Assessment of the relevance of intestinal Zonulin test for inflammatory conditions in an integrated clinical setting.

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Abstract

Human Zonulin is a protein that increases permeability in the epithelial layer of the small intestine by modulating the intercellular tight junctions. There is not sufficient information available about the participation of Zonulin in inflammatory conditions in a clinical setting. The aim of this study is to assess the relevance of integrating faecal Zonulin tests as part of our initial patient assessment. We therefore tested for the presence of elevated intestinal Zonulin and microbial infections in patients with a variety of inflammatory related conditions.

Background

Optimum function of tight junctions is necessary for maintaining normal physiologic processes in the intestinal tract. Human Zonulin, a 47-kDa protein, increases permeability in the epithelial layer of the small intestine [1]. Increased serum Zonulin levels have been identified in conditions such as Autism [2], coeliac disease [3], inflammatory bowel [4] in particular, Crohn’s disease [5], type1 & 2 diabetes [6], [7], and in insulin resistant obesity [8]. Plasma Zonulin has been suggested as a marker of intestinal permeability [9],[10],[11]. It has been suggested that the dysregulation of Zonulin could contribute to compromised intestinal barrier functions. A compromised intestinal barrier function could lead to the crossing of both food & microbial derived antigens into the blood of individuals with different immuno-pathological conditions such as allergies, infections of the gastrointestinal tract and autoimmune conditions [12], [13]. We therefore compared the levels of intestinal zonulin to the presence of intestinal
microbial overgrowth in patients with inflammatory or autoimmune conditions. The intestinal barrier can be disturbed by various causes, such as certain medicines, exercise, mast cell activation, high fat diet and stress [14]. This can lead to an increased permeability, allowing amongst others, enhanced entrance of lipopolysaccharides (LPS) into the body. LPS are parts of the outer membrane of gram-negative bacteria and are strong endotoxic compounds, which can cause the release of pro-inflammatory cytokines in the body, leading to inflammation.

Materials and Methods
33 individuals from patients of Integrated Immunology Clinics in both Europe and Middle East regions with a variety of inflammatory or auto-immune conditions were tested. Briefly, two samples of faeces were collected from each individual and were tested for either faecal zonulin or for general stool microscopy (microbiological assessment) & culture. Stool microbiology tests focused primarily on parasitic and fungal overgrowths. All Zonulin faecal samples were measured with standard procedures (Biovis Diagnostik, Germany). Fungal and parasitic overgrowth tests were conducted locally to the patient’s country of residence.

Results
Forty five percent of the total patients tested, had elevated Zonulin levels above the reference range of less than 55ng/ml. Seventy percent of patients with non-autoimmune inflammatory conditions tested positive for Zonulin compared with only sixteen percent of the auto-immune group (figure 1). We also tested the relationship between elevated intestinal Zonulin and intestinal fungal & parasitic overgrowth. Of the total elevated Zonulin positive individuals, sixty percent of individuals tested positive for yeast overgrowth, & thirty three percent had parasitic overgrowth with thirty three percent of individuals testing positive for both fungal and parasitic overgrowth (figure 2). Of the total individuals with a negative Zonulin level, forty one percent had an autoimmune diagnosis and fifty eight percent had a non-autoimmune inflammatory component (figure 3). Only thirteen percent of the elevated Zonulin positive individuals had an auto-immune component, while eighty six percent of individuals who were both Zonulin positive and had an inflammatory non-autoimmune component (figure 4).
INTESTINAL ZONULIN POSITIVE GROUPS (Figure 1)

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
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</thead>
<tbody>
<tr>
<td>Total Patients</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Autoimmune</td>
<td></td>
</tr>
<tr>
<td>Total Inflammatory</td>
<td></td>
</tr>
<tr>
<td>Positive Inflammatory</td>
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ZONULIN YEAST AND PARASITE POSITIVE GROUPS (Figure 2)

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Zonulin +/−</td>
<td></td>
</tr>
<tr>
<td>Yeast +/−</td>
<td></td>
</tr>
<tr>
<td>parasite +/−</td>
<td></td>
</tr>
<tr>
<td>Yeast &amp; parasite +/−</td>
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</tbody>
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ZONULIN NEGATIVE INDIVIDUAL DESCRIPTION (Figure 3)

- Psoriatic Arthritis
- Candida Infection
- Urticaria & Inflamed Lymph
- Anti-Thyroid Antibody Positive
- Chronic Fatigue
- Uricaria
- Depression
- Fatigue
- Graves Disease
- Hashimotos 1
- Hashimotos 2
- Hashimotos 3
- Taenia Infection
- Candida+ Parasite Infection
- Chronic Fatigue 2

ng/ML

0 10 20 30 40 50 60

NEGATIVE CONTROL

0 10 20 30 40 50 60

10 20 30 40 50 60

10 20 30 40 50 60

10 20 30 40 50 60

10 20 30 40 50 60

10 20 30 40 50 60

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Discussion
The primary aim of this investigation was to assess the relevance of intestinal permeability testing in our integrated immunology clinic, where we treat autoimmune and chronic inflammatory conditions. Recently, several studies have been published suggesting that faecal zonulin can be an indirect marker for the upregulation of intestinal permeability. In addition, intestinal permeability has so far been linked to Crohn’s and ulcerative colitis, type 1 diabetes, coronary heart disease, stress via downregulation of melatonin, coeliac disease plus Hashimoto’s thyroiditis. Surprisingly, only 2 of the total 11 autoimmune individuals who we tested were found to have elevated levels of Zonulin protein. This result may not reflect the zonulin levels at the time of autoimmune onset as most patients we see at the clinic have established autoimmune disease and many have made significant changes to their diet before commencing treatment at our integrated immunology clinic. Interestingly, most of the individuals who tested positive for elevated Zonulin had a non-autoimmune inflammatory component and also presented with a digestive component at the time of testing. A large number of which tested positive for intestinal candida albicans or a yeast overgrowth of unspecified type. Overall the results indicate that faecal zonulin test is a necessary
tool as part of our initial assessment for patients who display both inflammatory and digestive symptoms to their condition at time of presentation at the clinic.

References