Original Article

Mean platelet volume as short-term follow-up biomarker in children with celiac disease

Mohanaraj Ramachandran, Anurag Agarwal, R N Mandal Ravi, K Rajeshwari, Avinash Lomash, Seema Kapoor

From Department of Pediatrics, Maulana Azad Medical College and Associated Lok Nayak Hospital, University of Delhi, New Delhi, India

Correspondence to: Dr Anurag Agarwal, Associate Professor Deptt of pediatrics Maulana Azad Medical College Delhi. E-mail: doctoranuragagarwal@gmail.com

Received - 16 May 2017 Initial Review - 05 July 2017 Published Online - 10 September 2017

ABSTRACT

Objective: To assess the mean platelet volume (MPV) as a short-term follow-up biomarker in celiac disease (CD) and to compare it with anti-tissue transglutaminase antibody (TTGA) assay in Indian children. **Material and Methods:** Newly diagnosed 35 children aged <12 years who were positive for TTGA and further confirmed by intestinal biopsy with histological Grade 2 and 3 based on modified Marsh Classification were enrolled. TTGA, MPV, and clinical parameters were assessed at enrollment and after 3 months of gluten free diet (GFD). **Results:** Short stature (94.3%) and diarrhea (80%) were the most common presenting features. 33 (94.3%) children were found to have anemia. MPV reduced significantly from 9.28±1.88 fl to 8.55±1.10 fl after 3 months of GFD, ($p<0.001$). The mean TTG level reduced from 166.80±59.23 U/ml to 86.45±39.67 U/ml ($p<0.001$) after 3 months of GFD. **Conclusion:** MPV is one of the biomarkers that can be used to monitor dietary transgressions in CD in short term.

Key words: *Celiac disease, Gluten free diet, Mean platelet volume, Tissue transglutaminase antibody*

Celiac disease (CD) or gluten sensitive enteropathy is an immune-mediated disease in genetically susceptible individuals to gluten containing foods such as wheat, rye, barley, and oats. It is a known inflammatory disorder with a prevalence of 1-2% [1]. Intestinal biopsy is the ideal way to assess dietary compliance but is not feasible in most of the cases [2]. Search is on for an ideal biomarker, which is easily available and cost-effective. Tissue transglutaminase antibody (TTGA) and anti-endomysial antibody (EMA) have been used traditionally [3-5]. Limitations are high cost and lack of easy availability of TTGA as well as EMA in peripheral health centers. Besides in some cases with very high TTGA levels on initial diagnosis, there is delayed return to base line levels limiting its value as a biomarker for short-term follow-up.

Mean platelet volume (MPV) has recently been recognized as an inflammatory marker in various conditions including ulcerative colitis, acute pancreatitis, and myocardial infarction. Investigators have reported an association between increased MPV and disease severity [6-9]. The relationship between MPV and CD was first reported by O'Grady et al. [10]. Considering the inflammatory nature of the disease, we aimed to assess MPV as a biomarker for short-term follow-up as it is easily available from coulters placed even in primary care setups. We intended to compare it with traditional TTG assay in Indian children, for monitoring dietary compliance of gluten-free diet (GFD) in CD and to assess correlation between these two biomarkers.

MATERIAL AND METHODS

This observational study was conducted in a tertiary level hospital in North India from March 2013 to December 2014. Children aged <12 years who were diagnosed as CD on the basis of positive TTG and confirmed by intestinal biopsy based on modified Marsh classification were enrolled after informed parental consent and assent wherever applicable. The study was approved by the institutional review board. Children with pre-existing chronic pulmonary disease, ankylosing spondylitis, rheumatoid arthritis, ulcerative colitis, renal disease, cyanotic congenital heart disease, or acute or chronic infections, which could have altered the MPV, were excluded from the study [6-9].

Thirty-five consecutively presenting newly diagnosed children with CD were enrolled. Sample size was calculated based on a previous study demonstrating a mean difference of 0.4 fl in MPV before, and after GFD with alpha error of 5%, power of 80%, and with attrition of 20%, a total of 33 as a sample size was obtained [11]. Children were started on GFD and were followed up at 3 monthly intervals. The clinical parameters such as gain in weight and height, laboratory parameters such as TTG assay and MPV were measured at these time points. This period was selected based on a previous study at which changes were evident and attrition in the study group was unlikely.

