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Original Article

Mean platelet volume and red cell distribution width as potential new biomarkers in children with gastroesophageal reflux disease

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Abstract: The aim of this study was to calculate the corrected rate of reflux in children with gastroesophageal reflux (GER)-like complaints by 24-hour pH monitoring and esophagogastroduodenoscopy (EGD), and to determine the utility of mean platelet volume (MPV) and red cell distribution width (RDW) as diagnostic biomarkers of GER disease (GERD) in children. The subjects in this prospective study were 109 children, 6 to 18 years old. Of them, 74 subjects were with GER symptoms and 35 healthy controls. The subjects were divided into three groups: those who underwent 24-hour pH monitoring (Group 1), those who underwent EGD together with pH monitoring (Group 2), and the healthy controls (Group 3). The results of pH monitoring and EGD and hematological parameters with controls were compared between Groups 1 and 2. In Groups 1 and 2, the overall rate of reflux was 40%, of esophagitis was 27.8%, and of *Helicobacter pylori* infection was 31.2%. The MPV and RDW cut-offs in subjects with reflux were ≤ 8.97 (sensitivity 89%, specificity 89%) and ≤ 12.78 (sensitivity 80%, specificity 97%), with an area under the Receiver Operating Characteristic (ROC) curve ± standard error (AUC ± SE) = 0.917 ± 0.027 ($P < 0.001$) and AUC ± SE = 0.866 ± 0.036 ($P < 0.001$), respectively. The endoscopic procedures are not practical due to being invasive and expensive. However, hemogram is a simple test which can be performed in an outpatient clinic. MPV and RDW calculated in hemogram could be easy, cost-effective, and high sensitive new biomarkers that can be used in children with GERD.

Keywords: Gastroesophageal reflux disease, mean platelet volume, red cell distribution width, *Helicobacter pylori*

Introduction

Gastroesophageal reflux (GER) refers to the retrograde flow of the gastric contents toward the esophagus following a meal, which can cause severe symptoms and complications, collectively known as GER disease (GERD) [1, 2]. The epidemiology of GERD is mainly indeterminate due to the difficulties in defining the physiological and pathological symptoms and the lack of a gold standard diagnostic method. The current diagnostic approaches include 24-hour pH monitoring, multichannel intraluminal impedance (MII), scintigraphic imaging methods, and endoscopy with histology support [3-8].

However, since invasive diagnostic procedures are not recommended for children with GER-like complaints, anti-reflux or *Helicobacter pylori* (*H. pylori*) eradication therapy is promptly initiated, both of which are expensive and may be unnecessary. Therefore, a cost-effective and non-invasive diagnostic test is needed to confirm GERD and to plan the appropriate therapy. In this study, we compared the results of 24-hour pH monitoring with those of esophagogastroduodenoscopy (EGD) in children with reflux-like complaints and evaluated the presence of *H. pylori* antigen in both stool and endoscopic biopsy samples. Hemogram parameters of the healthy control group and the reflux groups were compared. Previous studies have confirmed the diagnostic value of mean platelet volume (MPV) and red cell distribution width (RDW) in gastrointestinal system diseases such as ulcerative colitis, Crohn's disease, and liver cirrhosis [9-13]. To the best of our knowledge, these parameters have not been studied in chil-

Table 1. DeMeester score

Measurement of lower esophageal acidity and, therefore, a surrogate of the severity of GER. A score of > 14.72 shows significant reflux.
Based on six parameters (scored in comparison to mean values in healthy subjects for each category below)
Supine reflux
Upright reflux
Total reflux
Number of episodes
Number of episodes longer than 5 min
Longest episode
See also esophageal manometer

dren with GERD. We calculated the cut-off, sensitivity, and specificity values of MPV and RDW in patients with reflux and performed ROC analysis, to determine the potential of MPV and RDW as new GERD biomarkers in children.

Materials and methods

Study design and patients

This prospective trial included 74 patients (age 6 to 18 years), who applied and were admitted to Karabuk Education and Research Hospital with GER symptoms, along with 35 healthy controls. The Clinical Research Ethics Committee approved the study, which was conducted according to the Declaration of Helsinki. The parents of the children were given detailed information about the study and signed consents were obtained. The patients who received medications that affect gastric acidity, motility, and lower esophageal sphincter pressure, *H. pylori* eradication therapy, proton pump inhibitors (PPIs), and antacid treatment, or acetylsalicylic and/or non-steroidal anti-inflammatory drugs within the last three months, or with endoscopic evidence of active gastrointestinal hemorrhage, presence of esophagitis due to esophageal stricture or systemic diseases, history of a gastric or esophageal surgery, acute or chronic infection, or hematological disorders were excluded from the study.

