkish form was established by Cakmakci (21).

The Clinical Global Impression-severity scale (CGI-S): CGI-S is a clinician-rated measure which is used for treatment-related changes in functioning (22). The CGI-S score varies between 1 (normal) and 7 (most severely patients). CGI-S is mostly used in Turkish Child and Adolescent Psychiatry outpatient clinics. There are many Turkish clinical studies which have used this scale. CGI-S was used to indicate symptom severity in the present study.

Statistical Analysis

Statistical analysis of the data for this study was carried out using the Statistical Package for the Social Sciences (SPSS 22.0) software. Number values and percentages were used in the evaluation of some study and control group socio-demographic and clinical categorical variables. Continuous variables are presented using summary statistics. This (unless otherwise stated) refers to the number of patients (n), mean and standard deviation. Categorical data are presented using either absolute or relative frequencies. Yates' and Fisher's corrections were applied when required. The Kolmogorov-Smirnov method was used for the evaluation of data distribution. As the data distribution was found to be normal, the Parametric t-test or one-way ANOVA, depending on group numbers, was used to evaluate paired groups. Pearson correlation analysis was used to determine the relationship between continuous variables. All tests were two-tailed with p values <0.05 considered significant.

Results

The records of 205 patients were analyzed. Of these, 99 (48.29%) patients were ADHD and 106 (51.71%) patients were ADHD-NOS. Comparison of sociodemographic data of the ADHD-NOS and ADHD groups is shown in Table I. T- DSM-IV-S-parent subscales, SCARED subscales and CGI-S scores between the two groups are presented in Table II.

Comorbid psychiatric disorder was detected in 144 cases (70.24%) of the 205 cases included in the study. It was found that conduct disorder (28.29%) and specific learning difficulties (21.95%) were the most common comorbid psychiatric disorders. While 71 of these 144 cases had one comorbid psychiatric disorder, others had multiple comorbid psychiatric disorders. It was found that 76 cases in the ADHD group and 68 cases in the ADHD-NOS group had comorbid psychiatric disorders. No statistically significant difference was found between the two groups in terms of comorbid psychiatric disorders (p=0.531).

Vitamin B12 levels were measured in 89 of 205 patients participating in the study. Folate levels were measured in 82 of 205 patients participating in the study. There was no

Table I. Comparison of sociodemographic data of the attention deficit and hyperactivity disorder and attention deficit and hyperactivity disorder-not otherwise specified groups

	ADHD-NOS group (n=99)	ADHD group (n=106)	p
Age	10.88±3.02	9.93±2.49	0.015
Gender			
Male	65	72	0.73
Female	34	34	
Family history			
Positive	54	45	0.095
Negative	45	61	
Medical disease history			
Positive	48	51	0.958
Negative	51	55	

ADHD: Attention deficit and hyperactivity disorder, ADHD-NOS: Attention deficit and hyperactivity disorder-not otherwise specified, n: Number of patients

Table II. Comparison of T-DSM-IV-S-parent subscales, screen for anxiety related emotional disorders subscales and clinical global impression-severity scale scores of the attention deficit and hyperactivity disorder-not otherwise specified and attention deficit and hyperactivity disorder groups

	ADHD-NOS group (n=99)	ADHD group (n=106)	p
T-DSM-IV-S-parent			
Attention	2.41±1.72	6.28±2.15	<0.001
Hyperactivity	1.40±1.85	4.52±3.18	<0.001
Opposition-defiance	1.45±2.02	3.56±2.99	**<0.001**
CD	**0.11±0.46**	**0.56±1.22**	**0.001**
Total score	**21.34±11.41**	**42.37±17.02**	**<0.001**
SCARED			
Somatic/panic	**1.85±2.31**	**2.29±2.47**	**0.441**
Generalized anxiety	**1.32±1.98**	**3.11±2.64**	**0.001**
Separation anxiety	1.43±1.68	2.68±1.92	0.003
Social anxiety	2.09±1.95	2.85±2.22	0.106
School phobia	0.53±1.31	1.15±.142	0.050
Total score	25.89±12.86	34.00±12.89	0.007
CGI-S	3.39±0.74	4.55±0.64	<0.001

ADHD: Attention deficit and hyperactivity disorder, ADHD-NOS: Attention deficit and hyperactivity disorder-not otherwise specified, n: Number of patients, SCARED: Screen for anxiety related emotional disorders, CGI-S: Clinical global impression-severity scale

statistically significant difference between the groups in terms of Vitamin B12 and folate levels (p=0.989, p=0.855; respectively) (Table III).

