Differential diagnosis of Crohn's disease using antibodies to glycoprotein 2 and Saccharomyces cerevisiae

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ABSTRACT

Background/Aims: *Glycoprotein 2 (GP2), the major autoantigen of Crohn's disease (CD)-specific pancreatic autoantibodies, is reportedly correlated with several characteristics of CD. We investigated this serological marker in Turkish patients with CD and assessed its utility in combination with anti-*Saccharomyces cerevisiae *antibodies (ASCAs) for differential diagnosis of CD.*

Materials and Methods: *A total of 60 patients with CD, 62 patients with ulcerative colitis (UC), and 46 healthy controls with a definite diagnosis who were similar in age and sex were enrolled in the study conducted from November 2011 to October 2012. ASCA and anti-GP2 levels were measured using commercially available kits.*

Results: *Anti-GP2 IgA and IgG levels were higher in patients with CD (25%) than in those with UC (5%) and controls (2%). The seroprevalence of anti-GP2 IgA was markedly higher than that of IgG in patients with CD in contrast to previous studies. The specificity and positive predictive value of seropositivity for both ASCA and anti-GP2 were 100%. ASCA IgA seropositivity was correlated with a complicated disease course and a history of surgery. There was no correlation between anti-GP2 seropositivity and disease location, disease behavior, or a history of surgery.*

Conclusion: *The combination of ASCA and anti-GP2 may enable differentiation of CD from UC. As ASCA seropositivity is associated with a more complicated disease course, patients seropositive for ASCA at the initial diagnosis should undergo more intense therapy.*

Keywords: *Crohn's disease, Anti-GP2, ASCA*

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are heterogeneous inflammatory bowel diseases (IBDs) with no certain cause that affect the gastrointestinal tract. With regard to etiopathogenesis, IBD is thought to develop as a result of dysfunction of the immune response to the bacterial microflora in individuals with genetic susceptibility (1). The tissue damage in IBD is mediated by immune mechanisms.

Autoimmune mechanisms play roles in the pathogenesis of IBD; pancreatic antibodies (PAB) are found in 31% of patients with CD (2,3). Glycoprotein 2 (GP2) is a major autoantigenic target of PAB in patients with CD (4).

Glycoprotein 2 is excessively glycosylated with N-linked carbohydrates and accounts for almost half of the zymogen granule membrane proteins in the acinar cells of the pancreas (5). As a result of neuronal and hormonal stimulation of the pancreas, acinar cells transport GP2 to the apical compartment; then, it is released together with zymogens into the pancreatic canal and finally passes into the duodenum (6). In addition, the expression of GP in inflamed tissues is higher in patients with CD than in those with UC. Therefore, this protein may play a pathophysiological role in the pathogenesis of CD (4). Approximately 20%-30% of patients with CD are anti-GP2 antibody positive (7-9). Anti-GP2 positivity is reportedly related to ileal and ileocolonic involvement, disease onset at an earlier than normal age, perianal disease, and stricturing disease (9-11).

We evaluated the relationship between anti-GP2 antibody positivity and the characteristics, severity, and levels of diagnostic markers in CD, as well as anti-*Saccharomyces cerevisiae* antibody (ASCA) positivity.

MATERIALS AND METHODS

A total of 60 patients with CD, 62 patients with UC, and 46 healthy controls with a definite diagnosis who were similar in age and sex were enrolled in the study conducted from November 2011 to October 2012. Patients

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without a definite diagnosis of CD or UC, who were <18 years old, pregnant, had other chronic autoimmune or inflammatory diseases, malignancy, or active infection were excluded from the study. Table 1 shows the clinical and demographic characteristics of the CD, UC, and control groups. Patients with CD were divided into groups according to disease behavior using the Montreal classification (12).

Ethics Committee of Kocaeli University School of Medicine approved the present study (2011/40 KAEK 14/4) in accordance with the Declaration of Helsinki. Written informed consent was obtained from each individual who participated in the study.

Measurement of anti-GP2 levels

Serum levels of anti-GP2 autoantibodies (IgA and IgG) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Generic Assays, Dahlewitz, Berlin, Germany). The test uses solid-phase recombinant human GP2 antigen expressed in the *Spodoptera frugiperda*-9 cells. The cut-off level for positivity was 20 AU/mL, according to the manufacturer's instructions.

