Association between serum zonulin level and severity of house dust mite allergic asthma

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Abstract

Background: Increased intestinal permeability, either due to the exposure to antigens in asthmatic patients or due to a barrier defect, play a critical role in susceptibility to environmental allergens. House dust mites allergy occurs more commonly than any other allergens among Egyptian asthmatic patients. Aim: To assess the relation between serum zonulin level as a marker of increased intestinal permeability and the severity of house dust mites allergic asthma. Methods: A case control study which included 96 subjects attending the allergy and immunology unit, microbiology and immunology department, Faculty of medicine, Zagazig University. They were divided into 48 house dust mites allergic asthma and 48 healthy control subjects. Results: On comparing the 2 studied groups, there was a statistically significant difference between the 2 groups concerning serum IgE and serum zonulin levels (p=0.000, 0.000 respectively)The mean serum zonulin was equal to 258.3±153.01 ng/ml in the asthmatic group and 80±13 ng/ml in the control group. Serum zonulin level significantly increased with the increase of asthma severity (p<0.001). The cut off value of serum zonulin was [?] 198 ng/ml, and the area under the curve was 0.76. It displayed sensitivity equal to 80% and specificity equal to 71.4%. Its negative predictive value was equal to 83.3%. Conclusion: Intestinal barrier dysfunction contributes in the pathogenesis of allergic asthma. Serum zonulin level reflects an increase in intestinal permeability and acts as prognostic factor of severity in Asthma. Correction of the gut barrier defect may be an additional novel approach for Asthma.

Association between serum zonulin level and severity of house dust mite allergic asthma

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Running Title: Zonulin- Intestinal permeability marker - is a predictor of Asthma severity

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Abstract

Background:

Increased intestinal permeability, either due to the exposure to antigens in asthmatic patients or due to a barrier defect, play a critical role in susceptibility to environmental allergens. House dust mites allergy occurs more commonly than any other allergens among Egyptian asthmatic patients.

Aim:

To assess the relation between serum zonulin level as a marker of increased intestinal permeability and the severity of house dust mites allergic asthma.

Methods:

A case control study which included 48 house dust mites allergic asthma and 48 healthy control subjects attending the allergy and immunology unit, microbiology and immunology department, Faculty of medicine, Zagazig University.

Results:

On comparing the 2 studied groups, there was a statistically significant difference between the 2 groups concerning serum IgE and serum zonulin levels (p=0.000, 0.000 respectively)The mean serum zonulin was equal to 258.3±153.01 ng/ml in the asthmatic group and 80±13 ng/ml in the control group. Serum zonulin level significantly increased with the increase of asthma severity (p<0.001). The cut off value of serum zonulin was [?] 198 ng/ml, and the area under the curve was 0.76. It displayed sensitivity equal to 80% and specificity equal to 71.4%. Its negative predictive value was equal to 83.3%.

Conclusion:

Intestinal barrier dysfunction contributes in the pathogenesis of allergic asthma. Serum zonulin level reflects an increase in intestinal permeability and acts as prognostic factor of severity in Asthma. Correction of the gut barrier defect may be an additional novel approach for Asthma.

Keywords:

Asthma; Zonulin; Asthma grade; Severity; House dust mites; Intestinal Barrier.

Introduction

Asthma is a common chronic airway disease characterized by airway inflammation, hyperresponsiveness, and variable airway obstruction, which is often attributed to gene-environment interactions [1].

Epidemiologic studies have shown that sensitization to indoor allergens is an important risk factor for the occurrence of acute attacks of asthma[2].

The most prevalent indoor allergens include house dust mites (HDMs), animal dander, moulds and cockroaches. Of these, HDMs, especially D. pteronyssinus and D. farinae, are considered the major perennial indoor allergen sources inducing allergic sensitization worldwide [3].

Environmental factors, microbiome, epithelial cells and immune cells show a dynamic cross talk at the skin and mucosal barriers in the development of atopic dermatitis, allergic rhinitis, eosinophilic esophagitis and asthma.[4]

Recent trends in targeting allergic disorders are directed towards personalized medicine approach which necessitates novel developments in the area of disease phenotyping , endotyping, and the development and application of reliable biomarkers.[4]

Increased intestinal permeability due to the exposure to antigens in asthmatic patients may play a role in susceptibility to environmental allergens. Therefore, correction of the gut barrier defect may be an additional novel approach for asthma treatment.[34]

Gut mucosal barrier depends on efficient tight junctions which are regulated by over 50 proteins. Zonulin is a 47 kDa protein that increases the permeability of the small intestine. It has a role in the intestinal mucosal innate immunity and it is the only physiological protein proven to reversibly modulate intestinal permeability. High serum levels of zonulin have been found in several autoimmune diseases, e.g., celiac disease as a marker of impaired intestinal barrier.[5]Chronic inflammation develops in response to environmental stimuli, it leads to up-regulated expression of zonulin and serum zonulin was found to be significantly associated with presence and severity of atopic dermatitis.[6]

Patients and methods:

This is a case control study which included 96 subjects attending the allergy and immunology unit, microbiology and immunology department, Faculty of medicine, Zagazig University.