When the relationship between B12 vitamin levels and comorbidity was evaluated, vitamin B12 levels were 355.02±157.32 in the non-comorbid group, 342.14±159.25 in the single comorbid group and, 294.22±120.38 in the multiple comorbid group. No statistically significant difference was determined between the three groups in terms of vitamin B12 levels (p=0.218). When the relationship between folate levels and comorbidity was evaluated, folate levels were 9.14±2.09 in the non-comorbid group, 7.91±2.38 in the single comorbid group and, 8.63±2.82 in the multiple comorbid group. There was no statistically significant difference between the three groups in terms of folate levels (p=0.217). When the relationship between the total number of diagnosis and vitamin B12, folate was assessed, a statistically significant negative correlation was found between the total number of diagnoses and vitamin B12 levels (r=-0.214, p=0.044), but the same relationship with folate levels was not obtained (p>0.05).

When the relationship between the symptoms of ADHD and vitamin B12 and folate was evaluated, no relationship was found between the symptoms of ADHD and either Vitamin B12 or folate (Table IV). However, when the relationship between SCARED scores and vitamin B12 and folate levels was evaluated, while no relationship was found between B12 levels and SCARED subscales and total scores (p>0.05), a significant relationship was found between folate and SCARED total score and the generalized anxiety disorder (GAD) subscale (respectively p=0.050, p=0.039) (Table V).

Table III. Comparison of vitamin B12 and folate levels of the attention deficit and hyperactivity disorder-not otherwise specified and attention deficit and hyperactivity disorder groups

	ADHD-NOS group	ADHD group	p
Vitamin B12, pg/mL (rr: 187-883 pg/mL)	328.28±126.97	327.84±159.61	0.989
Folate, ng/mL (rr: 3.1-20.5 ng/mL)	8.64±2.56	8.54±2.46	0.855

rr: Reference range, ADHD: Attention deficit and hyperactivity disorder, ADHD-NOS: Attention deficit and hyperactivity disorder-not otherwise specified

Table IV. Examination of the relationship between attention deficit and hyperactivity disorder symptoms and vitamin B12 and folate levels (Pearson correlation analysis)

		Vitamin B12 level	Folate level
T-DSM-IV-S-parent			
Attention	r p	-0.159 0.169	-0.157 0.192
Hyperactivity	r p	-0.092 0.428	-0.043 0.721
Total score	r p	-0.128 0.271	0.016 0.898

Table V. Examination of the relationship between screen for anxiety related emotional disorders subscales and total score and Vitamin B12 and folate levels (pearson correlation analysis)

		Vitamin B12 level	Folate level
SCARED			
Somatic/panic	r p	0.151 0.346	0.091 0.596
Generalized anxiety	r p	0.227 0.154	0.345 0.039
Separation anxiety	r p	0.163 0.309	0.320 0.057
Social anxiety	r p	0.162 0.311	0.322 0.055
School phobia	r p	0.223 0.172	0.194 0.265
Total score	r p	0.146 0.364	0.329 0.050

SCARED: Screen for anxiety related emotional disorders

Discussion

In this retrospective study, we aimed to compare the levels of vitamin B12 and folate in children with ADHD and ADHD-NOS, and to assess the relationship between ADHD symptom severity and anxiety symptom severity with vitamin B12 and folate levels. While we did not find a significant difference between ADHD and ADHD-NOS groups in terms of Vitamin B12 and folate levels, we found a statistically significant negative correlation between the total number of diagnoses and vitamin B12 levels. We also found a significant correlation between folate levels and the SCARED total score and GAD subscale.