MPV was measured at the time of enrollment using Sysmex automated cell counter by taking 1 ml of blood in ethylenediamine tetraacetic acid. Analysis was done within 2 h of blood sample

collection. The coulter principle is based on volumetric analysis. The cells in suspension pass through a small aperture between two chambers in which there is an electrical current. As each cell passes, it creates an impulse, which is considered to be proportional to the volume of the cell detected between the two electrodes. The normal reference range of MPV is 7.61 fl-10.19 fl with no significant differences for age [12]. TTG levels were done using DRG TTG-A enzyme-linked immunosorbent assay (ELISA) REF EIA-10503 kit. TTG antibody testing was done using sandwich ELISA (Euroimmune, Luebeck, Germany). The sensitivity of the assay was 0.26 U/ml. A simultaneous assay of the total IgA was done in all cases to exclude the possibility of missing a potential case with low IgA levels.

Patients with positive serology underwent 4-forked duodenal biopsy to confirm the diagnosis. A single observer did the histopathology grading on all the tissue specimens. Biopsy specimens comprising of at least four fragments with a forceps (open cup ~ 6 mm) were taken. Samples were carefully oriented on filter paper and fixed in 10% formalin. Histopathology was expressed according to the Marsh classification. A single pathologist who read all the biopsy specimens was blinded to the results obtained on serological testing. The study participants were followed up, and dietary adherence was indirectly assessed by the improvement in symptoms and gain in weight and height and observation of the diary maintained by them. Counseling was done at each visit to ensure compliance by a dedicated nutritionist.

All statistical tests were performed using the Statistical Package for Social Sciences Version 16. All data were given as mean values and standard deviation. Paired-t-test was used to analyze pre- and post-values for normal distribution and Wilcoxon sign rank test was used for non-normal distribution. Chi-square test was used for comparison of categorical variables. The $p<0.05$ was considered significant. Correlation coefficient was calculated to ascertain the relation between the two biomarkers.

RESULTS

Out of 35 children enrolled to the study, 32 completed the study. Six children were <3 years, 9 between 3 and 6 years, 11 between 6 and 9 years, and 9 between 9 and 12 years. 45% were males. Clinical presentation of the children is shown in Table 1.

33 (94.3%) children were anemic. Short stature was also found in 33 (94.3%) children. Weight of 27 (77.1%) children was less than −3SD for corresponding age and sex. Histologically, lesions of Marsh 3a was present in 1 child, whereas 3b in 20 (57.1%) children and 3c in 14 (40%) children.

The mean baseline MPV value at enrollment was 9.28±1.88 fL. Mean baseline MPV values for those cases had histopathology of Marsh 3c, 3b, and 3a are 9.03±0.99, 9.45±1.17, and 9.3±0.83, respectively. There was no significant difference in mean baseline MPV titers between different Marsh classes (p=0.56). MPV reduced significantly to 8.55±1.10 fl after GFD (p<0.001). Fig. 1 demonstrates the change in MPV at 3 months of institution of GFD. The mean TTG level of the total study population was 166.80±59.23 U/ml. There was statistically significant difference

Table 1: Clinical presentation of study population

Feature	Frequency (%)
Short stature	33 (94.3)
Diarrhea	28 (80.0)
Abdominal distension	15 (42.9)
Abdominal pain	15 (42.9)
Vomiting	5 (14.3)
Alopecia	1 (2.9)
Epilepsy	1 (2.9)

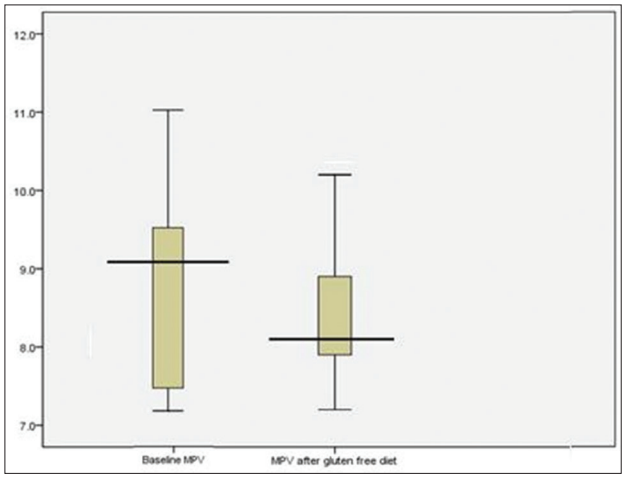

Figure 1: Box plot depicting baseline mean platelet volume (MPV) values and MPV values after gluten free diet

