Serologic markers of celiac disease in psoriatic patients

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Keywords
Anti-endomysial antibodies, anti-gliadin antibodies, anti-tissue transglutaminase antibodies, celiac disease, PASI, psoriasis

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Abstract

Background Aetiopathogenesis of psoriasis is complex and not yet well known. In recent years, it has been observed that psoriasis can coexist with clinically asymptomatic celiac disease and a gluten-free diet helps to obtain remission, even in patients with very chronic lesions.

Objective The aim of our work was to investigate how often the positive titres of antibodies characteristic for celiac disease occur in psoriatics’ serum in exacerbation in comparison with controls.

Patients/methods Serum samples from 67 patients with intensified psoriatic lesions were investigated. Serum from healthy people at a comparable age and with no familial predisposition to psoriasis and celiac disease was the control material. Antibodies against human tissue transglutaminase (recombinant antigen), against tissue transglutaminase isolated from guinea pig’s liver and against gliadin were determined by enzyme-linked immunosorbent assay technique. Anti-endomysial antibodies were determined by indirect immunofluorescence method.

Results Patients with psoriasis have significantly higher mean concentrations of antibodies against tissue transglutaminase (human recombinant and guinea pig-derived antigen) and against gliadin for IgA. IgA antibodies against tissue transglutaminase (both antigens) and gliadin positively correlate with psoriasis activity. No anti-endomysial antibodies for IgA were found in any serum.

Conclusions Our results seem to imply an association between psoriasis and asymptomatic celiac disease/gluten intolerance. High percentage of positive results to guinea pig-derived tTG could be due to cellular activity of tissue transglutaminase in psoriasis.

Introduction

Psoriasis is one of the most common chronic and recurrent skin diseases. Aetiopathogenesis of this dermatosis is complex and not yet well known. In recent years, attention has been paid to probability of primary intestinal absorption disorders in psoriatics and to IgA protective role. It has also been observed that psoriasis can coexist with clinically asymptomatic celiac disease and a gluten-free diet helps to obtain remission, even in patients with very chronic lesions. Celiac disease is an autoimmunological disease induced by gluten consumption by genetically predisposed people, which causes small intestine damage. It is characterized by various clinical symptoms – from latent course to evident enteropathy. Screening tests include an assessment of antibodies against gliadin (AGA), which correlate to the disease activity, and antibodies against smooth muscle endomysium (EMA), which are very characteristic for celiac disease. In 1997, an enzyme – tissue transglutaminase (tTG) – was found to be the main endomysial auto-antigen in celiac disease. Since then, a determination of antibodies against tissue transglutaminase (a-tTG) in serum (with the use of guinea pig antigen or human recombinant) has been used for diagnosis and monitoring of celiac disease as a method alternative to the
mentioned above. \textsuperscript{6,11–19} Determination of characteristic for celiac disease serologic markers is considered to be important for psoriasis, too. The aim of our work was to investigate how often the positive titres of these antibodies occur in psoriatics’ serum in exacerbation in comparison to controls.

**Patients and methods**

Study was made on archival serum samples stored at \(-85\, ^\circ C\) (max 24 months). Examined serum samples derived from 67 patients (27 females and 40 males; mean age: 36.73 ± 11.02) with psoriasis admitted to hospital due to intensified skin lesions [mean psoriasis area and severity index (PASI), 25.9 ± 14.9]. These patients had suffered from psoriasis for 122 ± 97 months (range, 1–372 months). In two cases, it was the first disease manifestation. Patients with psoriatic arthritis and with other diseases were excluded from the study. The patients studied were before any anti-psoriatic treatment. Sera from control group matched for sex and age and with no familial predisposition to psoriasis and celiac disease were the control material \(\left(n = 75\right)\) for determinations of antibodies against human recombinant tTG and gliadin and \(n = 30\) for EMA determinations). All persons, psoriatic patients and controls, were on gluten-containing diet, and all are residents of Upper Silesia (Poland). Blood samples were collected by venipuncture, following an overnight fast. Serum received from immediate centrifugation was frozen at \(-85\, ^\circ C\) until determinations could be performed.

Research protocol was accepted by the Local Bioethical Committee of the Medical University of Silesia in Katowice.