The main outcome of this study is a significant negative correlation between the total number of psychiatric disorder diagnoses with ADHD and ADHD-NOS and vitamin B12 levels. This means that as the vitamin B12 level decreases, the number of psychiatric diagnoses increases. Vitamin B12 deficiency affects many systems. It has been shown to cause megaloblastic anemia, glossitis, atrophic gastritis, neuropathy, and demyelination in the nervous system (23,24). Moreover, previous studies have demonstrated the association of deficiency in vitamin

B12 with numerous neurological and psychiatric disorders (25,26). The association between vitamin B12 deficiency and neurodevelopment was reported in observational studies in children (27-29). In North Indian children, it was found that their vitamin B12 level was related to lower scores on the mental development scale in a study (28). Ssonko et al. (30) found that low serum vitamin B12 was common among inpatient psychiatric patients. They also suggested routine screening of serum vitamin B12 for hospitalized psychiatric patients. Both the previous results and our findings suggest a probable relationship between vitamin B12 deficiency and psychopathology, including neurodevelopmental disorders such as ADHD.

We also observed a significant correlation between folate levels and the SCARED total score and GAD subscale. This means that as folate levels increase, anxiety symptom severity increases. Folate is a fundamental nutrient. It regulates apoptosis, neural stem cell proliferation and differentiation, various biochemical pathways such as DNA biosynthesis, neurotransmitter and myelin synthesis, regulation of gene expression, amino-acid synthesis and metabolism (31,32). In a meta-analysis study, it was suggested that low folate levels may be associated with depression (33). In previous studies, there were few studies examining the effect of serum vitamin B12 and folate levels in anxiety (34,35). Møllehave et al. (34) found no association between serum vitamin B12 and folate levels with depression and anxiety symptoms. In another study, it was found that while plasma folate level was related to depression, it was not related to anxiety (35). Although we found an association between folate levels and anxiety symptoms, there is a need for further study in this area because the number of samples and the information in the literature are inadequate.

Another finding we have in our study is that there is no significant difference between ADHD and ADHD-NOS groups concerning vitamin B12 and folate levels. Our study may be a contribution to the literature on this subject. There is no study in the literature assessing the association between ADHD and ADHD-NOS in terms of vitamin B12 and folate levels. In previous studies, ADHD was compared with autism spectrum disorder (ASD) and healthy controls with regard to vitamin B12 and folate levels (36,37). Bala et al. (36) compared vitamin and hormones levels in ADHD, ASD and healthy control groups. They found that the vitamin B12 level in the ADHD group was 371.72±160.63 [minimum (min)= 156/maximum (max)= 924], and the folate level in the ADHD group was 10.16±2.93 (min= 4/max= 15). They also found the lowest vitamin B12 level in the ASD group and statistically significant differences between the three groups. However, there was no difference in folate levels between the three groups. More studies are needed to evaluate the association of ADHD with vitamin B12 and folate levels.

Study Limitations

Our findings should be evaluated within the context of certain limitations. Firstly, this study was retrospective and depended on information recorded routinely in clinical records. This dependence led to missing data which may have affected our results. Secondly, this study was conducted on a clinical sample evaluated at a single center and may not reflect patient populations in other centers or the community. Thirdly, the laboratory evaluations were conducted as part of the baseline examination prior to commencing pharmacotherapy at the study center. However, due to a dependence on patient charts, we could not ascertain whether the patients were drug naive or were receiving treatment at the time of their evaluations. Despite these limitations, this is the only study that we are aware of evaluating the psychometric features and laboratory values of children diagnosed with ADHD and ADHD-NOS.

Conclusion

In conclusion, it has been shown in our study that there a negative relationship between the number of psychiatric diagnoses and vitamin B12 levels for ADHD patients. This finding suggests that assessing vitamin B12 levels in children with ADHD may be beneficial if the number of psychiatric diagnoses is high. We have also shown a positive relationship between folate levels and anxiety symptom severity. However, there is a need for further study in this area because our findings and literature information are inadequate to generalize our findings.