Table 1. Demographic and clinical characteristics of the CD, UC, and control groups

	CD (n=60)	UC (n=62)	Control (n=46)
Female/male	28/32	16/46	
Age (mean±SD) (years)	41±11.7	48±14.2	27/19
Duration (mean±SD) (months)	60±42	62±52	31±6.9
Age			
A2	32		
A3	28		
Area of involvement			
L1			
L2			
L3	32		
Proctitis	8	14	
Left-sided	20	26	
Pancolitis		22	
Perianal disease	9		
Upper GIS involvement	2		
Behavior			
B1	30		
B2	18		
B3	12		
Surgical history	20		

Measurement of ASCA levels

Anti-*Saccharomyces cerevisiae* IgG and IgA levels were quantified using a standardized ELISA method (QUANTA Lite; Inova Diagnostics, Inc., San Diego, CA, USA) using antibodies against mannan protein, which is present in the cell wall of *S. cerevisiae*. The cut-off level for positivity for both tests was 25 U/mL.

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics version 15 (SPSS Inc.; Chicago, IL, USA). The distribution of numeric data was assessed using the Kolmogorov-Smirnov test. The chi-square and Fisher's exact tests were used to compare the rates of antibody positivity. The relationships between antibody positivity and area of involvement, presence of perianal disease, disease behavior, and surgical therapy in patients with CD were assessed using the chi-square and Fisher's exact tests. The diagnostic values of anti-GP2 and ASCA antibodies in CD were evaluated using a receiver operating characteristic (ROC) curve analysis. Area under the ROC curve and 95% confidence interval values were obtained. A p value <0.05 was considered as statistically significant.

RESULTS

Anti-GP2 antibody levels

Anti-GP2 IgA and IgG levels were higher in patients with CD (15/60, 25%) than in those with UC (3/62, 5%) and controls (1/46, 2%) (Table 2). Three and two patients with CD and UC, respectively, were positive for both GP2 IgG and IgA. The rates of GP2 antibody positivity (IgA, IgG, and total) were similar in the UC and control groups (chi-square test, p>0.05 for all). The GP2 IgG levels were similar in the three groups (Kruskal-Wallis test, p=0.323).

ASCA

The ASCA positivity rate was higher in patients with CD (31/60, 52%) than in those with UC (14/62, 23%) and controls (1/46, 2%) (Table 2). The rate of ASCA IgA positivity was higher in patients with CD (25/60, 42%) than in those with UC (7/62, 11%) (p<0.001). Although the ASCA IgG positivity rate in patients with CD (19/60, 32%) was higher than that in those with UC (9/62, 15%), the difference was not statistically significant (p=0.24).

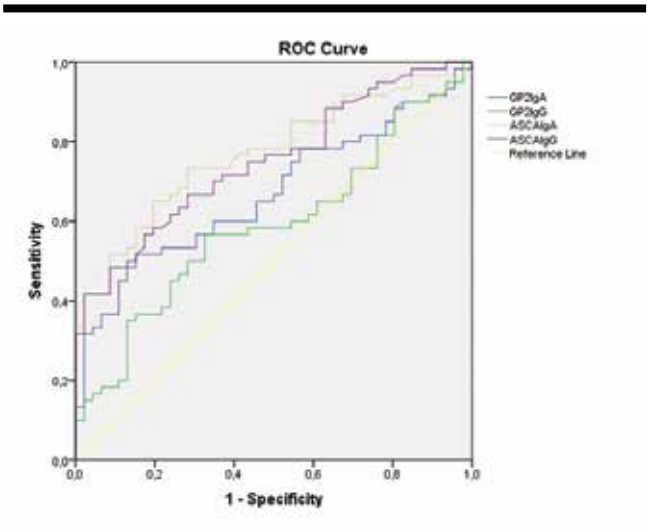

Figure 1. ROC curve for GP2 and ASCA antibodies in Crohn's disease and the control