Antibodies against human tissue transglutaminase (recombinant antigen; a-h-r-tTG) were determined for IgA with use of a Bindazyme tTG IgA enzyme-linked immunosorbent assay (ELISA) kit (The Binding Site, Great Britain). The anti-guinea pig tTG antibodies (a-GP-tTG) were assayed as previously described,\textsuperscript{12,17,20} with minor modification. The ELISA plate (Nunc Maxisorp, Nunc, Denmark) were coated overnight at 4 \(^\circ C\) with guinea pig tTG (Sigma, USA) solution \(10\, \mu g\) protein/mL, 100 \(\mu L\) per well) in 50 mmol/L carbonate buffer containing 5 mmol/L CaCl\(_2\) (pH 7.5). The plates were rinsed and blocked for 2 h at room temperature with wash buffer containing per litre: 10 mmol EDTA, 50 mmol TRIS, 1 mL Tween 20 and 0.2 g Tiomerosal (pH 7.5). Sera were diluted 250 times in wash solution and incubated on the plates for 1.5 h at room temperature. After washing, the bound immunoglobulins were detected by peroxidase labelled antibodies against human IgG and IgA (Dako-Cytomation, Denmark) at dilution proposed by manufacturer (1 h at room temperature, dilution in wash buffer). Activity of solid-phase bound enzyme was determined with Sigma TMB substrate solution for 10 to 20 min. The reaction was stopped by adding 0.5 mol/l sulphuric acid, and absorbances were read at 450 nm (reference wave 630 nm) in Power Wave XS plate reader (BioTek, USA). For calibration, we used the poll of celiac sera with high concentration of anti-tTG antibodies.

The anti-gliadin antibodies (AGA) were assayed by ELISA method. The ELISA plate (Nunc Maxisorp) were coated overnight at 4 \(^\circ C\) with 100 \(\mu L\) well with gliadin from wheat flour (ICN, USA) solution \(10\, \mu g/mL\) in 50 mmol/L carbonate buffer containing 20\% ethyl alcohol (pH 9.6). The plates were rinsed with phosphate buffered saline containing 0.5 mL/L Tween 20 (PBST, pH 7.2) and next blocked for 2 h at room temperature with PBST with 0.2\% teleostean gelatin (Sigma). Sera were diluted 250 times (for AGA IgG) or 100 times (for AGA IgA) in PBST-gelatin and incubated on the plates for 1 h at room temperature. The bound immunoglobulins were detected as described for anti-GP-tTG antibodies. For calibration of the IgG antibodies, we used the standards from Eurospital (Italy) and for IgA class – the poll of serum from celiac patients. Coefficients of intra-assay variation for all ELISA methods were below 10\%.

Anti-endomysial antibodies for IgA (IgAEmA) were determined in serum by indirect immunofluorescence method with application of the sets by The Binding Site (Great Britain). Scrapes of lower part of monkey’s oesophagus served as a substrate. A specific fluorescence pattern was examined under a microscope by Nikon (Japan) by two independent researchers. Specific endomysium fluorescence for 10-times diluted serum was recognized to be a positive result.

The results were presented with the use of basic parameters of descriptive statistics such as mean value, standard deviation and percentiles. Compatibility of variable distribution with normal distribution was checked with Szapiro-Wilk’s test. For group comparisons, the non-parametric tests by Kolmogorov-Smirnov, Wald-Wolfowitz and U Mann–Whitney were used. For correlations, the R Spearman test was used. A significance level \(P < 0.05\) was accepted as statistically significant. A computer programme STATISTICA was used for computations.

**Results**

Table 1 presents the results of antibodies assay: anti-tissue transglutaminase from guinea pig’s liver (IgA and IgG), anti-human recombinant tissue transglutaminase (IgA) and anti-gliadin (IgA and IgG) in psoriatics’ serum in exacerbation of disease and in controls’ serum. The analysis of data shows that patients with psoriasis have
The titres of antibodies against tissue transglutaminase from guinea pig liver (a-GP-tTG IgA and a-GP-tTG IgG) and against human recombinant tissue transglutaminase (a-h-r-tTG IgA) and against gliadin (AGA IgA and AGA IgG) in serum of psoriatic patients and healthy persons (control group) are shown in Table 1.

