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Detecting The Presence of Anti-HLA Antibodies in Autoimmune Diseases

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Abstract: By creating antibodies, the immune system protects the body from foreign antigens. The immune system may occasionally sustain damage that results in a loss of tolerance to its antigens, which triggers the creation of antibodies directed against those antigens. Another challenge in solid organ transplantation is the existence of these anti-HLA antibodies. Our goal was to find out if common autoimmune disorders such type 1 diabetes (T1D), Behçet's disease (BD), and ankylosing spondylitis (AS) result in the development of anti-HLA antibodies. 100 patients with AS, 100 patients with BD, 60 patients with T1D, and 100 healthy people were included in this study. PRA screening tests were performed on serum from blood samples taken from both patients and healthy individuals to look for the presence of anti-HLA antibodies. Of the AS patients, 5 were positive for PRA class II alone, 7 were positive for both PRA class I and class II, and 1 patient was positive for PRA class I. In BD, 3 patients was positive for both PRA class I and II, 7 patients was positive for PRA class II alone, and 1 patient was positive for PRA class I. In T1D, 1 patient were positive for PRA class I, 3 patients were positive for PRA class II alone, and 2 patients were positive for both. When three patient groups were compared with the control group, there was no statistically significant difference in the detection of anti-HLA antibodies between the patient groups and the control group. ©2024 NTMS.

Keywords: Ankylosing Spondylitis, Behçet's Disease; Type 1 Diabetes, Transplantation, PRA.

1. Introduction

It is believed that hereditary and environmental factors combine to cause autoimmune disorders. According to the hereditary foundation of these illnesses, those who have specific genetic variations are more likely to develop autoimmune diseases. A major element of this genetic tendency is Human Leukocyte Antigens (HLA). HLA genes are important for immune system regulation and have an impact on the onset of autoimmune disorders. Certain HLA alleles, for instance, have been linked strongly to the onset of autoimmune disorders, including Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), and Type1Diabetes (T1D) [1].

Autoimmune disorders demonstrate that the host's immune system can generate HLA antibodies against both foreign and internal HLA antigens. These anti-HLA antibodies can be found using a test known as Panel Reactive Antibody (PRA). When patients are waiting for an organ transplant, the PRA test is frequently done to find out if they have anti-HLA antibodies. Because the largest barrier to organ transplantation is the existence of anti-HLA antibodies. Nowadays, there is also questioning and scrutiny surrounding the occurrence of anti-HLA antibodies in liver and bone marrow transplants [2, 3].

Ankylosing spondylitis (AS), a subset of spondyloarthritis that is often referred to as Bechterew's disease, is characterized by aberrant bone remodeling and inflammation in the sacroiliac joints and spine. Even though the exact cause of AS is unknown, the genetic marker HLA-B27 is strongly linked to the condition. More than 90% of AS patients have the HLA-B27 gene, according to research [4].

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A systemic inflammatory illness, Behçet's disease (BD) is primarily characterized by painful oral ulcers that recur often. Other clinical symptoms that may be present include vaginal ulcers, erythema nodosum, or acneiform pustular lesions [5]. A genetic correlation was found in 1982 between BD and HLA-B51 [6].

An autoimmune condition known as type 1 diabetes (T1D) is typified by autoantibodies destroying beta cells in the pancreas known as Langerhans islets. According to Derrou et al. (2021) the death of cells results in an insulin deficit and chronic hyperglycemia. Geographic variations in the prevalence of T1D are caused by both genetic and environmental factors. The risk of having T1D is much higher in people who carry the HLA DR4-DQ8 and HLA DR3-DQ2 alleles [7].

Studies have shown that autoimmune disorders such systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are associated with elevated levels of anti-HLA antibodies [8, 9]. Finding out if AS, BD, and T1D illnesses result in the production of anti-HLA antibodies was our goal. To do this, antibody presence was determined using the standard Luminex PRA method, which is frequently used to identify the presence of antibodies in patients with chronic kidney disease (CKD) and organ transplantation.

2. Material and Methods

In addition to 100 healthy people without any chronic illnesses serving as the control group, the study comprised 100 patients with ankylosing spondylitis, 100 patients with Behçet's disease, and 60 patients with type 1 diabetes. The study comprised patients who tested positive for HLA-B51 in Behcet's patients and HLA-B27 in AS patients. Additionally, consent forms from people aged 18 to 59 were used to choose all included patients and healthy volunteers. The tissue typing laboratory of the Atatürk University Research Hospital served as the study's location.

2.1. Sera Collection

Using a Nüve NF 048 centrifuge, blood samples taken from patients and healthy individuals were centrifuged at 4100 RPM for five minutes in order to obtain serum samples. The top part was moved into Eppendorf tubes. The acquired serum samples were kept in the proper storage environments.