Ethical approval:

The study was approved by the institutional review board (IRB) no #6856/14-12-2020, Faculty of medicine, Zagazig University. An informed written consent was obtained from all parents at time of recruitment. This study was conducted in accordance with the Declaration of Helsinki.

Sample size:

Assuming that serum zonulin level in case group versus control group is (11.3 ± 3.7 ng/ml) and (9.3 ± 3.3 ng/ml) respectively with confidence level 95% and power 80%. So the sample size is 96 (48 in every group)

They will be divided into 2 groups.

Group 1 (Control group): includes 48 healthy controls who fulfilled the same exclusion criteria as the patients, and never had asthma or eczema.

Group 2 (Asthmatic patients): includes 48 adult ([?]18 years old) subjects with allergic asthma according to Global Initiative for asthma consensus report **(GINA), 2020** . [7]

Inclusion Criteria:

Allergic asthma patients were selected as having:

Obstructive pattern; Low FEV1/FVC on pulmonary function test:

Allergy as positive skin prick test for house dust mites.

Exclusion Criteria:

1-Evidence of digestive disease

2-Any other condition associated with increased intestinal permeability, such as cystic fibrosis,

3- Food allergy or patients having positive skin test against common food allergens.

The control subjects fulfilled the same exclusion criteria as the patients, and never had asthma or eczema.

4- Patient refusal to participate.

The control subjects fulfilled the same exclusion criteria as the patients, and never had asthma or eczema.

Each patient was subjected to the following:

1-Full detailed history and examination to exclude any comorbid condition that may affect the result of the study.

2-Full detailed allergic history and clinical examination of the respiratory system

3-Chest x ray was done to exclude other lung pathology.

4- Pulmonary function tests with special emphasis on FEV1, FEV1/FVC using SPIROMETRICS

5- Total serum IgE was done for each patient using enzyme-linked immunosorbent assay (ELISA).

6- Skin prick testing to common allergens.

7-Asthma severity score was calculated according to GINA, 2020. [7]

8- Serum zonulin level detection by ELISA

Asthma severity scoring:

Asthma was divided into grades I–IV (I being the mildest and IV the most severe). Grading was based on events that occurred over the past six months; including day and night symptoms, effect of asthma on daily activity, use of steroids, and peak expiratory flow rate as defined by**GINA guidelines 2020.** Pulmonary function tests were performed for all patients using a fully computerized Spirometer (Jaeger MasterScreen IOS, version 5.2 manufactured by VIASYS Healthcare GmbH, Hoechberg, Germany) Pulmonary functions were assessed using forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and the FEV1/FVC ratio, measured and expressed as a percentage of predicted values with a ratio higher than 0.8 being normal.

Skin testing:

- Skin testing was performed according **to *Bernstein et al., 2008*** . [8]
- Allergen extracts of skin testing were standardized allergen extracts provided by **Hamilton (Omega, Allergy OVERSEAS consultant Inc., Canada).** The test was performed using the following allergens: for aeroallergens: (house dust mite; Dermatophagoides (D.)pteronyssinus and Dermatophagoides (D.) farnae mites; grass, mixed pollens; mixed molds, tobacco, cotton, wool, cockroach, hay dust) and food allergens: (egg, fish, peanuts, cows' milk).
- Histamine dihydrochloride (10 mg/ml) was used as a positive control, while saline was used as a negative control.
- Subjects were asked to stop antihistamines a week before skin testing.
- The largest diameter of the wheal was measured, and it was considered positive if it was [?] 3 mm (**ASCIA guidelines, 2020).** [9]
- Those with positive tests for food allergy were excluded from the study.

Sample collection:

Five ml blood were collected by venipuncture under complete aseptic conditions. Samples were left to clot then centrifuged at 1000 xg for 15 minutes. Sera were collected and stored at -20 degC. Sera were used to measure serum levels of total IgE, and zonulin.