Table 1: Titres of antibodies against tissue transglutaminase from guinea pig liver (a-GP-tTG IgA and a-GP-tTG IgG) and against human recombinant tissue transglutaminase (a-h-r-tTG IgA) and against gliadin (AGA IgA and AGA IgG) in serum of psoriatic patients and healthy persons (control group)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Psoriatic patients (n = 67)</th>
<th>Controls (n = 75–85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-h-r-tTG IgA</td>
<td>0.943 ± 1.131</td>
<td>0.852 ± 0.576</td>
</tr>
<tr>
<td>a-GP-tTG IgA</td>
<td>426.515 ± 428.187</td>
<td>159.471 ± 91.930</td>
</tr>
<tr>
<td>a-GP-tTG IgG</td>
<td>757.880 ± 842.638</td>
<td>347.817 ± 178.062</td>
</tr>
<tr>
<td>AGA IgA</td>
<td>14.869 ± 9.015</td>
<td>5.720 ± 4.820</td>
</tr>
<tr>
<td>AGA IgG</td>
<td>0.376 ± 0.316</td>
<td>0.389 ± 0.320</td>
</tr>
</tbody>
</table>

*P < 0.05 in psoriatic patients compared to control group.

Table 2: Correlations between examined antibodies and PASI (Spearman rank R and P-values)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>a-h-r-tTG IgA</th>
<th>a-GP-tTG IgA</th>
<th>a-GP-tTG IgG</th>
<th>AGA IgA</th>
<th>AGA IgG</th>
<th>PASI</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-h-r-tTG IgA</td>
<td>R = 0.528775</td>
<td>R = 0.242918</td>
<td>R = 0.463192</td>
<td>R = 0.188564</td>
<td>R = 0.247133</td>
<td></td>
</tr>
<tr>
<td>a-GP-tTG IgA</td>
<td>R = 0.000004</td>
<td>R = 0.072126</td>
<td>R = 0.000079</td>
<td>R = 0.126473</td>
<td>R = 0.043780</td>
<td></td>
</tr>
<tr>
<td>a-GP-tTG IgG</td>
<td>R = 0.476422</td>
<td>R = 0.269830</td>
<td>R = 0.785987</td>
<td>R = 0.000000</td>
<td>R = 0.000000</td>
<td></td>
</tr>
<tr>
<td>AGA IgA</td>
<td>R = 0.000004</td>
<td>R = 0.020068</td>
<td>R = 0.294557</td>
<td>R = 0.322003</td>
<td>R = 0.066464</td>
<td></td>
</tr>
<tr>
<td>AGA IgG</td>
<td>R = 0.000079</td>
<td>R = 0.000000</td>
<td>R = 0.15536</td>
<td>R = 0.007877</td>
<td>R = 0.593074</td>
<td></td>
</tr>
<tr>
<td>PASI</td>
<td>R = 0.247133</td>
<td>R = 0.288735</td>
<td>R = 0.247133</td>
<td>R = 0.247133</td>
<td>R = 0.247133</td>
<td></td>
</tr>
</tbody>
</table>

Titres of antibodies, mean ± SD (U/mL)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Titres (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-h-r-tTG IgA</td>
<td>0.943 ± 1.131</td>
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<td>AGA IgG</td>
<td>0.376 ± 0.316</td>
</tr>
</tbody>
</table>

These observations are confirmed for IgA antibodies against human recombinant tissue transglutaminase (P < 0.001, P = 0.000000 and P = 0.000000 in Kolmogorov-Smirnov, U Mann–Whitney and Wald-Wolfowitz’s tests, respectively) and for IgG (P < 0.001, P = 0.000000 and P = 0.01 in Kolmogorov-Smirnov, U Mann–Whitney and Wald-Wolfowitz’s tests, respectively). These observations are confirmed for IgA antibodies against human recombinant tissue transglutaminase (P < 0.001, P = 0.036 and P = 0.002 in Kolmogorov-Smirnov, U Mann–Whitney and Wald-Wolfowitz’s tests, respectively). Titres of antibodies against gliadin differ significantly between psoriatics and controls’ sera only for IgA (P < 0.001, P = 0.000000 and P = 0.000000 in Kolmogorov-Smirnov, U Mann–Whitney and Wald-Wolfowitz’s tests, respectively) but not for IgG (P > 0.1, P = 0.75 and P = 0.244 in Kolmogorov-Smirnov, U Mann–Whitney and Wald-Wolfowitz’s tests, respectively). Table 2 presents correlations between examined antibodies and PASI (Spearman rank R and P-values): concentrations of a-h-r-tTG IgA positively correlated with concentrations of a-GP-tTG IgA, a-GP-tTG IgG and AGA IgA. Concentrations of a-h-r-tTG IgA, a-GP-tTG IgA and AGA IgA also positively correlated with PASI. Table 3 shows number of psoriatic patients with positivity for two or more serologic markers. Figure 1 presents the results in the controls and the patients for each of these antibodies as boxplots with 10th, 25th, 50th, 75th and 90th percentiles.