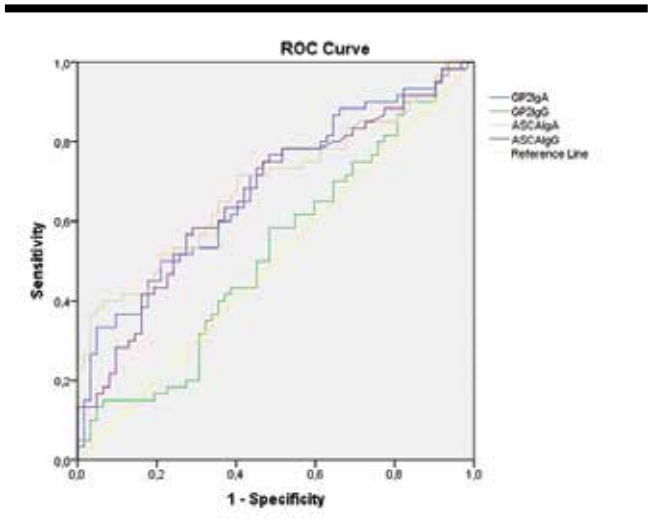

Figure 2. ROC curve for GP2 and ASCA antibodies in Crohn's disease and ulcerative colitis patients

Table 2. Rate of anti-GP2 antibody positivity in the UC, CD, and control groups

	UC (n=62)	CD (n=60)	Control (n=46)	p
GP2 IgA (+)	2 (3%)	15 (25%)	1 (2%)	<0.001
GP2 IgG (+)	3 (5%)	3 (5%)	0 / 46	0.309
GP2 total (+)	3 (5%)	15 (25%)	1 (2%)	<0.001
ASCA IgA (+)	7 (11%)	25 (42%)	1 (2%)	<0.001
ASCA IgG (+)	9 (15%)	19 (32%)	0/46	<0.001
ASCA total (+)	14 (23%)	31 (52%)	1 (2%)	<0.001

Chi-square test.

Table 3. ROC analysis for GP2 and ASCA antibodies between the patients with Crohn's disease and the control group.

	AUROC	p	95% CI
GP2 IgA	0.670	0.003	0.568-0.772
GP2 IgG	0.589	0.119	0.480-0.697
ASCA IgA	0.771	<0.001	0.683-0.859
ASCA IgG	0.750	<0.001	0.660-0.841

ROC curve analysis for GP2 and ASCA levels between the patients with CD and the control group showed that anti-GP2 IgG positivity was not different between the groups in terms of sensitivity and specificity. On the other hand, anti-GP2 IgA, ASCA IgA, and IgG antibodies were found to have moderate diagnostic value for CD compared with UC and the control group (Tables 3, 4; Figure 1, 2).

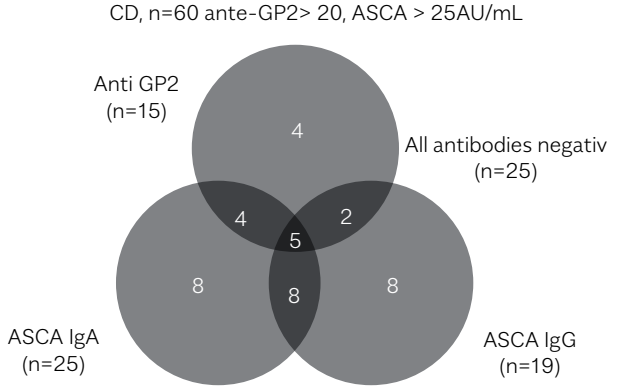

Figure 3. Combination of ASCA and anti-GP2 antibodies

Combination of ASCA and anti-GP2 antibodies

A kappa analysis showed weak to moderate agreement between the ASCA and anti-GP2 antibody test results (kappa test, κ=0.239, p=0.001). Of the patients with CD, 11 were seropositive for both ASCA and anti-GP2 antibodies. In other words, of the ASCA-positive (IgA and/or IgG) patients, 36% (11/30) were positive for anti-GP2 (IgA and/or IgG). Of the anti-GP2-positive patients, 73% (11/15) were positive for ASCA. Only 5 (8%) patients were positive for ASCA and anti-GP2 IgA and IgG (Figure 3).

