Red Cell Distribution Width Can Predict Intestinal Atrophy in Selected Patients With Celiac Disease

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Background: Red cell distribution width (RDW) has been shown as a distinctive marker of mortality and morbidity in a wide spectrum of conditions related to systemic inflammation or deficiency of antioxidant nutrients. **Objective:** We aimed to investigate the predictive value of RDW in detection of intestinal atrophy in celiac disease (CD). **Methods:** Iron indices and RDW were studied in 49 patients with CD to evaluate the utilization of RDW as a predictive marker for presence of intestinal atrophy. **Results:** Sixty-nine percent of patients had iron deficiency at initial presentation and 89% had abnormal RDW defined as >14 . Receiver operating charac-

teristics curves of RDW has been found to be a predictive of intestinal atrophy at levels higher than 17.25 (68% sensitivity and 85% specificity). In patients with transglutaminase antibody IgA titers >200 U/L, RDW level >17.75 showed 76% sensitivity and 100% specificity for intestinal atrophy. **Conclusions:** We suggest that RDW can be used as a surrogate marker of atrophy in patients with iron deficiency and suspected CD. In addition, the sensitivity, specificity, negative and positive predictive values of RDW increases when used in combination with high levels of transglutaminase IgA antibody. J. Clin. Lab. Anal. 26:497–502, 2012. © 2012 Wiley Periodicals, Inc.

Key words: celiac disease; red cell distribution width; intestinal atrophy; prediction

INTRODUCTION

Celiac disease (CD) is a form of enteropathy affecting the small intestinal portion of gastrointestinal tract, in genetically predisposed children and adults, which is also precipitated by the ingestion of gluten-containing foods (1). Current diagnostic criteria for diagnosis of CD in adult population is defined as demonstration of intestinal histological changes combined with positive serological tests (2). The presence of serological positivity and CD-associated symptoms in the absence of histological changes represent a special patient group defined as latent CD. The clear-cut difference between these two groups of patients is important since costs related with endoscopy plus histological examination and possible avoidance of unnecessary interventions can be excluded. The sensitivity of endoscopic findings is low (3) and interobserver consistency and reproducibility of histological examinations obtained from duodenum are also highly variable (4, 5). In order to reduce the dependency for intestinal biopsy, recent studies have shown that either higher cut-off level for serology (6–8) or use of clinical definition tool plus serology can perform

better as a diagnostic markers for atrophy preventing up to 58.5% of unnecessary duodenal biopsies (9).

Red cell distribution width (RDW) is widely available, cheap and reproducible marker that measures red blood cell (RBC) volume variability. RDW is most commonly used to differentiate iron deficiency anemia (IDA) from thalassemia (where RDW is low in IDA compared to normal RDW in thalassemia). Most recently, RDW has been found as an independent risk factor for mortality in patients with cardiac failure (10, 11), coronary heart disease (12, 13), in hospitalized patients (14), in intensive care unit (15, 16), and even in general population (17) irrespective of anemia status. In addition, RDW is also associated with inflammatory bowel disease activity (18) and surrogate markers of systemic inflammation (19). The mechanisms

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that result in a significant relationship between RDW and mortality can be explained by disturbed systemic inflammatory status and reduced overall oxidation capacity of blood that result in loss of membrane integrity of RBCs and eventually increased RDW (20).

Previous studies have shown that at level higher than 14%, RDW can predict presence of CD (relationship with status of intestinal atrophy is not studied; (21) irrespective of anemia status (22) and dietary response in CD patients (23). Other prediction studies have investigated the potential role of serological markers and increased tissue transglutaminase antibodies (tTGA) were found helpful in prediction of CD at specific cut-off levels (24, 25). According to the previous knowledge in the field, the purpose of this study was to determine that levels of RDW as a commonly used iron indices, were predictive of atrophy in CD patients.

