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Association of Inherited Genotype and Severity of Clinical Presentation in Subjects with Verified Pas III Disorder

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ABSTRACT

Background: Polyglandular autoimmune syndrome type III (PAS III) is combination two most common autoimmune disease: Diabetes mellytus type 1 (DM1) and autoimmune thyroid disease (AITD). **Objectives:** The aims of the study were a) to define conection between polymorphism of CTLA-4 gene, rs 231775 with PAS III; b) to establish the conection of inherited genotype with severity of clinical features; and c) to estimate the rate of risk for severe clinical presentation among subgroups in study population. **Metods**: This research included 50 subjects with diagnosed PAS III syndrome, wich are on treatment in clinic for Nuclear medicine and andocrinology KCUS. As methods of research has used: hystory of disesase AND clinical examination. As material is used blood sample. From blood sample DNA was isolated withn Qiamp- DNA- mini kit, with accopanied protocol. **Results**: In our study, 50 patients with polyglandular autoimmune syndrome type III (PAS III) were examined, and in the study population had 26 female subjects and 24 male subjects. The average age of the participants was 31.64 years, and in the subgroups: group GWT (G-wild type) the average age was 30.20 years, group GM (G-mutated) 32.40 years and group GH (G-heterozygote) 30 , 60 years. Using the Chi-square test, the association between the polymorphism rs231775 and PAS-III was demonstrated, χ^2 (2.100) = 18.258, where $p < 0.0001$. Using the Chi-square test, the association between the rs231775 polymorphism and the severity of the clinical picture, $\chi 2$ (2.50) = 8.531, where $p< 0.0140$ was proved. The CTLA-4 rs231775 genotypes were also assessed for disease severity. **Conclusion**: This study suggests that CTLA-4 expression plays a key role in balancing the immune system as well as the response against one's own tissues, and thus in the regulation of autoimmune diseases.

Keywords: pluriglandular disorder PAS III, severity of clinical picture, type 1 diabetes mellitus, hypothyroidism levothyroxine dosing.

1. BACKGROUND

Polyglandular autoimmune syndrome type III (PAS-III) is a combination of the two most common autoimmune diseases: Diabetes mellitus type 1 and autoimmune thyroid disease (AITD) (1). Thus, it consists of two diseases of autoimmune genesis, but with different pathogenesis. In general, type 1 diabetes mellitus occurs much earlier than autoimmune thyroid disease (2). While Diabetes type 1 is caused by autoimmune destruction of insulin-producing β-cells of the pancreas, autoimmune thyroid disease (AITD) includes hyperthyroidism (Graves 'disease), Hashimoto's thyroiditis (HT), or thyroid antibody positivity (3). Graves' disease is defined by the presence of hyperthyroidism together with the presence of autoantibodies to thyrotropic receptors, while Hashimoto's thyroiditis is defined as primary hypothyroidism, atrophy of the thyroid gland and increased levels of antibodies against thyroid peroxidase (TPO) (4). Finding genetic polymorphisms that lead to immune disorders and the appearance of a characteristic clinical picture will allow the development of new therapeutic and diagnostic methods that could recognize and stop PAS-III, long before the development of severe disease consequences (5). Using these polymorphisms as biomarkers for rapid scanning would allow early diagnosis of the disease, on the one hand, and blocking the disorders they cause with new therapeutic agents could stop the disease in its

early stages. (6, 7)

2. OBJECTIVES

The aims of this study were: a) to determine the association of CTLA-4 gene polymorphisms, rs231775 with PAS-III; b) to determine whether the type of inherited genotype is related to the severity of the clinical picture; c) to assess the risk ratio of clinical picture severity between subgroups in the study group.

3. PATIENTS AND METHODS

Patients

The study included 50 subjects diagnosed with PAS-III syndrome (Diabetes mellitus Type 1 and Hypothyroidism), who were treated at the Clinic of Nuclear Medicine and Endocrinology, Clinical Center of the University of Sarajevo (CCUS). Criteria for inclusion of patients in the study were: patients diagnosed with PAS-III syndrome, patients older than 18 years (age≥18) and patients who agreed to participate in the study.