Table 4. ROC analysis for GP2 and ASCA antibodies between the patients with Crohn's disease and UC

	AUROC	p	95% CI
GP2 IgA	0.683	<0.001	0.589-0.778
GP2 IgG	0.522	0.672	0.419-0.625
ASCA IgA	0.689	<0.001	0.594-0.784
ASCA IgG	0.664	0.002	0.568-0.761

Table 5. Rates of positivity for ASCA and anti-GP2 antibodies

	Negative for both antibodies	Positive for both antibodies	Only positive for ASCA	Only positive for GP2
CD (n=60)	25 (42%)	11 (18%)	20 (33%)	4 (7%)
UC (n=62)	48 (77%)	0	11 (18%)	3 (5%)
Control (n=46)	44 (96%)	0	1 (2%)	1 (2%)

Table 6. Sensitivity, specificity, PPV, and NPV for anti-GP2 and ASCA in patients with CD

CD	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Anti-GP2 IgA	25	97	88	55
Anti-GP2 IgG	5	97	62	50
Anti-GP2 total	25	96	78	69
ASCA IgA	42	92	84	61
ASCA IgG	32	91	79	57
ASCA total	50	86	78	63
Positivity for at least one antibody	58	82	76	66
Positivity for both antibodies	19	100	100	67

Overall, 25 (42%) patients with CD were negative for both anti-GP2 and ASCA, and 35 (58%) were positive for anti-GP2 or ASCA antibodies. No patients in the UC and control groups were positive for both anti-GP2 and ASCA antibodies (Table 5).

Assay performance

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in patients with CD were 25%, 97%, 88%, and 55% for anti-GP2 antibodies and 50%, 86%, 78%, and 63% for ASCA, respectively. For the combination of ASCA and anti-GP2 antibodies, the sensitivity, specificity, NPV, and PPV of positivity for at least one of anti-GP2 IgA, anti-GP2 IgG, ASCA IgA, and ASCA IgG were 58%, 82%, 76%, and 66%, respectively. Positivity for both anti-GP2 and ASCA reduced the sensitivity but increased the specificity and PPV to 100% (Table 6).

Correlation between ASCA and anti-GP2 and disease localization

The rates of positivity for all of the antibodies, with the exception of GP2 IgG, were similar in patients with CD with ileal, colonic, and ileocolonic involvement (Table 7). The frequency of GP2 IgG positivity was higher in patients with colonic involvement than in those with ileocolonic involvement.

Correlations between ASCA and anti-GP2 positivity and disease behavior

The GP2 antibody positivity rate was similar in patients with penetrating, stricturing, and non-stricturing non-penetrating CD (Table 5). According to two-way comparisons using chi-square tests with Bonferroni correction, the rates of total ASCA and ASCA IgA positivity differed significantly between penetrating and non-stricturing non-penetrating CD (Fisher's exact test, ASCA IgA

Table 7. Correlations between antibody positivity and disease behavior and area of involvement in patients with CD

	n	Anti-GP2 IgA (n)	Anti-GP2 IgG (n)	Anti-GP2 IgA or IgG	ASCA IgA	ASCA IgG	ASCA IgA or IgG
Ileal (L1)	32	7 (22%)	1 (3%)	7 (22%)	12 (37%)	10 (31%)	14 (44%)
Colonic (L2)	8	3 (37%)	2 (25%)	4 (50%)	3 (37%)	2 (25%)	3 (37%)
Ileocolonic (L3)	20	4 (20%)	0	4 (20%)	10 (50%)	7 (35%)	13 (65%)
		p=0.466	p=0.018	p=0.212	p=0.651	p=0.853	p=0.192
Non-stricturing	30	5 (17%)	1 (3%)	6 (20%)	7 (23%)	9 (30%)	11 (30%)
Non-penetrating	18	4 (22%)	0	4 (22%)	9 (50%)	6 (33%)	10 (56%)
Stricturing	12	5 (42%)	2 (17%)	5 (42%)	9 (75%)	4 (33%)	9 (75%)
Penetrating		p=0.222	p=0.102	p=0.324	p=0.006	p=0.962	p=0.07

Table 8. Correlations between perianal involvement and antibody positivity in patients with CD

	Perianal involvement present (n=9)	Perianal involvement absent (n=51)	p
Anti-GP2 positive	3 (33%)	12 (23%)	0.678¥
ASCA positive	5 (56%)	26 (51%)	0.544*