We used a 24-hour pH-metry test to diagnose the patients presenting with GER-like complaints. We also performed EGD in the patients detected to have severe reflux in pH-metry. The aim of performing EGD was to detect whether esophagitis has developed, in order to make the differential diagnosis for acid or bile reflux, to detect whether the reflux is due to any anatomic variation, and if present, to determine the extent of inflammation. In addition, biopsies were taken from gastric antrum and corpus to perform the bacteriological and histological examination. The patients who only underwent pH-metry were placed in Group 1, and those who also underwent EGD after severe reflux as detected in pH-metry were placed in Group 2. Healthy children without any complaints and who visited our clinic for routine follow-up were placed into Group 3, the control group. The control subjects did not undergo pH-metry or EGD.

Laboratory tests included complete blood counts and hematological parameters and identification of the presence of *H. pylori* antigen in stool samples and endoscopic biopsies.

24-hour monitoring of pH

The 24-h pH was monitored using the MMS Orion-II probe, which consisted of a catheter with two probes separated by 5 cm and 10 cm at two points, two calibration fluids (acid and alkali), a recorder, and the analysis software. The distal end of the pH meter probe containing the reference fluid and a glass pH electrode used for the measurement were inserted into the lower end of the esophagus through the nasal route. The localization of the probe was confirmed radiologically by posteroanterior pulmonary X-ray. The recording was initiated 30 min after the probe insertion to maximize salivation due to the feeling of foreign matter in the esophagus. The probe was calibrated before each measurement with two standard fluids, with pH values of four or seven, at room temperature.

The consumption of hot and cold foods and foods with pH < 5 were restricted before the pH measurement. An acid-reflux was reported when the pH at the lower end of esophagus

Table 2. The Los Angeles classification

Grade A: 5 mm mucosal break in mucosal folds
Grade B: 5 mm break in mucosal folds that is not continuous between the folds
Grade C: Mucosal break that is continuous between two or more mucosal folds but not all around the circumference
Grade D: All around mucosal breaks (involving more than 75% of the esophageal lumen)

Note: The presence of ulcer, stricture, Barrett's metaplasia should be indicated separately at every grade.

Table 3. Socio-demographic and some clinical characteristics of the groups

	Group 1 (n = 32)	Group 2 (n = 42)	Group 3 (n = 35)	*P*
Age	13.43 ± 3.19	14.11 ± 1.97	12.31 ± 1.67*	< 0.001*
Gender (F/M)	5/27	10/32	17/18**	0.004**
HGB	13.64 ± 0.83	13.66 ± 1.31	13.36 ± 1.04	0.962***
Monocyte	3.07 ± 3.41	1.71 ± 2.50	0.45 ± 0.12	< 0.001*
MCV	82.91 ± 4.21	82.24 ± 4.99	81.64 ± 6.61	0.596***
RDW	12.70 ± 4.93	12.24 ± 1.48	14.00 ± 1.11*	< 0.001*
MPV	7.67 ± 1.44	7.54 ± 1.27	9.93 ± 0.92	< 0.001*
EO	1.15 ± 2.10	0.78 ± 2.79	0.20 ± 0.16	0.234*

HGB: Hemoglobin, MCV: Mean corpuscular volume, RDW: Red cell distribution width, MPV: Mean platelet volume, EO: Eosinophil, *Kruskal-Wallis Test (Mann-Whitney U Test for post-hoc analysis), **Chi-square test, ***One Way ANOVA Test (Bonferroni corrected). One-way analysis of variance (ANOVA) with the Bonferroni correction was used to compare the normally distributed data across the three groups, and the Kruskal Wallis test (Mann-Whitney U test for further analysis) was used for non-normally distributed data. The chi-square test was used for inter-group comparison of the categorical data.

dropped below 4. The DeMeester score was used [14] (**Table 1**), and patients with high scores were diagnosed with GERD.

