

The Circulating Midkine in the Newly Diagnosed Celiac Disease: Clinical Implications

Abstract

Background: Celiac disease (CeD) is a chronic inflammatory small intestine disorder caused by an abnormal immune response to an array of the epitopes of the wheat gluten and related proteins of rye and barley in genetically susceptible individuals. Midkine (MK) is an angiogenic cytokine, chemotactic in the direction of polymorphonuclear neutrophils and macrophages, and a T-regulatory cell suppressor. So far, a possible relationship with CeD has not yet been explored. Diagnosis of CeD is based on serologic test in a clinical setting suggestive of CeD and confirmatory histologic examination of the duodenal biopsy. Sometimes, genetic testing of human leukocyte antigen (HLA)-DQ2 and HLA-DQ8 may be needed. The objective of this study was to measure and compare the circulating MK in the celiac patients and healthy individuals. **Materials and Methods:** Twenty newly untreated CeD cases and 20 normal controls were enrolled in this study. The enzyme-linked immunosorbent assay was used to measure the circulating MK in the celiac patients and controls. **Results:** There was insignificant difference in the circulating MK between the patients and controls ($P > 0.05$). **Conclusions:** The study results suggest that the MK marker does not have any diagnostic value in CeD activity to be used at the time of diagnosis or during follow-ups.

Keywords: Celiac disease, enzyme-linked immunosorbent assay, inflammation, midkine, serum, tissue transglutaminase

Introduction

Celiac disease (CeD) is an autoimmune disease initiated by gluten, a protein available in the wheat and some other grains in genetically predisposed individuals. As we know more than 99.5% of CeD cases have either human leukocyte antigen (HLA)-DQ2 or HLA-DQ8. It influences the upper part of the small intestine and initiates with an extra immune response to gluten. The inflammatory damage of the small intestine mucosa, occurring in the celiac patients after the ingestion of the wheat gluten or related rye and barley proteins, induces flattening of intestinal epithelial layer, resulting in an ineffective uptake of nutrients. CeD is treated with a gluten-free diet (GFD). It influences 0.6%–1% of the world's population, which differs widely throughout the world, and mostly related to the prevalence of HLA-DQ2 or HLA-DQ8 in the region. For instance, its prevalence rate in the Europe and the Mediterranean region is around 1%.^[1,2]

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CeD is common in the developing countries, especially in the North Africa and the Middle East, but is estimated to be about 1% in Iran. According to the unpublished observations by Emami MH *et al.*, the estimated prevalence rate of CeD in Isfahan is 0.8%.^[3,4] In some patients, large parts of the small intestine and rarely large bowel may also develop inflammation.^[1,5] Extensive mucosal lesions including changes in the structure of villi, crypt hyperplasia, infiltration of the lamina propria by inflammatory cells (lymphocytes, macrophages, and eosinophils), and an increase in the number of lymphocytes in the epithelial layer are seen in CeD.^[1,6]

The symptoms of CeD include diarrhoea, growth disorders, abdominal distension, anorexia, and growth retardation. These symptoms may have obviously lethal consequences. In <50% of adult patients in the United States, patients complain of diarrhoea and intestinal problems.^[1,7] Osteoporosis may also occur in these patients, even in patients with few mucosal changes.^[8] Dental enamel defect

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and rapid destruction of dentures are seen. In patients who have not been treated, the fertility may be compromised and even are prone to recurrent abortions. In a recent study in the United States, fatigue and joint pain were observed in most patients, and neurological symptoms including peripheral neuropathy, epilepsy, and motor imbalance (ataxia) were observed. CeD may be associated with other autoimmune diseases such as type 1 diabetes, thyroiditis, Addison's disease, and some other autoimmune diseases.^[1,9]

Anti-tissue transglutaminase (tTG) antibodies (immunoglobulin A [IgA] and IgG) are usually the first serologic tests recommended for suspicious cases. Upper gastroenterology with D1 and D2 biopsy is done to confirm the diagnosis. tTG (also called transglutaminase type 2, TG2) is a calcium-dependent ever-present enzyme which catalyses the post-translational modification of proteins and is released from the cells during inflammation. tTG is suggested to have, at least two critical roles in CeD: As a deamination enzyme, which augments the immunostimulatory effect of gluten, and as a target autoantigen in the immune response.^[1,5-8,10]