*Fisher's exact test
¥Two-sided Fisher's exact test

Table 9. Correlations between antibody positivity and surgical history in patients with CD

	Surgery (n=20)	No surgery (n=40)	p
GP2 IgA	6 (30%)	8 (20%)	0.519*
GP2 IgG	1 (5%)	2 (5%)	1*
GP2	6 (30%)	9 (22.5%)	0.528*
ASCA IgA	12 (60%)	13 (32.5%)	0.042¥
ASCA IgG	7 (35%)	12 (30%)	0.695¥
ASCA	12 (60%)	17 (43%)	0.143¥

*Fisher's exact test
¥Chi-square test

p=0.006; ASCA total p=0.008). Although the ASCA total and ASCA IgA positivity rates were higher in patients with stricturing and penetrating CD than in those with non-stricturing non-penetrating CD, the difference was not statistically significant (chi-square test, ASCA IgA p=0.058; ASCA total p=0.08). Perianal involvement was not correlated with ASCA or anti-GP2 positivity in patients with CD (Table 8).

Correlations between ASCA and anti-GP2 positivity and surgical history

The ASCA IgA positivity rate of patients with a history of surgery was higher than that of those with no history of surgery. The rate of positivity for the other antibodies was similar in those with and without a history of surgical treatment (Table 9).

DISCUSSION

We investigated the seroprevalence of ASCA and anti-GP2 antibodies and their association with the characteristics of CD. The anti-GP2 and ASCA antibody positivity rates were higher in patients with CD than in those with UC and controls. The positivity rate of anti-GP2 IgA was higher than that of anti-GP2 IgG. The ASCA IgA positivity rate was higher in patients with penetrating and structuring CD. Positivity for both ASCA and anti-GP2 antibodies had a high specificity and PPV for the diagnosis of CD.

The anti-GP2 antibody positivity rate was 25% in patients with CD, 5% in patients with UC, and 2% in the controls. These results are similar to previous studies (13,14,19). Of the patients, 15 (25%) were positive for anti-GP2 IgA and 3 (5%) for anti-GP2 IgG. By contrast, the rate of anti-GP2 IgG positivity was higher than that of IgA positivity in patients with CD (9%-18% and 1%-5%, respectively). Similarly, Nikolic et al. reported a higher rate of positivity for anti-GP2 IgA than IgG in patients with CD and suggested that this may be due to genetic differences (15).

The rate of ASCA positivity is 50%-70% in patients with CD, 15% in patients with UC, and 0%-5% in healthy individuals (16-18). In the present study, the rate of ASCA positivity was 52% in patients with CD, 23% in patients with UC, and 2% in controls.

Pavlidis et al. (11) reported ASCA and anti-GP2 positivity rates of approximately 35% in 70 patients with CD. While the levels of the two antibodies were not correlated, patients who were positive for anti-GP2 had higher ASCA levels. Roggenbuck et al. (7) identified a correlation between ACSA IgG and anti-GP2 IgG levels in patients with CD but no correlation between total antibody and IgA levels. Bogdanos et al. (10) demonstrated a significant correlation between ASCA and anti-GP2 levels. Similarly, in the present study, we found a positive correlation between ASCA and anti-GP2 antibody levels. In addition, 73% of anti-GP2-positive patients were positive for ASCA. The rate of positivity for either ASCA or anti-GP2 in patients with CD is reportedly 51% to 70% (7,10,11). This rate was 58% in the present study.

The rate of positivity for both ASCA and anti-GP2 antibodies is 22% (11), similar to our finding (18%). The rate of positivity for ASCA and anti-GP2 IgA and IgG is 4%-16% (10,11). This rate was 8% in the present study.

Roggenbuck et al. (7) reported that the presence of anti-GP2 antibodies has a low sensitivity and high specificity for the diagnosis of CD (13.5% and 98.5% for IgA and 29.2% and 95.8% for IgG, respectively). Our findings were similar (25% and 97% for IgA and 5% and 97% for IgG). In contrast to the study by Roggenbuck et al. (7), the sensitivity of IgA for the diagnosis of CD was higher than that of IgG. Moreover, in our study, the rate of positivity for IgA was higher than that for IgG. This difference may

be due to genetic traits in the studied populations. Our results support the use of anti-GP2 IgA in Turkey.