In recent years, some reports have appeared about a probable association of psoriasis with enteropathy, mainly celiac disease, and about a contribution of intestine process to skin lesions. Intestinal absorption disorders and enteropathy confirmed by intestine mucosa histology were observed in psoriatics. In addition, increased titres of antibodies typical for celiac disease, anti-gliadin for IgA, anti-reticulin (correlating to disease intensification), against tissue transglutaminase and smooth muscle endomysium for IgA, were found in psoriatics.

In patients with positive titres of antigliadin antibodies and chronic psoriatic lesions, a gluten-free diet application had a beneficial effect, which could be supported by the fact that after returning to normal diet the skin lesions

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Table 3  Number of psoriatic patients with positivity for two or more serologic markers

<table>
<thead>
<tr>
<th>Types of positive markers</th>
<th>Number of psoriatic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-h-r-tTG, a-GP-tTG-IgA and IgG, AGA IgA</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>a-GP-tTG-IgA and IgG, AGA IgA and IgG</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>a-h-r-tTG, a-GP-tTG-IgA, AGA IgA</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>a-h-r-tTG, a-GP-tTG-IgA and IgG</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>a-GP-tTG-IgA and IgG, AGA IgA</td>
<td>17 (25.3%)</td>
</tr>
<tr>
<td>a-GP-tTG-IgA, AGA IgA</td>
<td>9 (13.4%)</td>
</tr>
<tr>
<td>a-GP-tTG-IgA and IgG, AGA IgA</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>AGA IgA and IgG</td>
<td>1 (1.4%)</td>
</tr>
</tbody>
</table>

Fig. 1  Titres of CD-associated antibodies in serum of psoriatic patients and healthy persons.
intensified. The presence of CD-associated antibodies in psoriatic patients correlated with greater disease activity because a significantly higher proportion of patients with elevated CD-associated antibody levels was currently on or had previously required systemic immunosuppressants or PUVA therapy.

The aim of our studies was to estimate whether in psoriatic patients gluten intolerance appears more often in healthy persons. The analysis of the results showed that in psoriatics' serum mean concentrations of antibodies against tissue transglutaminase (guinea pig's antigen), both for IgA and IgG, were significantly higher than mean concentrations of these antibodies in controls; in addition, in 46% of cases for IgG, and as much as 66% of cases for IgA, titres of antibodies were higher than the 90th percentile of the control values. The above observation could be confirmed when human recombinant tissue transglutaminase was used as an antigen – mean titres of IgA were significantly higher in psoriatics than in controls (but only in 7.4% of cases, titres of a-h-r-tTG IgA were higher than the 90th percentile of the control values). Fabiani et al. proved that the anti-tissue transglutaminase specificity and sensitivity were 98% and 92% for guinea pig and 99.6% and 96% for human recombinant tissue transglutaminase, respectively. Authors suggest that the anti-human tissue transglutaminase test should definitely replace the anti-guinea pig-derived one as first-level screening tool. Zintzaras and Germenis are of the similar opinion. Their meta-analysis proved that ELISAs detecting IgA antibodies against human recombinant tissue transglutaminase and purified human tissue transglutaminase, but not guinea pig tissue transglutaminase, are working sufficiently in the initial diagnostic approach of celiac disease. In our psoriatic patients also, anti-gliadin IgA mean concentration was significantly higher than in controls (and 54% of cases were higher than the 90th percentile of the control values). Only anti-gliadin IgG mean concentration was similar in psoriatics and in controls. 5.7% of psoriatic patients had four serologic markers positive, 28.1% had three serologic markers positive and 17.7% had two serologic markers positive. We also found statistically significant positive correlations between concentrations of a-h-r-tTG IgA, a-GP-tTG IgA, a-GP-tTG IgG and AGA IgA. Concentrations of a-h-r-tTG IgA, a-GP-tTG IgA and AGA IgA also positively correlated with PASI. But, on the other hand, in any serum, no IgA, a-GP-tTG IgA and AGA IgA also positively correlated a-GP-tTG IgG and AGA IgA. Concentrations of a-h-r-tTG IgA, a-GP-tTG IgA, and a-h-r-tTG IgG more often has a higher specificity and a-h-r-tTG IgA more often has a higher sensitivity. Moreover, sensitivity of this test is higher in adults than in children. Authors conclude that a-h-r-tTG antibody is the preferred test for screening asymptomatic people. Mallbris et al. found EmA antibodies in 5 of the 400 psoriatic patients, which is 4.6 times more than in the general population.