Midkine (MK) or neurite growth-promoting factor 2, along with pleiotrophin (PTN, also known as heparin-binding brain mitogen), creates a separate family of the heparin-binding growth factors. It is a multifunctional cytokine due to upholding the tissue infiltration with pro-inflammatory cells; participation in chronic inflammation and angiogenesis, reinforced further by its mitogenic and differentiating properties. MK expresses transiently during the foetal development, and it is upregulated during the tissue repair and remodelling and in several pathologic conditions in the adult organism. Furthermore, MK causes infiltration of the pro-inflammatory cells, and elevation of circulating MK has been observed in the inflammatory conditions. Thus, it has been proposed as a marker in the diagnosis of inflammatory diseases or follow-ups of treatment. In adults, MK increases in the damaged and under reconstruction tissues. MK helps the leukocytes migration and induction of pro-inflammatory cytokines, and thus helps the inflammation process suppression of T-cells.^[11] Thus, MK can be used as a disease marker. MK inhibitors are expected to be used for the treatment of cancer, rheumatoid arthritis (RA), multiple sclerosis (MS), kidney disease, and high blood pressure after surgery.^[2,5,8,12,13]

It is noteworthy to know that in Crohn's disease, MK's association with disease activity has been seen as a good marker. Since CeD is an inflammatory disease of the small intestine and sometimes the large intestine, presently, the markers for monitoring the activity of CeD are limited. The diagnosis of disease activity in mild cases is usually dependent on endoscopy with biopsy and anti-tTG serology.^[14] Given the importance of MK diagnosis in the inflammatory and malignant diseases and especially inflammatory bowel diseases (Crohn's disease and ulcerative

colitis), MS and RA and the importance of this cytokine in inflammation, it can be concluded that MK may also be a good marker for CeD patients. However, its possible relationship with CeD has not yet been explored.^[2,5,8,12,13]

Thus, the objective of this study was to measure the circulating MK in the celiac patients and controls to find the possible relationship of MK with CeD and CeD activity.

Materials and Methods

Patients

In this case-control study, 20 confirmed CeD patients were identified by the Iranian Celiac Association Clinic at the Poursina Hakim Digestive Diseases Research Centre. This study was carried out from November 2018 to December 2019. CeD was diagnosed, based on the criteria of the American College of Gastroenterology (ACG), the presence of the clinical presentation, elevated CeD-related antibody-namely, tTG-IgA and at least, Marsh II or more of the histopathological classification.

The inclusion criteria

Newly diagnosed adult patients fulfilling definite criteria for CeD as approved by the gastrointestinal (GI) specialist of the CeD Clinic, based on the criteria of the ACG. In addition, they were not yet on GFD and were willing to enrol in this study by signing the informed written consent.

The exclusion criteria

Patients with the allergic disease, infection conditions, inflammatory bowel disease (IBD), previous gastrointestinal surgery or other GI or immune diseases, regular use of non-steroidal anti-inflammatory drugs (NSAIDs) (at least, one regular dose of any NSAIDs per week over the last 4 weeks), immunodeficiency syndrome, cancer, allograft transplantation and those, who were reluctant to donate the blood sample and undergo endoscopy.

Controls

There were 20 controls used to suffer from the symptoms of dyspepsia/heartburn without diarrhoea, and whom underwent upper GI endoscopy and duodenal biopsy showing normal histology. They had also negative serum tTG (IgA and IgG) assays. None of the controls had a family member or relative with the celiac patients. Furthermore, none of the controls had any malignant, autoimmune or allergic diseases. Five millilitres of the venous blood were taken from each individual after signing written consent and willingness to participate in this study; sera were separated and retained at -20°C for further assay

Enzyme-linked immunosorbent assay

Biovendor Human MK enzyme-linked immunosorbent assay (ELISA) (R&D) was used to measure circulating MK according to its protocol. Then, the standard curve was drawn using the results obtained [Figure 1].