The sensitivity and specificity of ASCA for the diagnosis of CD are 50%-70% and 80%-85%, respectively (12). Other studies have reported that ASCA has a low sensitivity but high specificity (93.3%-97.5%) and PPV (90%-97.5%) for the diagnosis of CD [19,20]. In the present study, the sensitivity, specificity, and PPV were lower at 50%, 86%, and 78%, respectively. Positivity for ASCA and anti-GP2 had similar sensitivity, specificity, PPV, and NPV at 58%, 82%, 76%, and 66%, respectively. While the sensitivity of positivity for both antibodies decreases significantly (19%), the specificity increases by a large amount (100%). No patients with UC or controls were positive for both ASCA and anti-GP2. If CD/UC cannot be distinguished clinically, radiologically, or histologically, the combination of ASCA and anti-GP2 antibodies is reportedly useful (8,25). Our results support this interpretation.

ASCA positivity is related to disease onset at a younger than normal age, ileal involvement, complicated disease, perianal disease, and the need for surgery in patients with CD (20,21). In the present study, ASCA (particularly IgA) positivity was correlated with surgical history and complicated disease (penetrating and stricturing types). However, ASCA positivity was not correlated with area of involvement, age of onset, or perianal disease.

Bogdanos et al. (10) reported that anti-GP2 antibody positivity is related to disease onset at a younger than normal age, ileocolonic involvement, stricturing behavior, and perianal disease. According to Pavlidis et al. (11), anti-GP2 positivity is related to the need for surgery and ileal involvement but not age of disease onset or disease duration in patients with CD. Interestingly, the anti-GP2 positivity rate is lower in patients with penetrating disease than in those without it. In the present study, anti-GP2 IgG positivity was correlated with colonic involvement but not ileal involvement. However, interpretation of this correlation is problematic because only three patients were positive for anti-GP2. In addition, the anti-GP2 antibody positivity rate was 50% in patients with CD with colonic involvement, 20% with ileal involvement, and 22% with ileocolonic involvement. GP2 is found in microfold cells (M cells) of the follicular-associated epithelium in intestinal Peyer's patches (22). While M cells are abundant in the small intestine, they are scarce in the large intestine (23). Although the relationship between GP2 autoantigenicity and its location on the apical surface of intestinal M cells remains to be confirmed, triggering of GP2 autoantibody production by ileal inflammation is widely accepted and is supported by the findings by Pavlidis et al. (11) and Bogdanos et al. (10). In the present study, anti-GP2 antibodies were predominantly IgA, which may explain for finding no correlation between anti-GP2 IgG positivity and ileal involvement. Therefore, our findings should be interpreted with caution.

The ASCA positivity rate and level are reportedly correlated with perianal involvement (20,21). In fact, Bogdanos et al. (10) reported that patients with CD with perianal disease have a lower rate of anti-GP2 IgG positivity that those without perianal involvement. In the present study, positivity for ASCA and anti-GP2 was not correlated with perianal involvement in patients with CD.

The higher rate of positivity for anti-GP2 IgA than anti-GP2 IgG in patients with CD reported in the present study may be due to genetic differences. Anti-GP2 IgA, ASCA IgA, and IgG antibodies were found to have moderate diagnostic value for CD compared with UC and the control group. Positivity for both ASCA and anti-GP2 has a high specificity and PPV, suggesting that positivity for both antibodies could facilitate discrimination of CD from UC. In addition, ASCA IgA positivity may be a marker of more aggressive disease; thus, early aggressive treatment may be appropriate in patients positive for ASCA IgA. The differences between our data and previous studies may be due to genetic factors. Further studies involving larger numbers of patients are required to confirm these data.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Kocaeli University School of Medicine (2011/40 KAEK 14/4).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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Author Contributions: Concept - A.E.D.; Design - A.E.D.; Supervision - S.H., H.M.K.; Materials - M.M.M.; Data Collection and/or Processing - H.Y., M.Ö., G.D., D.K., G.S.; Analysis and/or Interpretation - U.K.; Literature Review - A.E.D.; Writing Manuscript - A.E.D.; Critical Review - S.H., Ö.Ş., A.Ç.

Conflict of Interest: The authors have no conflicts of interest to declare.

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