Serum level of total IgE:

Quantitative measurement of serum level of total IgE was done using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) Kit supplied by **Chemux Bioscience, Inc. (CA, USA, Cat No.10602)** according to the manufacturer's instructions. The results were expressed in IU/mL. The total IgE level in a normal, allergy-free adult is less than 150 IU/ml of serum. The minimum detectable concentration of IgE by this assay is estimated to be 5.0 IU / ml. The Absorbance of standards and samples were measured at 450 nm using a microtiter plate ELISA reader **(Biotek, USA).**

Measurement of serumzonulin level:

Quantitative measurement of serum level of zonulin was done using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) Kit supplied by **MyBiosource, Inc. (USA, Cat No: MBS774026)** according to the manufacturer's instructions. The results were expressed in ng/ml. Absorbance of standards and samples were measured at 450 nm using a microtiter plate ELISA reader**(Biotek, USA).** The kit detection range was 20ng/ml - 800ng/ml.

Statistical analysis:

The collected data were processed and coded before being analyzed using SPSS program version 23. Quantitative data were presented as minimum, maximum, mean and SD.

Qualitative data were presented as count and percentage.Student t test was used to compare quantitative data between two groups and One Way ANOVA test was used to compare more than two groups.Pearson's correlation was used to measure linear relationship between two continuous variables.ROC curve was used to measure diagnostic validity of quantitative variables and linear regression test was used to measure independent effect of some factors on quantitative outcome variable.P value<0.05 was considered statistically significant.

Results:

The current study was a case control study which included 96 subjects attending the allergy and immunology unit, microbiology and immunology department, Faculty of medicine, Zagazig University during the year 2021.They were divided into 2 groups, group 1 included healthy controls with a median age of 28 [n=48] and group 2 included patients with allergic asthma according to Global Initiative for asthma consensus report (GINA), 2020 with a median age of 30 [n=48].

Regarding the asthmatic patients, the mean value of age was 30.67+-15.609, most of the patients were female (62.5%) and most of them lived in rural areas (54.16%). 39 patients (81.3%) had positive family history of atopy. All patients displayed positive skin prick test results to house dust mites, most of the patients (70.8%) were sensitive to D. pteronyssinus and 60.4% were sensitive to D. farina. 14 patients were mono sensitized to a single type of house dust mites and 34 patients were polysensitized to both types. As for the grade of asthma severity, 4 patients (8.3%) had grade 1severity, 24 patients (50%) had grade 2 asthma severity, 12 (25%) patients had grade 3 severity, and 8 patients (16.7%) had grade 3 asthma severity. (Table 1)

Concerning the control group, the mean value of age was 32.7+- 11.2, most patients were males (85.3%) and 70.8% of them lived in rural areas. As per the family history of atopy, only 11 (22.9%) patients had positive family history of atopy and all of them displayed negative skin prick test results.

On comparing serum total immunoglobulin E (IgE) level between asthmatic patients and control subjects, there was a highly statistically significant difference between both groups (p=0.000). Asthmatic patients displayed a higher statistically significant level of serum total IgE. The mean serum IgE was equal to 233.3+-103.4IU/ml in the asthmatic group and 84.4+-18.6 IU/ml in the control group. (Table 2)

Similarly, there was a high statistically significant difference between both groups as regards serum zonulin level. Asthmatic patients displayed a higher statistically significant level of serum zonulin than control subjects. The mean serum zonulin was equal to 258.3+-153.01 ng/ml in the asthmatic group and 80+-13 ng/ml in the control group. (Table 2)

Factors affecting serum total IgE level were male sex, asthma grade, and positive skin prick test. Male patients displayed a statistically significant higher serum total IgE level (p=0.03). The mean value of serum total IgE was 272.89+-141.33 among male patients and was 193.53+-56.38 IU/ml among female patients. Besides, post hoc test showed a significant difference between grade 1 vs grade 2 as regards serum total IgE. As the asthma severity increased there was a significant corresponding increase in the serum total IgE (p=0.02). Moreover, there was a highly statistically significant difference as regards the mean value of serum total IgE between patients displaying positive skin prick test results and those displaying negative skin prick test results (p=0.002) The mean value of serum total IgE among patients displaying positive skin prick test results was 244.41+-114.85 IU/ml. (Table 3)

With regard to the correlation between serum zonulin level and seum total IgE, it didn't reach statistical significance (p=0.34) . (Table 4) Figure 2

As for the factors affecting serum zonulin level, they were residence and asthma grade. There was a statistically significant difference in the serum zonulin level between patients living in Urban areas and those living in rural areas. (p=0.002) The mean value of serum zonulin was significantly higher in patients living in

urban areas and it equaled to 315.55+-157.34 ng/ml. Post hoc test showed a highly statistically significant difference between grade 1 vs grade 2, grade 1 vs grade 3, grade 1 vs grade 4, grade 2 vs grade 4 and grade 3 vs grade 4 as regards serum zonulin level ($p<0.001$). (Table 4) Figure 2

Grade of asthma had independent significant effect on zonulin level ($p<0.001$) while serum total IgE had no significant effect on zonulin level via linear regression analysis (p= 0.684 NS). (Table 5)