Ethics

Ethics Committee Approval: The Ethics Committee approval of the study was obtained from Bolu Abant İzzet Baysal University Clinical Trials Ethics Committee (date: 16.05.2018, number: 164).

Informed Consent: Retrospective study.

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Authorship Contributions

Surgical and Medical Practices: Z.T., N.D., Concept: Y.Ö., Design: Y.Ö., Z.T., Data Collection or Processing: Z.T., Y.Ö., N.D., Analysis or Interpretation: A.E.T., Literature Search: A.E.T., Writing: Y.Ö., Z.T., N.D.

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Case Report

A Rare Cause of Ascites: Eosinophilic Gastroenteritis

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ABSTRACT

Eosinophilic gastroenteropathies are rare disorders. An 18-year-old girl presented to the hospital with abdominal pain and distension. Abdominal tenderness and grade 2 ascites, serious peripheral eosinophilia and high immunoglobulin E level were found. Laparoscopic antrum biopsy showed subserosal eosinophilic infiltration. Eosinophilic gastroenteropathy was suspected, diet elimination was given, no steroid treatment was used.

Keywords: Eosinophilic gastroenteritis, eosinophilia, ascites, children

Introduction

Eosinophilic gastroenteropathies (EoGs) are rare and poorly defined diseases of the gastrointestinal tract (GIT). These disorders, which are usually associated with peripheral eosinophilia, involve recurrent eosinophilic infiltration of at least one organ within the GIT (1,2). Symptoms are similar to those of many systemic diseases. Gastroenterologists and general internal medicine specialists, therefore, should seek more awareness to ensure a proper diagnosis is made.

Case Presentation

An 18-year-old female presented to the hospital having had generalized abdominal pain and swelling for a week with no identified relation to food. Nausea and vomiting were reported during the previous three days. She did not demonstrate any fever, weight loss, night sweats, chest pain, or joint swelling and was not taking any prescribed medication or herbal compounds. Upon physical examination, increased intestinal sounds accompanied by slight abdominal swelling, abdominal tenderness without rebound and grade 2 ascites were noted. Her white blood cell count was found to be 15,200/µL with 58.4% being (8,900/µL) eosinophils. Biochemistry, thyroid function, sediment and urine analysis findings were all normal. Additionally, C-reactive protein, anti-endomysial antibodies, tissue transglutaminase antibody, anti-nuclear antibody, anti-ds DNA, anti-mitochondria antibody tests and urine culture results were all negative, while her immunoglobulin E (IgE) level was 1,321 IU/mL. Stool parasites tests and toxocara, echinococcus and faecal calprotectin tests were also negative. Flow cytometry of a peripheral blood sample

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did not reveal any myelolymphoproliferative findings. A contrasted abdominal computed tomography (CT) coronal section scan showed moderate ascites with thickened gastric antrum and small bowel walls (Figure 1). Abdominal paracentesis revealed that 95% of white blood cells were eosinophils (Figures 2a and 2b). Acid samples were as follows: pH= 7.47; lactate dehydrogenase= 401 mg/dL; and albumin= 3.18 g/dL (serum albumin= 4.0 g/dL). Skin prick test was negative. Esophagogastroduodenoscopy (EGD) demonstrated moderate pangastritis, bulbitis, and duodenitis. Histological appearance revealed 17 to 20 eosinophils per high-power field of the oesophagus and chronic non-atrophic gastritis (Figure 2c). Histological findings from the gastric mucosa did not reveal eosinophilic gastritis; thus, laparoscopy was performed. During the laparoscopic examination, a hyperaemic oedema area of 2 cm to 3 cm was detected on the front of the gastric antrum (Figure 3). In the biopsy evaluation of the stomach antrum, eosinophilic infiltration of the prominent muscularis propria and subserosa and associated mild acute inflammation reaction were detected (Figure 2d).

