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Zinc Deficiency is Associated with Meprin a in Iraqi Patients with Crohn's Disease

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Abstract

Zinc deficiency in Crohn's disease (CD)is considered a frequent finding and may exacerbate CD activity. We aimed to assess the prevalence of zinc deficiency in CD patients in clinical remission, its association with meprin and to analyze a potential impact on future disease course. Proper history with blood samples were collected from (30) healthy control group, (30) Crohn's disease patients have been respond to biological therapy (infliximab IFX) (response group) and (30) CD patients with (non-response group) to biological therapy undergoing surgical intervention for the estimation of zinc concentration and meprin activity. This study demonstrate a significant decrease in both zinc and meprin levels between (non- response group) and control group ($p< 0.01$). Similarly , zinc and meprin levels were decreased significantly ($p< 0.01$) in (non- response) group as compared with re sponse group. While there were no differences between control and CD patients that have been treated with infliximab only. Meprin α is a zinc metalloproteinase, and therefore a deficiency of zinc may result in a decrease of meprin level or activity. Thus, we should maintain the balance between the meprin α that is affected by the zinc concentration then may affect the Crohn disease.

Keyword: *Zinc, Crohn's disease, Infliximab, meprin α*

Introduction

Crohn*'s disease* CD is a chronic inflammatory disorder that could [1] involve any part of alimentary tract from mouth to anus. These disorders were first described by Dr. Burril Crohn's and his team in [2]1932. Although its aetiopathogenesis is still not clear, it has been well recognized that CD is one of the complicated disorders which result from [3]interaction of environmental, microbial, and genetic factors . Zinc is an essential trace element, which is absorbed in the small intestine and serves as a cofactor for numerous enzymes involved in growth, immune [4] function, and tissue repair . Zinc is a micronutrient, which has been linked to inflammatory diseases such as IBD,zinc levels are often low in patients with chronic diarrhea or malabsorptive disorders. Similarly, zinc deficiency (ZD) appears to compromise gastrointestinal barrier function, which can perpetuate different diseases such as celiac disease, chronic diarrhea or IBD, is common during disease and in remission, with a prevalence ranging from 15% to [5] 40%. Pre-clinical data as well as human studies support that zinc deficiency may contribute to mucosal inflammation in patients with IBD. In animal models, zinc deficiency exacerbates colitis and potentiates production of pro-inflammatory cytokines, including tumor necrosis [6, 7] factor α (TNFα). Furthermore, previous work indicates that a low zinc diet in healthy volunteers results in a decrease in the Th1, cytokines, IFN-γ and IL-2, as well as diminished lytic activity of natural killer cells[8]. In addition to the impact of zinc on immune function, studies involving both animal models of colitis and Crohn's disease (CD) patients have demonstrated improvement in mucosal permeability with [8,9] zinc supplementation. zinc plays a crucial role in the development [10] and function of cells mediating innate immunity has direct

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anti- inflammatory effects via zinc-finger protein, and has a positive effect on intestinal tight junctions and intestinal repair. Furthermore, zinc can act via metallothioneins (MTs). MTs are amfamily of small proteins with a high cysteine content at conserved positions that are rapidly up regulated in response to an [11] inflammatory stimulus such as tumor necrosis factor (TNF). [12] MT function seems to be dependent upon the presence of zinc. Effects of MTs include reduction of apoptosis and antimicrobial [(13,14)] activity. Zinc also is important for early and late autophagy Autophagy is thought to suppress inflammation via degradation of [15] inflammasomes and inflammasome-agonists . Zinc deficiency is common in CD, with up to one third of all patients presenting with [(16,17)] low serum zinc levels, even in patients in clinical remission. ZD may exacerbate CD by increasing mucosal permeability, leading to [18] neutrophil transmigration and luminal antigen permeation , such increased mucosal permeability has been shown to correlate with [(19,20)] both, CD activity and relapse probability . Despite highprevalence of ZD in IBD and its links to inflammation, so far nostudy investigated the role of serum zinc as a potential predictiveserum marker for future disease course and its potential causative role in patients with a low or absent inflammatory disease activity. Low–normal zinc values were defined as below the 30th percentile of the normal range. Meprin expression in the intestinal tract ishighest in the ileum and large intestine where host andmicroorganisms are in contact, and where intestinal inflammatory diseases develop. Meprins are zinc metalloproteinases that are highly expressed in the epithelial cells of the human and mouse intestine, and are found membrane bound and/or secreted into the [21] lumen of the intestine . In addition to the abundant expression in the epithelium, meprin alpha is expressed in human intestinal lamina propria leukocytes and in mouse mesenteric lymph nodes [22] both in the presence and absence of intestinal inflammation