Different scoring systems, such as the Boix-Ochoa, DeMeester, and Johnson-DeMeester score, are used for 24-hour pH-metry assessment. Boix-Ochoa scoring system is used for the pediatric age group in the literature, Demeester score has been found to have a very strong correlation with Boix-Ochoa score ($r = 0.94$, $P < 0.01$, 95% confidence interval) [15]. We, therefore, used the DeMeester scoring system in our study.

The test is considered positive if the pH falls below 4 for a period longer than 5 s [16]. In order to interpret the results, the following parameters were considered: total number of reflux episodes (normal < 2 episodes per hour on average), number of reflux episodes lasting more than 5 min (normal < 8 episodes), duration of the longest reflux episode (in a minute), reflux index (RI) = ratio between the total number of reflux episodes and recording time (normal < 4), Euler score = x+4y (where x is the number of reflux episodes with pH < 4 longer than 1 min and y is the number of episodes with pH < 4 longer than 5 min) [16]. The recorded data were converted to DeMeester scores (n < 14.72).

Esophagogastroduodenoscopy (EGD)

An EGD can distinguish between an acid or bile reflux and allows classification of reflux based on anatomical variation and the grade of inflammation, and can detect possible esophagitis or any anatomical anomaly of the gastroesophageal junction. Moreover, EGD enables biopsy to differentiate antral or corpus gastritis. Standard EGD was performed under intravenous sedation using Olympus and Pentax video pediatric gastroduodenoscopes in patients who had severe reflux as evaluated by pH monitoring. The Los Angeles classification of GERD [17] is given in **Table 2**.

The biopsied samples were taken from the gastric antrum and corpus for bacteriological and histological examination before the administration of any treatment, such as antibiotics, bismuth, or PPIs. A biopsy was considered positive for *H. pylori* if at least two out of four biopsies were positive. The biopsied samples taken from antrum were fixed in buffered 4% formalin, embedded in paraffin, and stained using the hematoxylin-eosin and modified Giemsa procedure. The stained sections were then examined for histopathological features and bacterial density.

Hematological measurements

Complete blood count (CBC) analyses were performed in the hematology laboratory of our hospital by a single technician using a BC-6800

Table 4. Comparisons of groups in terms of reflux positivity

		Group 1 (24 h pH meter)	Group 2 (24 h pH meter + Endoscopy)	Total	*P*
Reflux (-)	N	18	24	42	
	% within Group	42.9%	57.1%	100.0%	
	% within Reflux	56.3%	57.1%	56.8%	
Reflux (+)	N	14	18	32	
	% within Group	43.8%	56.3%	100.0%	0.939*
	% within Reflux	43.8%	42.9%	43.2%	
Total	N	32	42	74	
	% within Group	43.2%	56.8%	100.0%	
	% within Reflux	100.0%	100.0%	100.0%	

*Chi-square test (The chi-square test was used for inter-group comparison of the categorical data).

Table 5. Comparisons groups for H. pylori positivity

		Group 1 (24 h pH meter)	Group 2 (24 h pH meter + Endoscopy)	Total	*P*
Negative	N	28	32	60	
	% within Group	46.7%	53.3%	100.0%	
	% within *H. pylori*	87.5%	76.2%	81.1%	
Positive	N	4	10	14	0.218
	% within Group	28.6%	71.4%	100.0%	
	% within *H. pylori*	12.5%	23.8%	18.9%	
Total	N	32	42	74	
	% within Group	43.2%	56.8%	100.0%	
	% within *H. pylori*	100.0%	100.0%	100.0%	