As a consequence, a diagnosis of eosinophilic gastroenteritis was made. She was treated via the elimination of milk and milk products from the diet and proton pump inhibitor administration. Her abdominal pain, acid and examination findings were completely resolved after two weeks of milk and dairy products being eliminated. Her absolute eosinophil count at this point was 1,900/µL (25.7%). Additionally, her IgE level was decreased to 202 IU/mL and abdominal ultrasonography showed complete resolution of ascites. By the second month of treatment, she was asymptomatic while continuing the elimination diet. Her peripheral blood count revealed an absolute eosinophil count of 300/µL (8.3%).

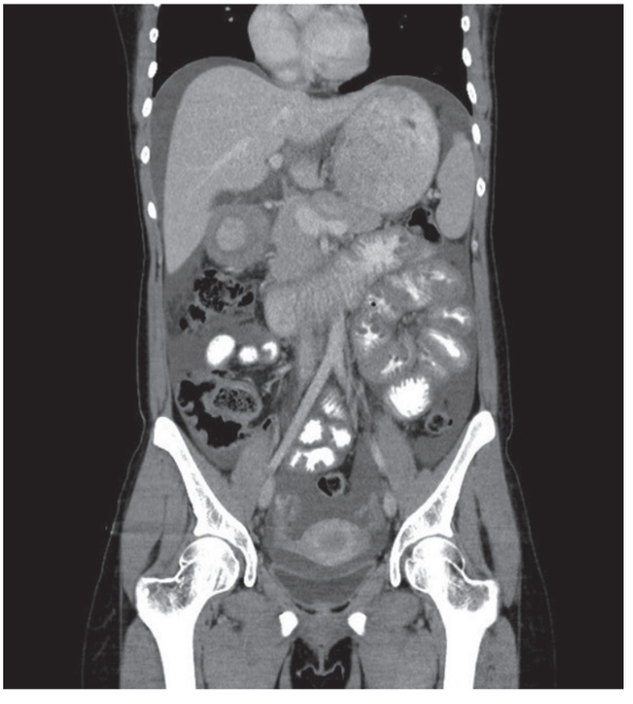

Figure 1. Abdominal computed tomography showed moderate ascites with thickening of the gastric antrum and small bowel walls

Discussion

EoGs are an infrequently observed disease characterized by recurrent eosinophilic infiltration of various sections of the GIT. Eosinophilic tissue infiltration may affect any of the three layers (i.e., mucosal, muscular, subserosal) in the digestive tract wall, with symptoms typically varying according to the affected layer (3). Of the three types, the mucosal form is the most common, while the serosal form is the least common (1). Often, symptoms of EoGs include colonic abdominal pain, bloating, diarrhoea, weight loss, and vomiting (4,5). Other features of severe EoGs are gastrointestinal bleeding, iron deficiency anaemia, hypoalbuminemia, protein-losing enteropathy, and growth disturbance (5). Patient symptoms in the case of the mucosal form of EoGs are usually abdominal pain, nausea, vomiting, and diarrhoea. The muscular form in contrast demonstrates a thickened intestine that match the symptoms of intestinal obstruction (6). Serosal infiltration accompanied by eosinophils and associated abdominal distension have rarely been reported with eosinophilic ascites and bowel perforation (4,7-9).