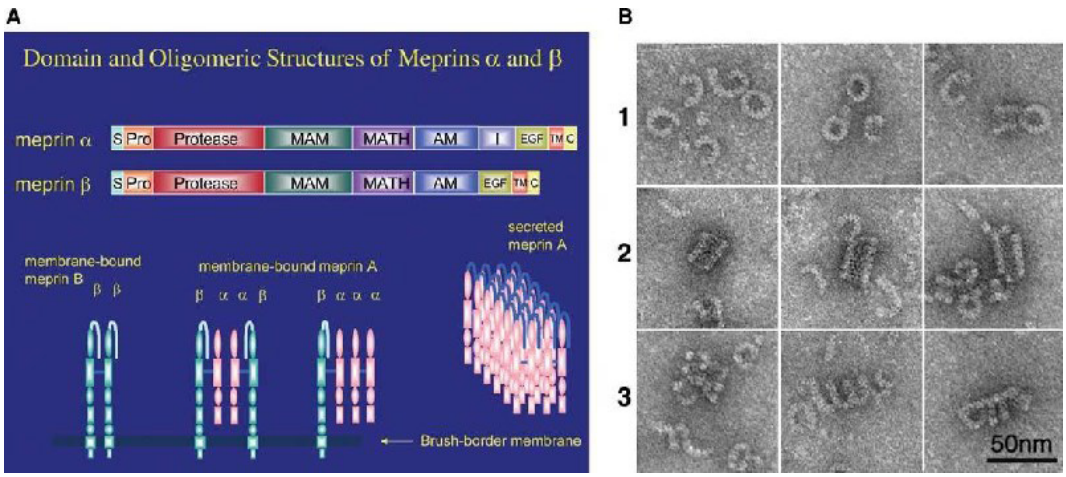

Fig. 1. (A) Domain and oligomeric structure of meprins a and b. Domains are S, signal sequence; Pro, prosequence; Protease, catalytic domain; MAM, meprin, A5 protein, protein tyrosine phosphatase l; MATH, meprin and TRAF homology; AM, after MATH; I, inserted domain; EGF, epidermal growth factor-like, TM, transmembrane-spanning; C, cytoplasmic domain. The MATH and AM domains are now designated the TRAF domain .

(B) Electron micrograph of various forms of secreted, latent homooligomeric meprin A demonstrating high molecular masscomplexes. Row 1, closed rings and crescents (containing about 10 to12 meprin a subunits); rows 2 and 3 show tubes and spirals containing up to 100 subunits.

Inflammatory bowel diseases (IBDs) are a collection of clinically heterogeneous intestinal diseases that result in damage to intestinal [27] epithelium and affect million people in the USA

Material and Method

Study subject:

This research has been approved by the Ethics committee, department of chemistry, college of

science , Mustansiriyah University , Bagdad , Iraq. and the Iraqi Ministry of Health approved this work as well. The blood samples were taken after informed consent of participant were recruited from Gastroenterology and Hepatology teaching hospital at Bagdad Medical city, while the healthy group were volunteers. All the patients were diagnosed by senior doctors specialist in gastroenterology field, (60) sixty unrelated Iraqi Crohn's disease patient's divided in to two groups according to response to biological therapy (infliximab) the first group (30) patients was respond to infliximab according to classical regimen (loading dose 5mg/kg at week 0,2,and 6 followed by repeated infusion of 5mg/kg every 8 weeks) and (30) patients not respond to infliximab undergoing surgical intervention as well as (30) unrelated healthy person termed as control group without any systemic disease. All the patients and control aged between 18 and 64 years. Five milliliters of venous blood was obtained from patients and control group by 5 ml disposable syringe (without tourniquet) drained into get plain tubes and left in room temperature (25C°) for 15 minutes, Then it was centrifuged at 2000 xg for 10 minutes in order to collect sera. Sera aliquots were placed in eppendorf tubes and stored at -40C° until used.

Biochemical analysis

The human Serum MEP1A(Meprin A subunit alpha were using ELISA Kit obtained from Mybiosource using the sandwich enzyme linked immune sorbent (ELISA) assay technology method according to manufactures instruction (Cat No. MBS 765586, My bio source / USA). While Zinc Serum concentration determined by Atomic Absorption /flame spectrophotometer(AA 680G) (SHIMADZU, Japan).