hematology analyzer (Mindray, China). A blood sample of 2 mL was taken from each subject into a tube containing 40 μL ethylene-diaminetetraacetic acid (EDTA). Since the MPV increases over time in EDTA due to platelet swelling, the MPV and RDW were measured within two hours after the blood sampling [11, 18]. The hematological parameters which involved MPV with a range of 6.5-12 fL and RDW with a range of 11-16% were analyzed by standard methods with a time-to-result of approximately 5 min.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation and categorical data as number and percentage. Normal distribution was tested using the Kolmogorov-Smirnov Goodness of Fit test. One-way analysis of variance (ANOVA) with the Bonferroni correction was used to compare the normally distributed data across the three groups, and the Kruskal Wallis test (Mann-Whitney U test for further analysis) was used for non-normally distributed data. The chi-square test was used for inter-group comparison of the categorical data, and intra-group analysis of categorical data was performed using McNemar's test. For MPV and RDW, the receiver operating characteristic (ROC) curve analysis was used to estimate the area under the ROC curve with a confidence interval. All analyses were performed using IBM SPSS Package Software version 24.0 (IBM Corporation, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Significant differences in monocyte counts, RDW, and MPV were seen between Groups 1 and 2, and MPV and RDW were significantly lower in these groups than in the healthy controls (Group 3) ($P < 0.001$). The hemoglobin, mean corpuscular volume, and eosinophil values were similar across the groups (**Table 3**). Reflux positivity was not significantly different between Groups 1 and 2, at approximately 40% (**Table 4**). *H. pylori* positivity rate was almost double in Group 2 (23.8%) compared to Group 1 (12.5%) (odds ratio (OR) = 2.18; CI = 0.617-7.755) (**Table 5**). When all subjects in Groups 1 and 2 were compared for *H. pylori* positivity, 31.2% of the subjects with reflux and 9.5% of those without reflux were found to be infected with the bacteria ($P < 0.05$) (**Table 6**). Thus, *H. pylori* infection was 4.3-fold higher in subjects with reflux than in the subjects without reflux (OR = 4.31; CI = 1.209-15.421). In Group 2, 27.8% of the patients with reflux were also positive for esophagitis, and this rate was 21.7% in those without reflux ($P > 0.05$) (**Table 7**), corresponding to only a 1.3-

Table 6. Comparisons of the presence of reflux with H. pylori positivity

		Reflux (-)	Reflux (+)	Total	*P*
Negative	N	38	22	60	
	% within Reflux	63.3%	36.7%	100.0%	
	% within *H. pylori*	90.5%	68.8%	81.1%	
Positive	N	4	10	14	0.033*
	% within Reflux	28.6%	71.4%	100.0%	
	% within *H. pylori*	9.5%	31.2%	18.9%	
Total	N	42	32	74	
	% within Reflux	56.8%	43.2%	100.0%	
	% within *H. pylori*	100.0%	100.0%	100.0%	

*Chi-square test (Fisher's exact test) (Fisher's Exact test is a test of significance that is used in the place of chi square test in 2 × 2 Tables, especially in cases of small samples).

Table 7. Comparisons of the presence of reflux in terms of endoscopic esophagitis

			Reflux (-)	Reflux (+)	Total	*P*
Endoscopic Esophagitis	(-)	N	18	13	31	
		% within Reflux	58.1%	41.9%	100.0%	
		% within EE	78.3%	72.2%	75.6%	
	(+)	N	5	5	10	0.096*
		% within Reflux	50.0%	50.0%	100.0%	
		% within EE	21.7%	27.8%	24.4%	
Total		N	23	18	41	
		% within Reflux	56.1%	43.9%	100.0%	
		% within EE	100.0%	100.0%	100.0%	

*Mc Nemar Test (The McNemar's test is used for intra-group analysis of categorical data).

Table 8. Comparisons of the presence of reflux in pathological diagnosis

			Reflux (-)	Reflux (+)	Total	*P*
Pathology	Normal	N	12	2	14	0.005*
		% within Reflux	85.7%	14.3%	100.0%	
		% within Pathology	50.0%	11.1%	33.3%	
	Chronic infection	N	3	8	11	
		% within Reflux	27.3%	72.7%	100.0%	
		% within Pathology	12.5%	44.4%	26.2%	
	Chronic infection + Atrophy	N	9	5	14	
		% within Reflux	64.3%	35.7%	100.0%	
		% within Pathology	37.5%	27.8%	33.3%	
	Metaplasia	N	0	3	3	
		% within Reflux	0.0%	100.0%	100.0%	
		% within Pathology	0.0%	16.7%	7.1%	
Total		N	24	18	42	
		% within Reflux	57.1%	42.9%	100.0%	
		% within Pathology	100.0%	100.0%	100.0%	

*Chi-square test (The chi-square test was used for inter-group comparison of the categorical data).

fold difference (OR = 1.385; CI = 0.331-5.787).