(p=0.047) in mean baseline titers of TTG levels between different Marsh classes. The mean TTG levels were 86.45±39.67 U/ml after 3 months of GFD (p<0.001).

Pre- and post-GFD values of MPV had a good correlation of r=0.75. Pre- and post-GFD values of TTG had a good correlation of r=0.50 (p=0.022). The correlation between the values of TTG and MPV both pre- and post-intervention did not correlate well with pre r=0.05 and post r=0.02.

DISCUSSION

This hospital-based observational study showed a statistically significant decline in MPV and TTGA in patients on GFD which could be used as a monitoring tool to assess dietary compliance in CD patients. The mean baseline MPV reduced significantly (9.28±1.88 fl-8.55±1.10 fl, (p<0.001) after 3 months of GFD. Purnak et al., in a similarly designed study, evaluated 60 newly diagnosed CD patients and 40 healthy controls and observed that in the compliant group, 3 months after initiation of GFD, a significant decrease in MPV from base-line values was seen, (8.56 fl-8.2 fl; p=0.008) [11]. The difference in MPV values could be due to difference in the type of counter used. In the same cohort of patients, after 3 months of GFD, the mean value of TTGA reduced significantly (p<0.01). The time period to complete normalization of TTG titers is longer and could be even up to 2 years. The fall in antibody titers along with clinical improvement suggested dietary compliance. This result was consistent with previous studies [13].

As MPV is a marker of inflammation it rapidly changes when inflammation subsides, whereas TTGA being an immune marker takes longer to reach baseline levels.

The mean TTG level of the study population (166.80±59.23 U/ml) was comparable with the study conducted by Rawal et al. (164.24 U/ml) [14]. Tursi et al. showed that the mean serum value of anti-TTG ranged from 3.6 U/ml in Marsh 1 lesions to 74.95 U/ml in Marsh 3c lesions, respectively; however, the values were not comparable owing to different cutoffs and different kits used in the study [15]. It was observed that the mean TTG levels also varied among different Marsh classes. The difference was statistically significant (p=0.047). This is supported by various previous studies depicting higher TTGA levels with a higher Marsh score [15]. The difference in TTGA values in different Marsh classes was explained by the severity of inflammation in different classes.

Correlation coefficient for MPV and TTG showed good significance individually but on comparison of both correlation coefficients did not correlate well. This could be due to smaller sample size as sample size was calculated to assess fall in MPV after 3 months GFD in CD. This could also be because fall in TTG occurs slowly over a span of 2 years in children on GFD. It is also plausible that the grade of inflammation may not correlate with the enterocyte loss. Follow-up of antibody titers has been proposed as a good indicator of transgressions in CD patients. However, there are some limitations in routine clinical practice with TTGA. Some patients may have extremely high base-line antibody titers on initial diagnosis, and a delayed return to normal levels in such cases may mislead clinicians.

MPV is one of the biomarkers that has been sparingly used to monitor CD. O'Grady et al. first reported the relationship between MPV and CD. They compared the MPV values of three groups (CD patients, normal controls, and splenectomised patients). The authors discovered that CD patients with intact spleens had higher MPV values and platelet counts [10]. This is understandable as platelet size is regulated at the level of megakaryocytes and is influenced by IL-3 and IL-6 levels. This leads to production of more reactive and large platelets [16]. However, these investigators did not evaluate the effect of a gluten-free diet on MPV.