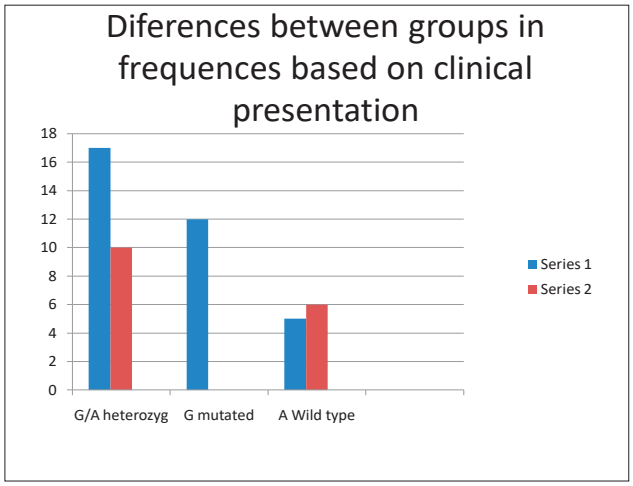
The anamnesis, clinical examination and peripheral blood samples of the patients were used as the parameters of the study. DNA was isolated from the blood sample taken using the Qiamp-DNA mini kit, using the attached protocol.

Procedures

Isolated DNA was quantified using the ABI Quantifiler kit and the Real-Time Polymerase Chain Reaction (Real-Time PCR) method (RT-PCR) prepared according to the kit used in the quantification. Quant studio 7. DNA concentration was determined by Nanodrop (ND-1000 spectrophotometer) and all samples were equated to a concentration of up to 10 ng/µl Single nucleotide polymorphisms (SNPs) were analyzed: rs231775 (+ 49AG) and rs886041906 CTLA-4. rs231775 (+ 49AG) two specific primers were used - 5'-GCTCTACTTCTTGAAGACCT-3 'and 5'-AGTCTCACTCACCTTTGCAG-3'. Jena Biosciences Taq Master High Yield PCR Master Mix was used to amplify the isolated DNA. The isolated DNA was transferred to plates of 50 samples, in which amplification of the desired segments was performed. Detection and documentation of the results were performed on a device for amplification of DNA fragments in real-time (Real-Time PCR) type ABI 7300 (Applied Biosystems, Life Technologies Corp., USA).

The reaction substrates are 5.0 µl. genomic DNA, 5x Taq master mix High Yield 7.5 µl., 4 pmol solid primer F and primer R antisense (20 pmol/1.0 µl each) and Rnase released (free) water. Reactions were performed at 94°C for 5 min, then 37 cycles of denaturation (94°C for 20 s), annealing (25-60°C for 40 s) and elongation (72°C for 40 s). PCR products were separated by agarose gel electrophoresis with ethidium bromide dyes. The last step was to gel and take a photograph, using a camera (DS-34 Direct Screen Camera) on Polaroid film, under UV radiation (Transilluminator 4000, Stratagene). The photos were scanned and saved as a graphic file with the jpeg plugin. Genetic analyzes were performed at the AGC Alea Genetic Center in Sarajevo (8, 9).

Statistical processing included descriptive statistics and the X2 test.