When comparing reflux positivity by pathological diagnosis, we found that, among the sub-

Table 9. Comparisons of H. pylori positivity versus pathological diagnosis

			H. pylori negative	*H. pylori* positive	Total	*P*
	Normal	N	9	5	14	
		% within *H. pylori*	64.3%	35.7%	100.0%	
		% within Pathology	28.1%	50.0%	33.3%	
	Chronic infection	N	9	2	11	
		% within *H. pylori*	81.8%	18.2%	100.0%	
		% within Pathology	28.1%	20.0%	26.2%	0.091*
Pathology	Chronic infection + Atrophy	N	13	1	14	
		% within *H. pylori*	92.9%	7.1%	100.0%	
		% within Pathology	40.6%	10.0%	33.3%	
	Metaplasia	N	1	2	3	
		% within *H. pylori*	33.3%	66.7%	100.0%	
		% within Pathology	3.1%	20.0%	7.1%	
Total		N	32	10	42	
		% within *H. pylori*	76.2%	23.8%	100.0%	
		% within Pathology	100.0%	100.0%	100.0%	

*Chi-square test (The chi-square test was used for inter-group comparison of the categorical data).

Table 10. Cut-off value and ROC curve results of MPV for reflux

	Cut-off	*Diagnostic test*		ROC curve			95% CI	P
		Sensitivity	*Specificity*	PPV	NPV	AUC		
MPV	≤ 8.97	89.00	89.00	94.30	79.50	0.917	0.864-0.970	< 0.001**

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, CI: Confidence interval, **ROC Curve Analysis Test. Accuracy is measured by AUC. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. The accuracy of the test depends on how well the test separates those with and without the disease in question. This table above shows a ROC curve representing an excellent test that the area under the MPV ROC curve is 0.91. By the cuff-off point of ≤ 8.97, the MPV would be considered to be "excellent" at separating patients who have reflux or not.

jects with reflux positivity, 44.4% had chronic infection, 27.8% had chronic infection and atrophy, and 16.7% had metaplasia (P = 0.005) (**Table 8**). There was no statistically significant relationship between *H. pylori* positivity and pathological diagnosis (P = 0.091) (**Table 9**). According to ROC curve analysis, in which MPV and RDW values of the patients (Group 1 and Group 2) determined by pH test and endoscopy were compared with the control group (Group 3), the MPV cut-off was ≤ 8.97 (sensitivity 89%, specificity 89%) with area under the ROC curve ± standard error (AUC ± SE) = 0.917 ± 0.027 (P < 0.001) (**Table 10**; **Figure 1**), and the RDW cut-off was ≤ 12.78 (sensitivity 80%, specificity 97%) with AUC ± SE = 0.866 ± 0.036 (P < 0.001) (**Table 11**; **Figure 2**).

Mean MPV was 8.26 ± 0.46 in patients with coexisting reflux and endoscopic esophagitis (n = 5), it was 7.34 ± 1.49 in patients without esophagitis (n = 17) (P = 0.058), on the other hand mean RDW was 11.51 ± 0.59 and 12.31 ± 1.72, respectively (P = 0.329).

A statistically significant *p*-value could not be found in the ROC analysis either for MPV or RDW, and the sample size was inadequate to determine a cut-off value. ROC analysis was performed for MPV and RDW, in case of coexisting reflux and endoscopic esophagitis; however, since the sample size was inadequate, no significant result was obtained.

We did not perform an esophageal biopsy in this study. Therefore, we could not distinguish the type of esophagitis and did not classify the esophagitis by severity either. The patients were grouped only on the basis of the presence of esophagitis. No statistically significant difference was detected between the subjects with and without esophagitis in terms of hematological parameters (P > 0.05) (**Table 12**).

Discussion

In this study, the reflux rate was approximately 40% in both the pH meter and the EGD groups.