Roc curve was done to assess the validity of zonulin level for differentiation between (grade 1 or 2) vs (grade 3 or 4) ($p=0.002$). It displayed sensitivity equal to 80% and specificity equal to 71.4%. Its positive predictive value was equal to 66.7% and negative predictive value was equal to 83.3%. The cut off value of serum zonulin was [?] 198 ng/ml. The area under the curve was 0.76. (Table 6) Figure 3

Discussion:

Asthma is a chronic inflammatory lung disease which is characterized by airway inflammation, intermittent airflow obstruction, and bronchial hyper-responsiveness.[10] Allergic asthma can be sparked by allergen sensitization in which house dust mites (HDM) are one of the most prevalent indoor allergens.[11] The prevalence of allergic sensitization is rising in developing countries, a phenomenon related to rapid urbanization and an adult's migration from a rural to an urban area increases their risk of sensitization to mite allergens. It also could contribute to a rapid increase of chronic respiratory diseases (CRD). Exposure to dust mite allergens is a risk factor for clinical asthma in sensitized subjects.[12]

The relationship between allergic asthma and intestinal permeability is a subject of research interest. An increase in the intestinal permeability may result in facilitating the entry of allergenic proteins from the intestinal lumen into the systemic circulation. Consequently, activation of the adaptive immune system, allergen sensitization and/or extra-intestinal inflammation occurs.[13]

The current study is a case control study that aimed at assessing the relation between serum zonulin level as a marker of increased intestinal permeability and the severity of house dust mites allergic asthma. Our study included 96 patients; 48 asthmatic patients and 48 control subjects. The mean age of the asthmatic patients (n=48) was 30.67±15.609, 30 (62.5%) were females and 18 (37.5%) were males and most of them, 26 patients, lived in rural areas (54.16%). 39 patients (81.3%) had a positive family history of atopy. All patients displayed positive skin prick test results to house dust mites, most of the patients (70.8%) were sensitive to D. pteronyssinus and 60.4% were sensitive to D. farina . 14 patients were mono sensitized to a single type of house dust mites and 34 patients were polysensitized to both types. As for the grade of asthma severity, 4 patients (8.3%) had grade 1 severity, 24 patients (50%) had grade 2 asthma severity , 12 (25%) patients had grade 3 severity, and 8 patients (16.7%) had grade 3 asthma severity.

Several studies supported these current results indicating that bronchial asthma was associated with females with an obvious sex bias.**Ricciardolo FL et al., 2020** conducted a cross-sectional study on 499 asthmatic patients to assess the potential difference between asthmatic males and females in a real-life setting. Their study also displayed a female predominance in which 301 patients 60.32% were females.[14] In addition, **Sabry, 2011** reported that 20–40% of asthmatic females of reproductive age suffer from worsening of their symptoms during their menstrual period, suggesting that sex hormones may have a major role in the biologic sex difference. [15]**.**

These results could be attributed to hormonal factors, environmental exposure, and the presence of comorbidities. Since estrogen receptors (ERs) are highly expressed in the lungs and their smooth muscles with a special role in bronchoconstriction/dilatation, this explains the gender bias. Moreover, another possible hormonal factor is the lack of male sex hormone which plays an integral role in downregulation of innate and adaptive immune response.[16] Genetic factors may also be involved. The Cyclooxygenase (COX) pathways play an important role in the course of bronchial asthma and Cyclooxygenase-2(COX-2) gene homozygosity is related to females.[17]

In fact, rural residence has higher prevalence of parental smoking, higher number of siblings, advanced overcrowding rates, higher humidity levels and greater exposure to chemicals and farm animals.[18] Moreover,

hot climatic conditions and high population density are crucial for the development and reproduction of mites[19] . Hence, the geographical situation of Egypt and its favorable climatic conditions together with its overpopulation contribute to the abundance of HDM especially in rural areas.[21]

The current results displayed that asthma patients were more commonly living in rural areas (54%vs 45.84% urban asthmatics) and all of them had positive skin prick test response to house dust mites, DP;Df (70%,60% respectively). **Lawson et al., 2017** likewise reported that children with asthma who lived in rural areas were more likely to wheeze or have more severe symptoms of wheeze. [20] Furthermore, this also agreed with **Müsken et al., 2002** study which concluded that sensitization to storage mites in Germany was more frequently sensitive to D. pteronyssinus and the prevalence of positive skin test results to storage mites was greater in rural than in city dwellers. Also, In vitro sensitization to B. tjibodas mites was also significantly greater in rural than in city dwellers.[42] Besides, **Hassan and Hagrass 2017** reported that house dust mites (HDM) allergy occurs more commonly than any other allergens among Egyptian asthmatic patients. [21]