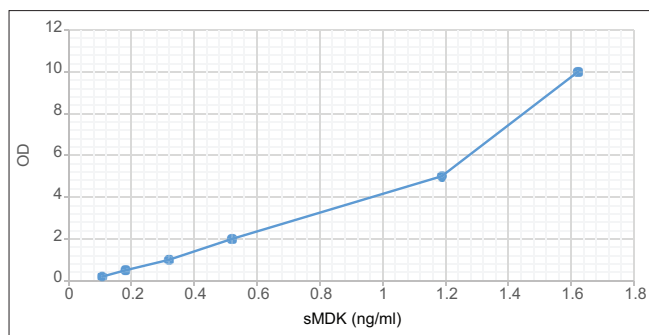


Figure 1: The standard curve of the circulating midkine

Statistical analysis

Statistical analysis was carried out using the SPSS software version 19 statistical package (SPSS Inc., Chicago, IL, USA). Data were expressed as mean values \pm standard deviation of the mean, and statistical comparisons are made using the independent-samples *t*-test. The Chi-square test of independence (also known as the Pearson Chi-square test) was used to show the difference between two groups concerning the gender distribution. Similarly, the Kolmogorov–Smirnov normality test was used to normalize the data.

Ethical standards

This study was carried out in accordance with the ethical guidelines of the 1975 declaration of Helsinki as reflected in a prior approval by the Institution's Human Research Committee. Accordingly, this study ethically approved by the Isfahan University of Medical Sciences and the Iranian Celiac Association, Ethical Committee for Clinical Research, and the participants gave their written informed consent once the researchers explained to them the objective and protocol of the study.

Results

Patients and controls

Twenty untreated celiac patients (8 males, 12 females, the mean age 36.1 years,) and 20 controls (5 males, 15 females, and mean age 33.4 years) were studied [Tables 1 and 2, respectively]. The Chi-square test showed that there was insignificant difference between two groups concerning the gender distribution [Table 1, $P = 0.31$]. These patients participated in the active phase before having any treatment or GFD regimen (positive serum tTG-IgA [Table 2], and Marsh III classification identified by a flat mucosa), along with 20 controls.

Measurement of the circulating midkine in the celiac patients and controls

Based on the standard curve [Figure 1] and optical absorption spectra of each celiac patients and controls, obtained by ELISA Reader (Stat Fax 303 Plus), the means levels of MK

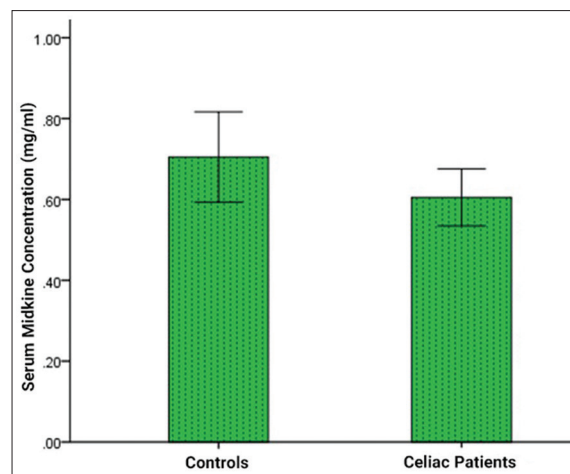


Figure 2: The circulating midkine in the controls and celiac patients. There was insignificant difference in the circulating midkine of the two groups ($P = 0.454$)

Table 1: The gender distribution in the controls and celiac patients

Gender	Controls, n (%)	Celiac patients, n (%)
Male	5 (25)	8 (40)
Female	15 (75)	12 (60)
Total	20 (100)	20 (100)

Table 2: Baseline demographic and results of the serological assay in the controls and celiac patients

Variable	Mean \pm SD		<i>P</i>
	Controls	Celiac patients	
tTG-IgA (U/ml)	0.7 \pm 0.09	87.1 \pm 18.8	0.001
Age (years)	33.4 \pm 9.8	36.1 \pm 12.8	0.45

tTG-IgA: Tissue-type transglutaminase immunoglobulin A, SD: Standard deviation

were determined. The study results showed that the means serum levels of MK in the controls and celiac patients were 0.7 and 0.6 ng/ml, respectively [Figure 2]. This finding showed that the circulating MK in the celiac patients was insignificant from that of the controls ($P > 0.05$), which was obtained by independent *t*-test (0.445).