The limitation of MPV would be its fallacy to detect adherence to GFD in the face of any cause for acute inflammation like a concomitant gastrointestinal illness or acute diarrhea. If this were to be excluded, this marker appears to be promising for short-term follow-up. However, initially it does not correlate well with grade of enterocyte loss; hence, can only be used for follow-up. Different analyzer devices might produce different MPV results leading to difficulty in the generalization of results. Hence, one needs to establish their own cutoffs in their setting. The study sample size was small and the follow-up in this study with MPV was done for only 3 months. Following these, children further could have helped us evaluate its utility in the longterm. Using a control group not on GFD would have substantiated our hypothesis further but was not ethically feasible.

CONCLUSION

MPV could be useful for short-term follow-up when compared to other available biomarkers due to its low cost, easy availability even in primary health centers and requirement of low blood volumes, a very important aspect in pediatric patients in following in these patients short term.

REFERENCES

1. Bhattacharya M, Dubey AP, Mathur NB. Prevalence of celiac disease in north Indian children. Indian Pediatr. 2009;46(5):415-7.
2. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: Slow and incomplete recovery. Am J Clin Pathol. 2002;118(3):459-63.
3. Liu E, Bao F, Barriga K, Miao D, Yu L, Erlich HA, et al. Fluctuating transglutaminase autoantibodies are related to histologic features of celiac disease. Clin Gastroenterol Hepatol. 2003;1(5):356-2.
4. Dipper CR, Maitra S, Thomas R, Lamb CA, McLean-Tooke AP, Ward R, et al. Anti-tissue transglutaminase antibodies in the follow-up of adult coeliac disease. Aliment Pharmacol Ther. 2009;30(4):236-44.
5. Vahedi K, Mascart F, Mary JY, Laberenne JE, Bouhnik Y, Morin MC, et al. Reliability of antitransglutaminase antibodies as predictors of gluten-free diet compliance in adult celiac disease. Am J Gastroenterol. 2003;98(5):1079-87.
6. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, et al. Mean platelet volume: A useful marker of inflammatory bowel disease activity. Am J Gastroenterol. 2001;96(3):776-81.
7. Yüksel O, Helvaci K, Basar O, Köklü S, Caner S, Helvaci N, et al. An overlooked indicator of disease activity in ulcerative colitis: Mean platelet volume. Platelets. 2009;20(4):277-81.
8. Danese S, Motte Cd Cde L, Fiocchi C. Platelets in inflammatory bowel disease: Clinical, pathogenic, and therapeutic implications. Am J Gastroenterol. 2004;99(5):938-45.
9. Kisacik B, Tufan A, Kalyoncu U, Karadag O, Akdogan A, Ozturk MA, et al. Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. Joint Bone Spine. 2008;75(3):291-4.
10. O'Grady JG, Harding B, Stevens FM, Egan EL, McCarthy CF. Influence of splenectomy and the functional hyposplenism of coeliac disease on platelet count and volume. Scand J Haematol. 1985;34(5):425-8.
11. Purnak T, Efe C, Yuksel O, Beyazit Y, Ozaslan E, Altiparmak E. Mean platelet volume could be a promising biomarker to monitor dietary compliance in celiac disease. Ups J Med Sci. 2011;116(3):208-11.
12. Lanzkowsky P. Manual of Pediatric Hematology and Oncology. 4th ed. California: Elsevier; 2005.
13. Bürgin-Wolff A, Dahlbom I, Hadziselimovic F, Petersson CJ. Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. Scand J Gastroenterol. 2002;37(6):685-91.
14. Rawal P, Thapa BR, Nain CK, Prasad KK, Singh K. Changing spectrum of celiac disease in India. Iran J Pediatr. 2010;20(4):459-65.
15. Tursi A, Brandimarte G, Giorgetti GM. Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease. J Clin Gastroenterol. 2003;36(3):219-1.
16. Kapoor A, Patwari AK, Kumar P, Jain A, Narayan S. Serum soluble interleukin-2 receptor, interleukin-6 and tumor necrosis factor alpha as markers of celiac disease activity. Indian J Pediatr. 2013;80(2):108-3.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Ramachandran M, Agarwal A, Ravi RNM, Rajeshwari K, Lomash A, Kapoor S. Mean platelet volume as short-term follow-up biomarker in children with celiac disease. Indian J Child Health. 2017; 4(4):515-517.