Several studies disclosed that the high frequency of family history of allergy is common among asthmatics. Familial assemblage of asthma and allergic disease has frequently been observed, indicating that a positive familial history of atopy may be considered as an identifiable risk factor of asthma. This may be due to the evidence hinting that genetics play an essential role in the pathogenesis of asthma. In alliance, **Antonios et al. 2012** reported 60% of cases had a positive family history and displayed 95% positive reactions to both D. pteronyssinus and D. farinae allergens using SPT among asthmatic patients in Gharbia Governorate, Egypt. [22] Similar results were reached by **Haggag MG et al, 2017** .Their study revealed that 62.5% of patients had positive family history.[23]

A detailed clinical history and physical examination followed by the detection of total serum IgE level and IgE immunoreactivity against specific allergens still represents the cornerstone in approaching allergic disorders.[4]

On comparing serum total immunoglobulin E (IgE) level between asthmatic patients and control subjects in the present study, asthmatic patients displayed a higher statistically significant level of serum total IgE. It was statistically significantly higher among male asthmatic patients. There was a positive correlation between total serum IgE level and asthma severity grade and skin prick test results.

Several studies disclosed high serum IgE levels among asthmatic patients. This is in alliance with **Kim et al. 2013** who stated that the total IgE levels were significantly higher in males.[24]**Fereidouni M et al.,2009** study uncovered that the mean total IgE serum was significantly high among asthmatics. Males had higher mean total IgE values than females (305 vs 252 IU/mL, P = 0.6), but the difference was not significant.[25]

On the contrary, **Somani ,2008** demonstrated that total IgE level is higher in female patients and they attributed their results to the possibility that total levels of IgE might be inheritable, especially in females, because of the existence of two alleles at the X-linked locus.[26]

The disparity in the development of total IgE between males and females is the consequence of the fact that the levels of total IgE depend on many other factors; such as parasitic infestations, smoking, pollution, local diet and different genetic background in which males are at greater risk of exposure.[25]

IgE is an antibody linked to allergic reactions and airway inflammation which is associated with asthma severity. IgE is also linked to airway hyperresponsiveness, and lower pulmonary function. Additionally, the use of monoclonal anti-IgE has resulted in decreasing asthma severity.

Concerning the correlation between level of total IgE and asthma severity, **Kovač K et al,2007** also discerned that the greater the asthma severity the greater the total serum total IgE level (>288.0 kIU/L) and specific IgE to D pteronyssinus (>44.1 kIUA/L). They conducted a study to evaluate the correlation between serum total IgE level and asthma severity in asthmatic children sensitive to D pteronyssinus.[27]

Kenawy et al ., **2017** found a highly significant increase in serum level of IgE in patient with severe

asthma than those with mild asthma with P values < 0.001.[28] Likewise, a study done by **Rotsides and his coworkers (2010)** reported a strong positive association between high IgE level and asthma severity in children and they proposed that serum IgE level is a strong predictor for allergy in asthmatic children. [29]

Regarding the relation between serum IgE level and the positivity of skin prick test, **Baldacci S et al., 1996** concluded that the higher IgE levels the greater the positivity of SPT regardless the gender .[30] **Rose et al., 1996** likewise assumed that there is some inverse relationship between the quantitative level of IgE antibody and the level of allergen necessary to cause symptoms of asthma.[31]

The impairment of the epithelial barrier is a corner stone in the development of allergic diseases. Increased intestinal permeability, either due to the exposure to antigens in asthmatic patients or due to a barrier defect, play a critical role in susceptibility to environmental allergens.[32] The mechanisms responsible for increased intestinal permeability in asthma remain unclear. It seems that intestinal permeability may be a "bronchus to gut" consequence of bronchial inflammatory process because a correlation with the severity of the disease, airflow obstruction, could be demonstrated [33] This supports that a primary general mucosal defect could be present simultaneously in several organs and clinically expressed in a single organ after an exogenous stimulation, such as antigenic or environmental factors [41]. Duodenal histological changes mimicking those observed in bronchial mucosa have been shown. In addition, gastrointestinal abnormalities have been also reported in patients with asthma.[5]

Therefore, correction of the gut barrier defect may be an additional novel approach for asthma treatment [34] via reduction of epithelial susceptibility to damage and consequent inflammatory and remodeling responses. In addition to growth factors, several other peptides have been identified that have the ability to restore barrier function. One such peptide is AT-1001, a peptide inhibitor of zonulin [35]. Zonulin is a prehaptoglobulin protein and a biomarker for gut barrier leakiness that downregulates tight junction function, and it has been proposed to play a role in several autoimmune diseases [8] and asthma in children [32]. It would therefore be of interest to examine zonulin expression in airways and its regulation in patients with asthma.[36]