Discussion

This study aimed to measure the circulating MK in the celiac patients and controls to find the possible relationship of MK with CeD and CeD activity. To the best of our knowledge, this is the first report concerning MK in CeD.

CeD is an intestinal autoimmune disorder with the genetic background activated by gluten in the diet. In the gastrointestinal tract, the small intestine villi are damaged, causing mild-to-severe malabsorption of either micro or macronutrients with a variety of presentations.^[1,2] Symptoms of CeD include diarrhoea, abdominal distension, anorexia, growth retardation, and several autoimmune

diseases. These symptoms and long-term complications may have obviously lethal or disabling consequences.^[1,6] MK, along with PTN, forms a cytokine linked to heparin, which causes inflammatory infiltration and increases in the serum levels in inflammatory diseases.^[11] MK participates in inflammatory reactions due to the migration of leukocytes and induction of cytokines and suppression of T-cells. Increasing the incidence of inflammatory diseases has been observed, and the use of MK as a marker of disease has been proposed. MK inhibitors are expected to be effective in the treatment of cancer, RA, MS, kidney disease, and high blood pressure after surgery. It also helps angiogenesis.^[15]

The study results showed that there was insignificant difference between the circulating MK in the newly diagnosed CeD cases and the controls ($P = 0.454$). However, Krzystek–Korpaczka *et al.* have shown that the circulating MK increases in Crohn's disease ($P < 0.05$), which is well consistent with the disease activity indicating the intensity of the inflammatory response and the intensification of pathologic angiogenesis. In Crohn's disease, MK's function is better than C-reactive protein as an inflammatory marker.^[16,17] Similarly, in ulcerative colitis, an increase in the circulating MK is consistent with the clinical disease activity as well as endoscopic and angiogenesis activity.^[17]

In a study done on patients with RA, MK levels both in the serum and synovial fluid were higher in the patients than the controls. Thus, these findings suggest that MK may play a role in the pathogenesis of RA.

In MS patients, MK decreased the Treg cell population by suppressing the phosphorylation of STAT5, which is essential for the expression of Foxp3, the master transcriptional factor of Treg cell differentiation. In an animal model of MS (experimental autoimmune encephalomyelitis, EAE), MK has worsened the EAE, through a decrease in the number of regulatory T-cells (CD4⁺CD25⁺Foxp3). The regulatory T-cells regulate the incidence of the autoimmune responses.^[13] Furthermore, MK decreases the DCreg cell population by inhibiting the phosphorylation of STAT3, which is crucial for DCreg development. Blockade of MK signalling by a specific RNA aptamer significantly raised the population of DCreg and Treg cells and ameliorated EAE without obvious adverse effects. Furthermore, in patients with cancer, MK inhibition also causes tumour suppression, and the MK level has increased remarkably, compared to the normal tissue. In fact, the MK produced in inflammatory conditions inhibits the development of tolerogenic dendritic cells, and these cells propagate the evolution of the regulatory T-cells.^[13,17-19]

It is noteworthy to stress that the development and function of regulatory T-cells is closely related to dendritic cells that play an important role in the activation and

reactivation of encephalitogenic cells in the central nervous system. Dendritic and regulatory T-cells have a close two-way relationship, and in conjunction with other factors and cells, certain dendritic cells have the ability to induce the regulatory T-cells. In other words, CD4⁺CD25⁺FoxP3⁺regulatory T-cell (Treg) plays a pivotal role in autoimmune tolerance, and tolerogenic dendritic cells (DCreg) drive the development of inducible Treg cells.^[13,17-19]

Study limitations and strengths

The limitations of this study were limited numbers of the newly diagnosed celiac patients who were eligible to enrol in this study. Nevertheless, the first strength point of this study is the novelty of measurement of the circulating MK as an inflammatory marker in the celiac patients, a disease with inflammatory nature.

Conclusions

Collectively, the study results showed that there was insignificant difference between the circulating MK in the celiac patients and controls. Thus, the utility of MK as an inflammatory marker is not suggested in CeD at the time of diagnosis or follow-ups.

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Conflicts of interest

There are no conflicts of interest.

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