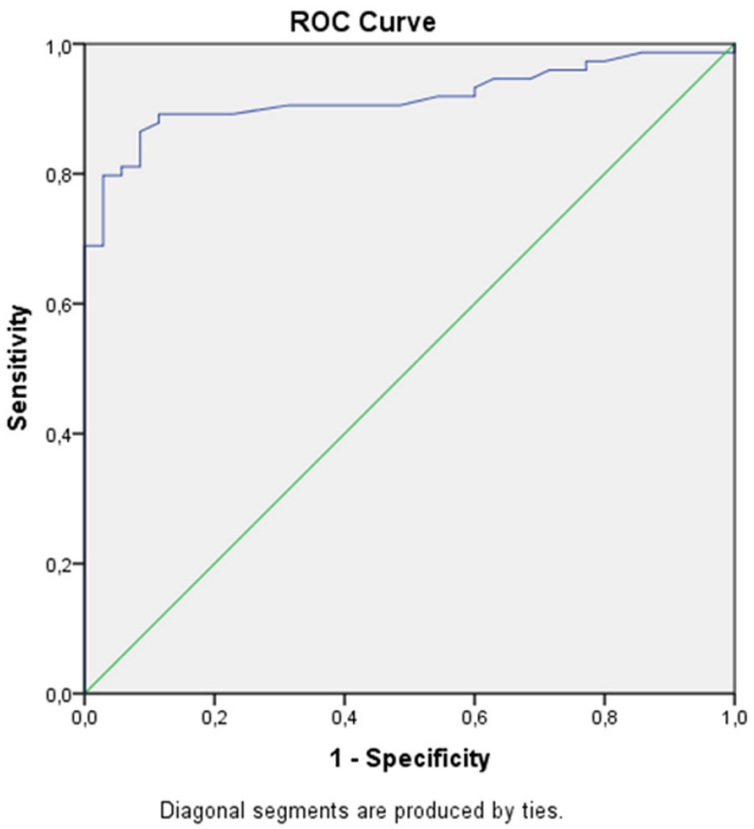

Figure 1. The area under the ROC curve (AUC) for MPV in patients with reflux. Accuracy is measured by AUC. The more the curve follows the left-hand border and then the top border of the AUC space, the more accurate the test is. An area corresponding to 1 represents a perfect test; an area of 5 represents a worthless test. The accuracy of the test depends on how well the test separates those with and without the disease in question. This graph shows a ROC curve representing a good test, where the area under the MPV ROC curve is 91. By the cuff-off point of ≤ 8.97, the MPV would be considered to be "excellent" in separating patients with and without reflux.

The frequency of *H. pylori* infection in children with positive reflux was 31.2%, and 27.8% of the children who were diagnosed with reflux by endoscopy also had esophagitis. Moreover, MPV and RDW were significantly lower in the patients compared to the healthy controls. In the children with reflux, MPV cut-off was ≤ 8.97 (sensitivity 89%, specificity 89%), and the RDW cut-off was ≤ 12.78 (sensitivity 80%, specificity 97%).

Platelets (PLT), in addition to hemostasis and thrombosis, play a key role in inflammatory diseases. Matowicka-Karna et al. found a strong correlation between platelet dysfunction and inflammatory bowel disease (IBD) in patients showing reactive thrombocytosis, decreased MPV, and increased production and secretion of platelets granular contents [9]. Öztürk et al. also detected significantly lower MPV levels in IBD patients during both the active and remission periods compared to levels in the healthy controls [11]. Furthermore, Lida et al. confirmed the MPV/PLT ratio as a predictive marker of liver cirrhosis [12]. RDW is another hematological factor associated with gastrointestinal diseases. Turcato et al. showed a significant correlation between RDW and the clinical severity of acutely decompensated liver cirrhosis and found it to be an independent prognostic predictor associated with 1.2- to 2.3-fold higher risk of one-month mortality in the cirrhotic patients [13]. Consistent with the findings of the present study, the children who were diagnosed with reflux via EGD and 24-h pH monitoring had significantly lower MPV and RDW than those in the healthy controls, and both biomarkers had a diagnostic sensitivity and specificity of > 80% for reflux. Therefore, the measurement of MPV and RDW could serve as a simple, non-invasive, and cost-effective diagnostic test for GERD in children, as it is for liver cirrhosis and IBD. Since it is difficult to determine physiological and pathological reflux in children who have reflux-like complaints, a non-invasive diagnostic method such simple hemogram tests that could be applied by a physician in an outpatient clinic would be definitive to start medical treatment for GERD.