Statistical analysis [28]

The statistical analysis system SAS program has been utilized to compare between control and two CD patients groups (response and non- response to biological therapy) in study parameters. (Analysis of variation- ANOVA) was used to compare between means (P value of 0.05 and 0.01 has been considered to be statistically significant).

Result and Discussion

Mean ± SD value of zinc and meprin α were recorded from all subscribers as shown in table 1. Results of this study shown a significant decrease in both zinc and meprin levels between (non- response group) and control group ($p< 0.01$). Similarly , zinc and meprin levels were decreased significantly ($p< 0.01$) in (non- response) group as compared with response group as shown in table (2) .While there were no differences between control and CD patients that have been treated with infliximab only. According to the currently accepted hypothesis, both UC and CD result from a dysregulated response of the intestinal immune system to antigens of microbial origin or pathogenic bacteria in genetically predisposed individuals. MEP1A has been identified as a genetic [23,25] susceptibility factor for IBD . It encodes meprin α, a metalloprotease highly expressed in the intestine. Meprin a is secreted into the intestinal lumen or accumulates at the apical brush border membrane of polarized epithelial cells retained by meprin β. Thus any decrease in meprin α or β expression can lead to similar defects in the host. In this study we determine the levels of serum meprin α in Iraqi patients with Crohn Disease which include two main groups (respond and non respond to biological thearpy), as well as the correlation between zinc with serum meprin α in these groups. The results showed strongly significant association between meprin α and zinc.

A total of 60 patients with Crohn's disease (CD), (30) patients treated with infliximab and (30) with surgical treatment were included in the analysis. zinc deficiency was associated with an increased risk of surgeries in patients with CD Normalization of zinc was associated with improvement in these outcomes in patients with both CD. Meprin is involved in inflammation by the release and maturation of cytokines [26,29] and proteoglycans, it induce extracellular matrix assembly and fibrosis, and enhance cancer progression through trans-activation of [25] EGF receptors, which is reflected by defined cleavage specificity [30] and structural features unique among all proteases. Meprin α is shed by furin during the secretory pathway and secreted into extracellular space . Interestingly, this show that meprin α tends to oligomerize to huge complexes up to the mega Dalton range, which [26] makes it the largest extracellular protease (See Fig. 1). These fascinating ring and chain like structures can easily be visualized by transmission electron microscopy (TEM), but structure-function relationships are still ambiguous, meprin α was found to b differentially expressed in the small and large intestine, leucocytes, [31] and several tumors. In normal dermal

skin, meprin α is higher expressed than meprin β, and are highly up-regulated in keloid tissue [32]

Our results and those of others have led to the hypothesis that meprin α play a role in the pathogenesis of IBDs. Data indicate that meprin α influence CD by affecting intestinal leukocyte dissemination to inflammatory sites in the gut, by interacting with bacteria at the epithelial surface, by degradation of compounds such as defenses that kill bacteria, or by exacerbating host tissue damage in the inflamed gut. Previous studies had demonstrated high expression of meprin subunits in leukocytes of [22] the lamina propria of human inflammatory sites. This observation, plus the known ability of Meprin α to hydrolyze extracellular matrix proteins, led to the speculation that Meprin α play a role in the movement of macrophages to inflammatory sites. Our study has several strengths and some limitations. We provide the first study evaluating an association of serum zinc levels and meprin in CD patients.

Table 1 : Statistical analysis of meprin alpha and zinc concentration distributed among patients and control groups. 95% C.I. for

Parameter	Group	Mean±SD	SE	Mean		Min.	Max.
				L.b.	U.b.		
Zinc µg/dL	A	3.910±2.551	0.473	2.939	4.881	0.489	8.63
	B	3.275±1.492	0.272	2.717	3.832	0.418	6.72
	C	1.117±1.571	0.720	0.286	0.531	1.704	5.51
Meprin ng/ml	A	83.306±16.334	3.033	77.093	89.519	53.70	114.30
	B	87.940±12.214	2.230	83.379	92.501	71.60	116.40
	C	50.058±7.622	1.391	47.212	52.904	36.90	66.61

Table 2: Multiple comparison significant (ANOVA) for parameter among the different groups.

Groups	Zinc µg/dL	Me ng/ml
	P- Value	
A & C	0.0011 **	0.0011 **
B & C	0.0010 **	0.0012 **
A & B	NS	NS

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines.

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