Graph 1. Clinical picture severity in subgroups: G-heterozygous, G-mutated, A Wild type. Serises A – Severe clinical presentation. Serises B- Easier clinical presentation

4. RESULTS

In our study, 50 patients with polyglandular autoimmune syndrome type 3 (PAS III) were examined, and in the study population, there were 26 female and 24 male subjects. The average age of the respondents was 31.64 years, and in the subgroups: group GWT (G-wild type) the average age was 30.20 years, group GM (G-mutated) 32.40 years and group GH (G-heterozygote) 30, 60 years. Using the chi-square test, the association between the polymorphism rs231775 and PAS-III was demonstrated, X2 (2.100) = 18.258, where $p < 0.0001$. Using the chi-square test, the association between the rs231775 polymorphism and the severity of the clinical presentation, X2 (2.50) = 8.531, where $p < 0.0140$ was demonstrated. The CTLA-4 rs231775 genotypes were also assessed for disease severity. Patients with PAS III were divided according to the severity of the clinical picture into the mild, moderate and severe clinical pictures. The division was based on the dosage of insulin and levothyroxine for each subject titrated to adequate substitution and disease control. The severity of the clinical picture was determined by the total insulin dose and the total levothyroxine dose. A milder clinical picture included a dose of levothyroxine less than or equal to 50 µg and a total insulin dose less than 50 IU per day. A moderate clinical picture included a dose of levothyroxine less than or equal to 75 µg and a total insulin dose between 50 and 80 IU per day. The more severe clinical picture included a total dose of levothyroxine over 75 µg and a total insulin dose of more than 80 IU per day. After statistical processing, it was proved that the heavier clinical picture is significantly more present in the GH and GM groups compared to the WT group (X2 (2.50) = 8.531, where $p < 0.0140$. Risk ratio (Odds ratio) was applied in to assess the risk of inherited genotype and the severity of the clinical picture of the subjects in the group, the Odds ratio test showed a difference between the rs231775 polymorphism and the severity of the clinical picture, the highest risk was recorded in the % CI 1.404-621.57, where $p < 0.0294$.

5. DISCUSSION

Polyglandular autoimmune syndrome type III (PAS-III) is a combination of the two most common autoimmune dis-

eases: Diabetes mellitus type 1 and autoimmune thyroid disease (AITD). Thus, it consists of two diseases of autoimmune genesis, but with different pathogenesis. In general, type 1 diabetes mellitus occurs much earlier than autoimmune thyroid disease (9). According to Patel et al., there is a strong association between T-lymphocytes and antigen 4 (CTLA-4) with thyroglobulin and genetic variants of autoimmune hypothyroidism (10). CTLA-4 has been described as a vital regulator for reducing T-cell activation resulting in peripheral tolerance and is also a negative regulator of T / B, T-cell activation, and related T/monocyte-macrophage interactions. In this context, decreased regulation of CTLA-4 may contribute to an overemphasized T cell response. Thus, the G allele is thought to reduce the ability to control T-cell proliferation, which can cause impaired CTLA4 function, resulting in higher T-cell activity, a stronger immune response, and a higher likelihood of autoimmunity, according to Zhao et al., 12). It has been previously shown that individuals with mutations in one CTLA-4 allele are highly susceptible to autoimmunity, which increases the possibility, that more subtle variations in CTLA-4 levels in the general population may affect response to therapy. Thus, nonpathogenic polymorphisms in CTLA-4 that alter CTLA-4 expression may affect an individual's lifetime risk for the development of malignant and autoimmune disorders as stated by Anjos et al. (13). Together, these findings illustrate that CTLA-4 expression plays a key role in balancing the immune system as well as responses against one's own tissues, and thus in regulating autoimmune anticancer responses, as reported by Sun T. et al. (14).

6. CONCLUSION

The chi-square test (X2) was used to assess the association between the inherited genotype and the severity of the clinical picture of subjects in the group with verified PAS III pluriglandular disorder. Using the chi-square test, the association between the rs231775 polymorphism and the severity of the clinical picture, X2 (2.50) = 8.531, where $p < 0.0140$ was demonstrated. The risk ratio (Odds ratio) was used to assess the risk of inherited genotype and the severity of the clinical picture of subjects in subgroups. The difference between the rs231775 polymorphism and the severity of the clinical picture was demonstrated, the highest risk was observed in the subgroup with G mutated genotype compared to Wild type OR = 29.544, 95% CI 1.404-621.57, where $p < 0.0294$.

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