High percentage of a-GP-tTG IgA and IgG positive psoriatic sera is also worth considering. This observation may suggest that in psoriatics some immunological processes depending on specific expression, due to disease, of tissue transglutaminase occur. This enzyme is an intracellular protein which catalyses the covalent and irreversible cross-linking of a protein-bound glutamine residue with the lysine residue of a second protein or with the amino group of primary amines.

Proteins rich in lysine (e.g. extracellular matrix proteins) are specific substrates for tTG. Due to cellular membrane damage, tTG is released from the cell and activated, which leads to cross-linking of matrix proteins and introducing of transglutaminase molecules into space structure of nascent network. In the result, the neoepitopes of matrix are formed and specific, directed against tTG and tTG-containing protein polymers, antibodies are induced. Up until now, a close relationship between the presence of anti-tTG antibodies in serum for IgA and celiac disease has been generally accepted and tTG has even been suggested to be a main endomysial antigen. Moreover, an increase in concentration of anti-tTG antibodies for IgG was observed in celiac disease, but it was not as specific as for IgA. More and more reports suggest immunological response to tTG as being independent of endomysium and quite frequent in other diseases (e.g. autoimmune diseases). There is much evidence that psoriatic lesions start in dermis and endothelium cells proliferation in peaks of dermal papillas is one of the first detectable pathological processes. tTG is an enzyme closely connected with proliferating blood vessels. Clearance of psoriatic lesions during gluten-free diet (applied as the only treating method in patients with anti-gliadin antibodies) was accompanied by a decrease in transglutaminase expression (that was intensive before), parallel to a reduction in number of proliferating cells Ki67+ in endothelium. tTG coding genes were identified to be effector elements of apoptotic cell death. Under normal conditions, this enzyme is practically undetectable in healthy epidermis. Bianchi et al. showed a significant reduction in amount of apoptosis blocking gene Bcl-2 in basal layer of psoriatic epidermis and a presence of tissue transglutaminase in it – specifically placed in cytoplasm of epidermal apoptotic cells. Thus, significantly high titres of antibodies against tTG in psoriatics are likely to result from the response to this protein expression in psoriatic lesions. Positive correlation between anti-tTG IgA antibodies and PASI can partially confirm this suggestion. On the other hand,
the results of IgA determinations with the use of animal antigen were not fully compatible with the determinations when human recombinant tissue transglutaminase was used (66% vs. 7.4% of positive sera, respectively, but positive correlation between concentrations of a-h-r-TTG and a-GP-tTG: $R = 0.528775$, $P = 0.000004$). This observation can be explained by the fact that human antigen, which is a recombinant protein, cannot be processed after translation which is believed to modify antigenic activity. It is quite possible that there is the existence of a close antigen relationship between epidermal transglutaminase, the expression of which is activated in the psoriatic epidermis, and tTG isolated from guinea pig liver in comparison to the recombinant tTG that eventually affects the strong binding of tTG antibodies with guinea pig liver antigen. Probably, the results of our experiment can also be affected by insufficient purity of commercially available tTG preparation isolated from guinea pig liver, which was also described by other authors. Some unidentified compounds can constitute an antigen for the antibodies presented in sericid serum and eventually result in false-positive results.

Our results seem to imply an association between psoriasis and asymptomatic celiac disease/gluten intolerance. High percentage of positive results to guinea pig-derived tTG could be due to cellular activity of tissue transglutaminase in psoriasis. Any attempts to verify this hypothesis may lead not only to better knowledge about the nature and role of this protein, but also better understanding of psoriasis pathogenesis, particularly contribution to it of angiogenesis and apoptosis.

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