The present study disclosed that asthmatic patients displayed a higher statistically significant level of serum zonulin than control subjects. Patients living in rural areas had a significantly higher levels on serum zonulin. Furthermore, there was a significant correlation between zonulin level and asthma grade . The higher the zonulin level the greater the grade of asthma severity. Nevertheless, we found that there was no difference in serum levels of zonulin subjects regarding gender and age. The cut of value of serum zonulin level to deffrentiate between grade 1-2 asthma severity and grade 3-4 asthma severity. In addition, serum zonulin showed a sensitivity equal to 80%, a specificity equal to 71.4%, a positive predictive value equal to 66.7% and a negative predictive value equal to 83.3%. There was no statistically significant correlation to serum total IgE concentration. Grade of asthma had independent significant effect on zonulin level while serum total IgE had no significant effect on zonulin level via linear regression analysis.

Benard and colleagues in France were the first study in the literature on intestinal permeability in asthma. They showed increased intestinal permeability in adult patients with bronchial asthma compared with patients with chronic obstructive pulmonary disease and healthy control subjects. In their study, they used radioactive material (CrEDTA) which was administered orally, and estimated its urinary recovery. They noticed that both patients with allergic asthma and those with nonallergic asthma were significantly different from the control groups and reported that intestinal permeability was not correlated with the severity of asthma as judged by FEV˜ measurement. Unlike the current results, they disclosed that intestinal permeability did not significantly vary according to asthma severity score or steroid treatment [37] This may be due to difference in method used to assess intestinal permeability.

Furthermore, a study performed by **Cervantes-García D and collaborators** aiming at assessing the outcome of oral Lactococcus lactis NZ9000 use on airway inflammation and lung remodeling in asthmatic rats and its relation to the preservation of the intestinal barrier, concluded that oral L. lactis could be used for asthma prevention through its maintenance of an adequately functioning intestinal barrier.[38]

Preliminary data suggest that a subset of asthmatic patients have increased serum zonulin levels and increased

intestinal permeability [39]. These data suggest that both the lung and intestinal mucosa may be routes through which specific antigens can gain access to the submucosa with subsequent exposure to the immune system leading to lung inflammation [40].

Moreover, a study performed by **Yamaide F et al. 2020** to examine the differences between serum zonulin level among allergic children and non- allergic children concluded that serum zonulin was significantly greater in children with allergy (Food allergy, Bronchial Asthma). In addition, it was significantly higher in patients with food allergy than in bronchial asthma patients. However, their results are not completely reliable as food allergy can be the main reason for increased intestinal permeability and this explains the higher increase in zonulin level among food allergy patients than among asthmatic ones. [32]

Up to the present moment no studies have been conducted to assess the relationship between serum zonulin level and asthma severity or to assess its correlation to different residential distribution. A study performed by Sheen et al to assess serum zonulin level in atopic dermatitis (AD) and its correlation to disease severity. They found that AD group had a greater median serum zonulin level than the control group and serum zonulin level had significantly positive correlations with age and the SCORAD index, but not with total IgE, total eosinophil count (TEC), or the number of allergens to which a child is sensitized. And concluded that each 1 ng/mL increase in serum zonulin was associated with a 15% greater risk of moderate-severe AD [6]. Their results agree with the current results as regards the correlation between serum zonulin level and total serum IgE level. Both revealed no statistically significant correlation.

Conclusion:

Intestinal barrier dysfunction contributes in the pathogenesis of allergic asthma. Serum zonulin level reflects an increase in intestinal permeability and acts as prognostic factor of severity in Asthma. Correction of the gut barrier defect may be an additional novel approach for Asthma.

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Conflicts of interest: none.

Authors' contributions

Shereen A. Baioumy, Shereen Mahmoud Ibrahim, Aya Elgendy and Shaimaa Fouad designed the study. Shereen A. Baioumy and Shaimaa Fouad Fouad shared in sample collection. Shereen Mahmoud Ibrahim and Aya Elgendy shared in sample collection and did the statistical analysis. Shaimaa Hani Fouad wrote the draft. Shereen A. Baioumy and Aya Elgendy performed the critical review of the manuscript. All authors reviewed and approved the final version. All authors read and approved the final manuscript.

Availability of data and materials

All the data needed to support the current findings could be found in a supporting sheet.