MATERIALS AND METHODS

Subjects

The clinical charts of patients with a newly diagnosed CD at University Medical Center between January 2005 and May 2011 were analyzed retrospectively. All of the patients with gluten sensitivity and CD patients already on a gluten-free diet, patients with inappropriate intestinal biopsy specimens showing disorientation of intestinal villi or patients with conditions that might influence RDW values (such as uncontrolled systemic inflammation, renal failure and recent malignancy) were excluded. As RDW is closely related with iron status, any patient without initial iron studies was also excluded. The presenting symptoms, reason of referral for CD screening and biopsy findings at time of initial diagnosis were noted.

Laboratory Studies

The blood studies were performed following an 8-hr overnight fasting period. All of the patients had full biochemical studies and standard complete automated blood counts at initial presentation. Hemoglobin (reference range: male: 13–15.5 g/dl, female: 12–14 g/dl), mean corpuscular volume (reference range: 80.4–95.9 fl) and RDW (reference range: 11.7–14.6 %) values are examined and noted. RDW is calculated by dividing the standard deviation (SD) by the mean corpuscle volume (MCV) and then multiplying that result by 100. The SD represents the volume of erythrocytes or RBCs that are in the blood smear. According to Beckman Coulter, the equation for the RDW calculation is $RDW = SD/MCV \times 100$. Also, serum iron parameters such as serum iron levels (reference range: male: 65–177 µg/dl, female: 50–170 µg/dl), total iron-binding capacity (reference range: 250–370 µg/dl)

and serum ferritin (reference range: 13–150 ng/ml) levels were studied in all of the patients at initial presentation before initiation of gluten-free diet. When available, the levels of folic acid (reference range: 3–17 ng/ml) and vitamin B12 (reference range: 200–860 pg/ml; studied in some cases for investigation of anemia at initial presentation) were also noted. The antiendomysial antibody and tTGA was studied by indirect immunofluorescence (Euroimmun Labordiagnostika AG, Luebeck, Germany) and microchip-ELISA (Euroimmun Labordiagnostika AG), respectively, as described by the manufacturer's recommendations.

Histological Examination

Intestinal specimens from subjects were obtained during the upper endoscopic procedure or upper enteroscopic examination. At least two specimens from first or second parts of duodenum with normal villi orientation was accepted as proper biopsies in all of the patients. The specimens were stained by hematoxylin-eosin and examined by experienced gastrointestinal pathologist. In order to test our hypothesis, the histological findings were classified according to Marsh-Oberhuber criteria (0: normal, 1: intraepithelial lymphocyte infiltration, 2: intraepithelial lymphocyte infiltration plus crypt hyperplasia, 3: villous atrophy) (26, 27) as group 1 (atrophy not present, representing Marsh 1 and 2 lesions) and group 2 (atrophy present, representing Marsh 3).

Statistical Analysis

All numerical continuous data in patient population and defined subgroups were first tested for normality by Shapiro–Wilk analysis and $P > 0.05$ indicated normal-Gaussian distribution. Mann–Whitney U -test was used for comparison of skewed variables between two groups and Wilcoxon signed-ranked test was used for comparison of continuous data. The differences between nominal and ordinal data were tested by χ^2 or Fisher's exact test. The correlations between continuous data were tested by Spearman correlation analysis. In order to evaluate the sensitivity and specificity for significant values detecting the presence of intestinal atrophy, receiver operating characteristics (ROC) curves were calculated for RDW and iron indices. Statistical analyses were performed by SPSS (Statistical Package for the Social Sciences) 15.0 and P values <0.05 were considered as significant.

RESULTS

A total of 49 patients with newly diagnosed CD (35 females, 14 males; median age 38 years, range 17–61) were included to this study. The presenting symptoms are

TABLE 1. Initial symptoms in patient population

Presenting symptom		Frequency	Percentage (%)
Initial presentation	IDA	16	32.7
IDA related	IDA + CD related symptom ^a	15	30.6
Initial presentation	CD related symptom ^a	18	36.7
not IDA related	Total	49	100.0

^aChronic diarrhea, colicky abdominal pain, weight loss, male-osteoporosis, premenopausal osteoporosis, and osteomalacia.

summarized in Table 1. Upon histological examination no atrophy was found in 14 subjects (group 1) and 35 subjects had atrophy (group 2). The serological diagnosis was made by either EMA and/or tTGA positivity. The serological tests have resulted in EMA positivity in 40 patients where tTGA was studied in 37 patients (5 had negative result, 5 had titers between 1 and 100 U/ml, 2 had titers between 100 and 200 U/ml, and 25 patients had titer >200 U/ml). There was no significant difference in distribution of serological tests between groups 1 and 2. In 25 patients who had tTGA titer >200 U/ml, the histological examination found no atrophy in 4 patients.