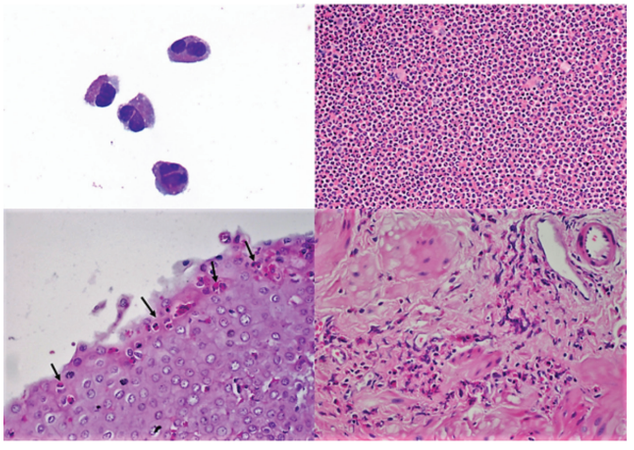

Figure 2. Figure 2a: High-power oil immersion view of the peripheral blood film shows eosinophils (Wright-Giemsastain; x1000). Figure 2b: Diagnostic paracentesis demonstrates ascitic fluid rich in eosinophils (H&E, x40). Figure 2c: Eosinophiles scattered towards the surface of the mucosa in the endoscopic biopsyspecimen of esophageal mucosa (H&E, x40). Figure 2d: Prominent eosinophilic infiltration in the intersitisiel connective tissue in the laparoscopic biopsy

Peripheral eosinophilia is a very common finding in EoG (9). Eosinophilia was determined to be 58.4% in our case due to serosal involvement. When searching for other eosinophilia causes, the results of the relevant tests were negative. The patient was not taking any prescribed medications such as aspirin, penicillin, cephalosporins, cotrimoxazole, or carbamazepine. The patient refused colonoscopy, which would have been the next step in the clinical workup of eosinophilic gastroenteritis. Clinical, laboratory, and histological findings of our patient were not compatible with Crohn's disease. Ascites does not appear in most patients at the time of exacerbation of Crohn's disease (10).

An EoGs diagnosis is based on high clinical suspicion with histopathological findings. If the diagnosis is uncertain, CT imaging can help to determine the location of the thickened GIT areas. The presentation of ascites that develops in serosal involvement is exudative and eosinophil-rich, as detected in this patient (9). EGD examination can detect hyperaemia, ulceration, stricture, nodular appearance and ulcer in EoGs. Pangastritis, bulbitis and duodenitis were seen in this case. A full-thickness biopsy is taken laparoscopically for subserosal involvement and is sometimes necessary for diagnosis (1,3). In this case, a seromuscular biopsy was obtained and the presence of a dense eosinophil infiltration in the biopsy specimen revealed a diagnosis of EoG (Figure 3).

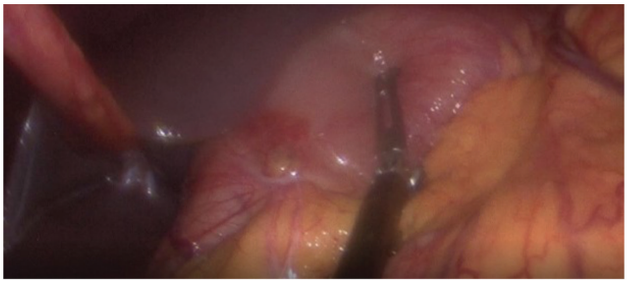

Figure 3. Laporoscopic evaluation of the patient revealed serosal inflammation involving the portion of antrum

Conclusion

The first-line treatment for EoG is the removal of the responsible food from the diet (1). However, nutritional elimination treatment is only valid in some patients, as there are difficulties inherent in identifying the responsible food (4). We treated the present case with food elimination only. Subsequently, ascites and eosinophilia reduction occurred. This is a different outcome from other studies that required steroid therapy (7,9). Corticosteroids are an important treatment option if dietary therapy is unsuccessful in promoting remission, although they should be used with caution due to associated side effects and it should be kept in mind that relapses may occur at a later time after medication discontinuation (1). Therefore, nutritional elimination should be tried prior to the introduction of steroid therapy in clinically appropriate patients. Since an outcome similar to our case who responded to the elimination of nutrients alone had not been previously reported, we would like to present our report as documentation to contribute to the literature.

Ethics

Informed Consent: Informed consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Medical Practices: G.D., Ö.Y., E.K., Concept: G.D., H.G., Design: G.D., F.D., Data Collection or Processing: G.D., F.D., S.A., Analysis or Interpretation: G.D., S.A., H.G., Literature Search: G.D., E.K., Writing: G.D., F.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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