There are also some studies suggesting that gastrointestinal system disorders, such as *H. pylori* infection, gastritis, gastric ulcer, etc., cause a decrease in vitamin B12, iron, and certain hematological parameters including hemoglobin, mean corpuscular volume (MCV), MPV, RDW [19-21]. Since gastritis is also a cause of inflammation, the hematological parameters could be affected. However, we did not perform any assessment for gastritis in our study. There was no statistically significant difference be-

Table 11. Cut-off value and ROC curve results of RDW for reflux

	Cut-off	*Diagnostic test*		ROC curve			95% CI	P
		Sensitivity	*Specificity*	PPV	NPV	AUC		
RDW	≤ 12.78	80.00	97.00	98.30	69.4	0.866	0.796-0.936	< 0.001**

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, CI: Confidence interval, **ROC Curve Analysis Test. Accuracy is measured by AUC. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. The accuracy of the test depends on how well the test separates those with and without the disease in question. This table above shows a ROC curve representing a good test that the area under the RDW ROC curve is 0.866. By the cuff-off point of ≤ 12.78, the RDW would be considered to be "good" at separating patients who have reflux or not.

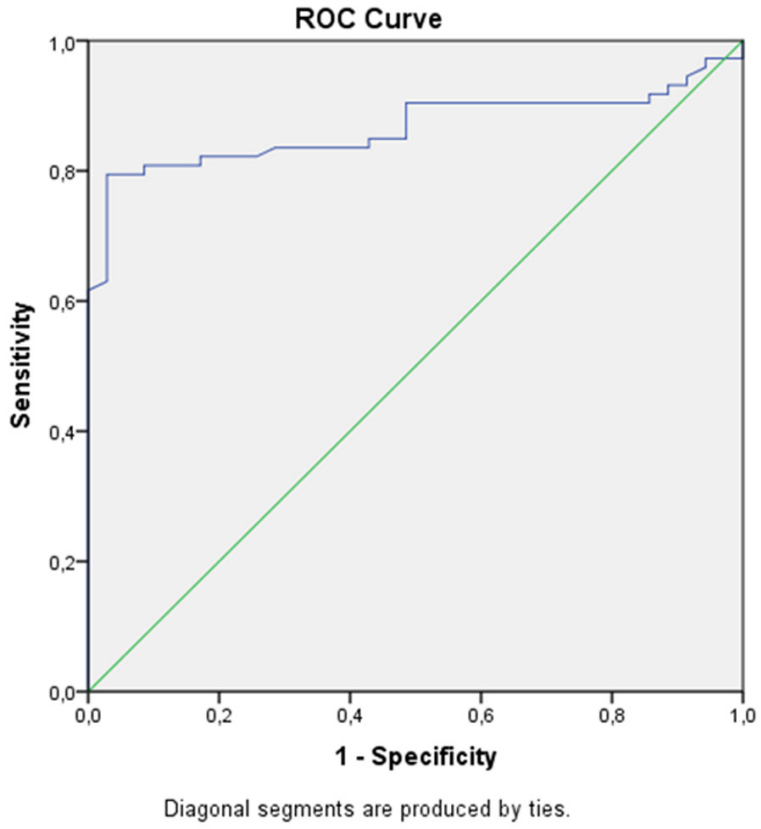

Figure 2. The area under the ROC curve (AUC) for RDW in patients with reflux. The accuracy is measured by AUC. The more the curve follows closely the left-hand border and then the top border of the AUC space, the more accurate the test is. An area corresponding to 1 represents a perfect test; while an area of 5 represents a worthless test. The accuracy of the test depends on how well the test separates those with and without the disease in question. This graph shows a ROC curve representing a good test as the area under the RDW ROC curve is 86. By the cuff-off point of ≤ 12.78, the RDW would be considered to be "good" in separating patients with and without reflux.

tween the subjects with and without esophagitis in terms of hematological parameters.

In a study conducted in the USA, of 1576 children and adolescents, who were screened for reflux-esophagitis, only 19.7% of them had complaints typical to GERD. According to the endoscopic data of children, the rate of reflux-esophagitis was 18.7% in 2005 and 18.8% in 2010 [22]. In a Korean study, the cases of children with reflux symptoms were reviewed retrospectively between 2001 and 2014 [23]. The overall prevalence of endoscopically-proven reflux esophagitis was found to be 28.7% (978/3413) and increased from 11.8% between 2001 and 2007 to 37.7% between 2008 and 2014. In a study conducted in Turkey, pre-school children with recurrent hospitalizations due to wheezing episodes were evaluated and GERD was detected in 32% of them [24]. Gül et al. monitored pH for 24 h in 109 pediatric patients with reflux-like complaints and GERD was detected in only 28.4% of the patients [25]. The rate of reflux detected in our study was slightly higher at 40%. All these results, when compared with each other, showed that routine tests often give a negative diagnosis of GERD, and therapy is usually unnecessarily initiated in the majority of pediatric patients with reflux-like symptoms.