The comparison of both groups in terms of blood counts and laboratory parameters are shown in Table 2. It must be noted that although hemoglobin, MCV, and ferritin values are comparable, there are significant differences in serum iron, transferrin saturation and RDW values in favor of lower iron indices and greater RDW in group 2. The RDW was >14% in 41 patients (89%) with a comparable distribution between two groups at this cut-off level. There are more male patients in group 2 but odds ratio is not significant ($P = 0.130$, 95% CI: 0.015–1.114) when computed for atrophy. After continuation of gluten-free diet for at least 6 months, the control levels of hemoglobin, MCV, ferritin, and RDW showed statistically significant normalization ($P < 0.01$, control parameters available only in 36 patients).

We have also analyzed the ROC curves for RDW, serum iron, and transferrin saturation because they showed significant difference between two groups. Another ROC curve was created for 25 patients with tTGA titers >200 U/l to analyze a special population. ROC analysis revealed that RDW was the most significant indicator of atrophy in all patients (RDW value at level >17.25) and similar result was found in patients with high titers of tTGA (RDW value at level >17.75; see Figure 1 and Table 3).

DISCUSSION

This retrospective cohort study suggests that a simple and widely available test can be used as an adjunctive

Fig. 1. ROC curve of RDW ($P = 0.003$) in intestinal atrophy.

modality to predict the presence of atrophy in CD patients at a cut-off level of 17.25 and its power increases if combined with tTGA.

The study population was mostly derived from referred patients other outpatient clinics to our department with IDA and this potential bias reflected itself by more male patients in group 2. Another point of interest in patient selection is the completeness of iron studies in the inclusion criteria. However, in order to study a marker (RDW) directly related to iron indices, we inevitably formed such a selected group. Although this criteria might have resulted in selection bias, we believe this is inevitable because 50% of adult CD patients already present with IDA and it is one of the most common reasons of CD screening (28,29).

The search for a noninvasive diagnostic tool to predict the histological changes observed in CD is appealing due to increasing costs and medical risks related to endoscopy, histological examination, and anesthesia. For this purpose, previous studies found that setting cut-off level of tTGA titers to a higher level can be used as predictor of atrophy in both adult and pediatric populations (6,8,24,25). IDA in CD can be explained by malabsorption and occult blood loss that are parallel to degree of villous atrophy (30–32). Furthermore, IDA is observed in adults more than children and up to 50% of adult CD patients have been reported to have IDA (29). As a result of early iron deficiency and subsequent IDA, RDW increases because of variability of RBC populations. In addition to being a sensitive marker for iron deficiency, it has been suggested that RDW can also be used as a surrogate marker of systemic inflammation and it can serve as a