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Table (1): Description of studied subjects: :

Variable	Control Group (n=48)	Percentage%	Asthmatic patients (n=48)	Percentage%
Sex: Male Female	28 20	85.3% 14.7%	18 30	37.5% 62.5%
Age (years): X ± SD Median	32.7 ± 11.2 28		30.67±15.609 30	
Residence: Rural Urban	34 14	70.8% 29.2%	26 22	54.16% 45.84%

Variable	Control Group (n=48)	Percentage%	Asthmatic patients (n=48)	Percentage%
Family history of atopy Positive: Negative:	11 37	22.9% 77%	39 9	81.3% 18.8%
Skin prick: negative positive	48 0	100% 0%	0 48	0% 100%
House dust Mites D. pteronyssinus D. farina Both	---	---	48 34 29 34	100% 70.8% 60.4% 70.8%
Asthma grade	asthma grade 1	asthma grade 1	4	8.3%
	asthma grade 2	asthma grade 2	24	50.0%
	asthma grade 3	asthma grade 3	12	25.0%
	asthma grade 4	asthma grade 4	8	16.7%

Table (2): Comparison between Total IgE levels and zonulin levels among both control and case groups:

Variable	Control Group 1 (n=48)	Asthmatic patients Group 2 (n=48)	F	P
Total IgE level (IU/ml) Mean ± SD	84.4 ± 18.6	223.3 ± 103.4	**4.8**	**0.000HS**
Zounlin (ng/ml) Mean ± SD	80 ± 13	258.3 ± 153.01	**6.12**	**0.000HS**

Table (3) Factors affecting Total IgE level:

Variables	Variables	Total IgE Mean	Total IgE SD	Test value	P value
Sex	**male**	272.89	141.33	2.29*	**0.03 S**
	female	193.53	56.38		
Residence	**urban**	219.27	93.30	0.25*	**0.81 NS**
	rural	226.69	112.99		
Age	**<30 Years**	240.00	120.07	1.12*	**0.27 NS**
	>30 years	206.58	82.78		
Asthma grade	**asthma grade 1**	154.50	.58	5.77**	**0.02 S**
	asthma grade 2	205.87	88.23		
	asthma grade 3	241.08	113.93		
	asthma grade 4	283.25	132.03		
Skin prick	**negative**	172.00	34.37	3.33*	**0.002 HS**

positive	244.41	114.85	

*Student t test **One Way ANOVA test (post hoc test shows significant difference between grade 1 vs grade 2)

Table (4) Factors affecting Zonulin level:

		Zonulin level Mean	**Zonulin level SD**	**Test value**	**P value**
Sex	**male**	290.11	163.89	1.12*	0.27 NS
	female	239.27	145.59		
Residence	**urban**	315.55	157.34	2.51*	0.02 S
	rural	209.92	133.81		
Age	**<30 Years**	261.67	152.41	0.15*	0.88 NS
	>30 years	255.00	156.83		
Asthma grade	**asthma grade 1**	127.50	8.66	64.54**	<0.001 HS
	asthma grade 2	209.50	103.91		
	asthma grade 3	218.50	75.50		
	asthma grade 4	530.00	87.83		
Skin prick	**negative**	299.29	182.05	1.20*	0.24 NS
	positive	241.47	138.88		
		Zonulin level	**Zonulin level**		
Total IgE	**Pearson Correlation**	0.14	0.14		
	P value	0.34NS	0.34NS		

*Student t test **One Way ANOVA test (post hoc test shows significant difference between grade 1 vs grade 2, grade 1 vs grade 3, grade 1 vs grade 4, grade 2 vs grade 4 and grade 3 vs grade 4)

Table 5: Linear regression analysis for factors affecting Zonulin level:

	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	Sig.	95.0% Lower
Grade 3 or 4	167.317	29.962	0.838	<0.001 HS	107.0
Total IgE	0.075	0.183	0.062	0.684 NS	-.293

Table (6): Validity ofZonulin level for differentiation between (grade 1 or 2) vs (grade 3 or 4)

Parameters	**Cut off point**	**AUC**	**Sig**	**Sensitivity**	**Specificity**	**+PV**	**-PV**	**95% Confidence Inter Lower Bound**
Zonulin	> 198	0.764	.002 HS	80%	71.4%	66.7%	83.3%	0.622