Several mechanisms are involved in the etiopathogenesis of GERD. It has long been known that there is also a genetic component. A possible genetic predisposition was detected for reflux development in the family members of patients with Barrett's esophagus and esophagus adenocarcinoma. It has been found that

Table 12. The comparison of hematologic parameters between the subjects with and without esophagitis

	Subjects with Esophagitis (n = 10)	Subjects without Esophagitis (n = 31)	*P**
HGB	14.03 ± 0.70	13.51 ± 1.45	0.170
Monocyte	3.12 ± 3.61	1.29 ± 1.93	0.089
MCV	84.37 ± 3.73	81.47 ± 5.24	0.078
RDW	11.66 ± 0.78	12.50 ± 1.59	0.217
MPV	7.85 ± 0.90	7.48 ± 1.37	0.167
EO	0.55 ± 0.63	0.88 ± 3.23	0.230

HGB: Hemoglobin, MCV: Mean corpuscular volume, RDW: Red cell distribution width, MPV: Mean platelet volume, EO: Eosinophil, *Mann-Whitney U Test (Mann-Whitney U test was used for non-normally distributed data).

environmental factors are more important than genetic factors in uncomplicated reflux esophagitis [26, 27]. According to a large trial in monozygotic and dizygotic twins, carried out in Sweden and the United Kingdom, genetic factors were found to play an important role in GERD etiology [28, 29]. The studies determining the gene loci related to GERD are limited and 30 different possibly responsible gene loci have been detected so far [30].

H. pylori infection plays an important pathogenic role in many gastrointestinal diseases, including chronic gastroenteritis, peptic ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Epidemiological studies have shown 30-90% prevalence of *H. pylori* infection in GERD patients, with a median rate of 35% [31]. Similarly, we detected *H. pylori* in 31.2% of the subjects with reflux. Moreover, 27.8% of the children were accompanied by esophagitis with reflux.

The role of *H. pylori* in reflux esophagitis or GERD has not been fully elucidated. Several studies show beneficial effects of *H. pylori* on acid reflux due to the alkalinization of gastric secretions by this bacterium [32-35]. *H. pylori* infection has also been considered a protective factor for GERD. In a Romanian study, however, gastric biopsies of 72 children diagnosed with GERD using 24-hour pH monitoring showed the presence of *H. pylori* in only 26.39% (n = 19) of the children. Interestingly, the severity of esophagitis was lower in the children with *H. pylori* infection [3]. In our study, in *H. pylori* positive patients, normal histology in 50%, chronic infection in 20%, chronic infection, and atrophy in 10%, and metaplasia in 20% were observed. The rate of metaplasia was higher in the children with *H. pylori* infection than in those negative for *H. pylori*.

In a study by Haqiwara et al., *H. pylori*-infected Mongolian gerbils given long-term PPI treatment showed significantly higher neutrophil and lymphocyte infiltration compared to those in the untreated infected gerbils. In addition, the PPI-treated gerbils had higher corpus atrophy scores and showed the development of adenocarcinoma. Therefore, patients receiving long-term PPI treatment should be screened for *H. pylori* infection and be treated with the appropriate antibiotics if tested positive [36]. There is some evidence correlating *H. pylori* eradication and chronic acid suppression in pre-cancerous atrophic gastritis [36, 37]. Mukaisho et al. examined the association between bile acids, pH, and *H. pylori* infection in GERD patients and found that corpus dominant gastritis often followed PPI treatment in these patients [38]. Therefore, PPI treatment should be prescribed only after serious consideration due to the serious consequences of treatment.

Conclusion

It is common to come across reflux-like symptoms among children but to diagnose GERD properly is difficult because the current diagnosis tests involve invasive procedures that are not recommended and not frequently used for children. Endoscopic procedures are not practical due to being invasive and expensive. However, hemogram is a simple test which can be performed in outpatient clinics by a single physician. MPV and RDW calculated in hemogram could be easy, cost-effective, and high sensitive new biomarkers that can be used for GERD, as used in other gastrointestinal system diseases such as liver cirrhosis and inflammatory bowel diseases.

Disclosure of conflict of interest

None.

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