TABLE 2. Overall findings and comparison of groups 1 and 2

Variable	All cases (n = 49)	Group 1 (n = 14)	Group 2 (n = 35)	Comparison of Groups 1 and 2 (P value)
Age (years)	38 (17–61)	45.5 (17–57)	34 (17–61)	0.203
Sex (n; female/male)	35/14	13/1	22/13	0.03
Hb (g/dl)	11.8 (7.8–14.9)	11.5 (7.8–14.3)	11.9 (7.8–14.9)	0.595
MCV (fl)	79.1 (56.1–96.7)	80.4 (61.3–96.1)	77.5 (56.1–96.7)	0.388
Ferritin (ng/ml)	6.1 (0.9–299)	8.9 (1.8–299)	5.4 (1–191)	0.701
Serum iron (µg/dl)	31 (6–106)	48 (13–97)	29 (6–106)	0.03
Transferrin saturation (%)	8.9 (2–29.95)	16.3 (2.6–29)	7.1 (2–29)	0.01
Iron deficiency (n)	34	8	26	0.201
RDW level	17.6 (12–37)	15.3 (13.3–18.4)	18.1 (12–37)	0.003
Patients with RDW higher than 14%	41 (83.6%)	11 (78.5%)	30 (85.7%)	0.672
Vitamin B12 deficiency (n) (3 cases combined with iron deficiency)	7	2	5	0.626
Folic acid deficiency (n) (3 cases combined with iron deficiency)	5	0	5	0.174

Data are presented either as *median* (min–max results) or *number of cases* (n) when applicable.

marker of mortality in a wide spectrum of patient populations as described earlier. RDW has also been associated with anemia of chronic disease (33).

In relation to CD, previous studies have accepted RDW as abnormal when its level is higher than 14 (21, 22). However, 89% of our total patient population has RDW level higher than 14 with no difference in groups studied (with respect to number of patients with levels higher than 14). This is valuable since it suggests the homogenous distribution of groups studied (RDW levels >14, low ferritin and low hemoglobin in both groups).

We hypothesized that RDW, a cheap and widely available test, can predict the presence of villous atrophy in patients with suspected CD. This hypothesis was based on the fact that there is growing evidence about close relationship between RDW and systemic inflammation. The decreased oxidation capacity secondary to malabsorption of nutrients also contributes to this relationship. However, the heavy odds in use of RDW as a surrogate marker in CD are iron deficiency and IDA, which are frequently observed in adult CD patients. IDA was present in 63% of our study population and further analysis revealed that ferritin level, hemoglobin level, or presence of anemia (defined as hemoglobin <12g/dl in female and <13g/dl in male) in groups 1 and 2 was comparable while serum

iron and transferrin saturation were lower and RDW was higher in group 2. If we assume that degree of villous atrophy was the sole determinant of iron deficiency, then we should have observed lower ferritin and lower hemoglobin levels in group 2 in contrary to our findings. The presence of lower serum iron indices but comparable ferritin and hemoglobin levels in group-2 patients can be explained by predisposition to a higher level of systemic inflammation related to presence of atrophy in CD (a similar mechanism observed in anemia of chronic disease). This finding is also suggested by a previous study that showed similar frequencies of anemia in patient subgroups with varying degree of villous atrophy (34). The presence of systemic inflammatory response and anemia of chronic disease leading to increased ferritin levels in CD have been also observed (34, 35).

The tTG class antibodies have been showed as a marker of prediction of atrophy in CD (7, 8, 25, 36). The major concerns about tTG antibodies can be summarized as fluctuation of levels over time, loss of positivity, or reach to very high levels spontaneously (37, 38). In order to test the previous findings, we tested the RDW in patients with very high tTGA levels as a marker of prediction of atrophy. We found that if RDW level is combined with high tTGA titers, the prediction of atrophy reaches to sensitivity of

TABLE 3. Data of validity for factors tested in ROC curve analyses for prediction of mucosal atrophy

Factor tested	Significant level for diagnosis confirmation	Area curve (AUC)	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)	P value
RDW (n = 49)	17.25	0.771	68	85	52	92	0.003
Serum iron (µg/dl) (n = 49)	37.5 µg/dl	0.706	68	69	47	84	0.03
Transferrin saturation (%) (n = 49)	7.5 %	0.746	56	92	46	95	0.01
RDW in patients with tTGA titers >200 U/l (n = 25)	17.75	0.845	76	100	79	100	0.03

76%, specificity of 100%, negative predictive value of 79% and positive predictive value of 100%.

Therefore, we suggest that RDW can be used as a surrogate marker of atrophy in patients with iron deficiency and suspected CD. In addition, the sensitivity, specificity, negative, and positive predictive value of RDW increases when used in combination with high levels of tTGA.

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