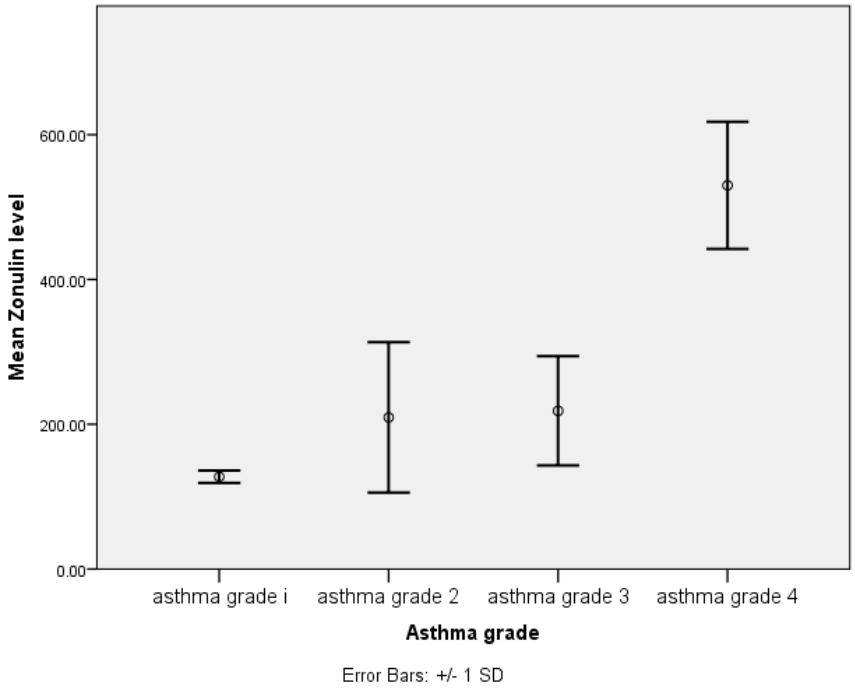

Figure1.Correlation between asthma grade of severity and serum zonulin level.

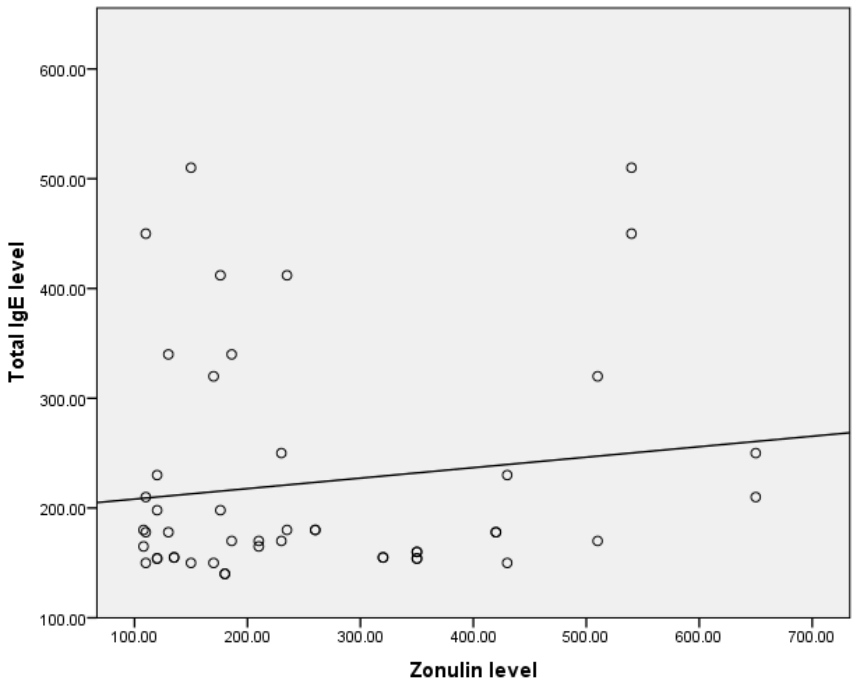

Figure 2. Correlation between serum zonulin level and total serum IgE level.

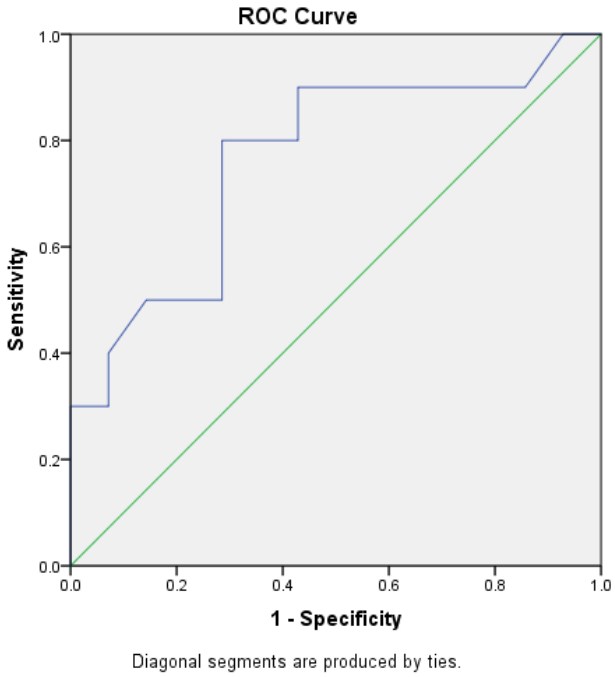

Figure 3. Roc curve of Zonulin level for differentiation between (grade 1 or 2) vs (grade 3 or 4)