2.2. Luminex PRA Test

The Luminex PRA TestThe IMMUCOR LIFECODES LifeScreen Deluxe kit was used to conduct the PRA screening test. Initially, 300 microliters of purified water were introduced into every well to moisten the filters beneath the filtered plate, and then the mixture was allowed to sit for five minutes. After that, each well was filled with 40 µl of wash buffer, 12 µl of patient serum, and 5 µl of LifeScreen Deluxe Beads from the kit. The wells were then left in the dark for 30 minutes. Following incubation, the wells were twice washed with 250 µl and 100 µl of wash buffer. After that, 50 µl of the conjugate solution that had already been made was added, and the mixture was once more incubated for 30 minutes in the dark. Following the incubation period, the wells were twice cleaned using 250 µl and 100 µl of wash buffer. After that, 30 minutes of dark incubation were spent again after adding 50 µl of the conjugate solution that had already been made. Following the incubation period, 130 µl of wash buffer was introduced into each well, and the Luminex Labscan 200 instrument was utilized for analysis. The Matchit Antibody program was used to examine the data, and MFI (Median Fluorescence Intensity) values larger than 1000 were regarded as positive.

2.3. Statistical Analyses

The mean, standard deviation, percentage, and count of the data were displayed. In 2x2 tables, the Pearson chi-square test was utilized to compare categorical variables if the expected value was below 20% and (>5), the Yates chi-square test was employed to compare values below 20% and (3-5), and the Fisher's Exact test was utilized to compare values if the expected value was (<3). When comparing categorical variables in tables bigger than 2x2, the Fisher-Freeman-Halton test was utilized when the expected value was less than 5, and the Pearson chi-square test was employed when the expected value was greater than 5. P-values were regarded as statistically significant if they were less than 0.05. One-way ANOVA was used to statistically analyze the MFI readings in the PRA screening test.

3. Results

There were 100 volunteers in the control group, 59 of them were female and 41 were male. The average age of the male population was found to be 39.17±10.3 years, and the female population was found to be 30.42±8.94 years. Three female volunteers tested positive for PRA class I, one positive for PRA class II, and one positive for both PRA class I and II, per the results of the PRA screening. Out of the male subjects, one tested positive for PRA class I and two tested positive for PRA class II. Within the control group, statistical analysis showed no discernible difference between male and female volunteers (Table 1).

Table 1: PRA results and mean ages of the control group.

	Female (n=59)	Male (n=41)	p
Number of participants	59	41	-
Mean Age (Years)	30.42±8.94	39.17±10.3	-
Positive PRA Class I	3 (5.08%)	1 (2.43%)	0.642
Positive PRA Class II	1 (1.69%)	2 (4.87%)	0.566
PRA Class I and II Positive	1 (1.69%)	0	0.402
Total Positive PRA	5 (6.78%)	3 (7.31%)	0.713

There were 52 women and 48 men among the 100 individuals with AS diagnoses in the research. The mean age of the male and female patients was 34.74±13.36 years and 38.72 ± 14.17 years, respectively. Of the patients who were female, none found positive for PRA class I; four found positive for PRA class II, and four found positive for both PRA class I and II. One patient was positive for PRA class I, one for PRA class II, and two for both PRA class I and II in the case of the male patients. The statistical analysis revealed that the results of the PRA screening test did not significantly differ between males and females in AS. PRA screening results and a thorough statistical analysis are shown in Table 2.

Table 2: Number of AS patients, mean age, and results of PRA screening.

	Female (n=52)	Male (n=48)	p
Number of participants	52	48	-
Mean Age (Years)	38.72±14.17	34.74±13.36	-
Positive PRA Class I	0	1 (2.08%)	0.296
Positive PRA Class II	4 (7.69%)	1 (2.08%)	0.199
PRA Class I and II Positive	4 (7.69%)	3 (6.25%)	0.778
Total Positive PRA	8 (15.38%)	5 (10.41%)	0.278

Although statistical analysis did not reveal statistically significant differences, numerical differences were noted between the AS patients and the control group in the groups positive for PRA class II and both PRA class I and PRA class II. Details of the results are offered in Table 3.

Table 3: Comparison of the AS patient group's and the control group's PRA screening findings.

	AS Groups (n=100)	Control Groups (n=100)	p
Positive PRA Class I	1 (1%)	4 (4%)	1.00
Positive PRA Class II	5 (5%)	3 (3%)	1.00
PRA Class I and II Positive	7 (7%)	1 (1%)	0.783
Total Positive PRA	13 (13%)	8 (8%)	0.847

There were 49 male and 51 female patients with Behçet's disease overall in the study. Male patients had a mean age of 36.55±12.03 years, whereas female patients had a mean age of 34.37± 9.94 years. Just two female patients among those with Behçet's disease tested positive for PRA class II, and two patients tested positive for both PRA class I and PRA class II. No patient tested positive for PRA class I alone, according to the findings of the PRA screening. There was one positive PRA class I patient, five positive PRA class II patients, and one positive patient for both PRA classes I and II among the male patients. The statistical analysis revealed that there was no discernible difference in any group's PRA screening test results between males and females (Table 4).

Table 4: Number of patients of Behçet's disease, median age, and results of PRA screening.

	Female (n=49)	Male (n=51)	p
Number of participants	49	51	-
Mean Age (Years)	34.37±9.94	36.55±12.03	-
Positive PRA Class I	0	1 (1.96%)	1.00
Positive PRA Class II	2 (4.08%)	5 (9.8%0)	0.437
PRA Class I and II Positive	2 (4.0%8)	1 (1.96%)	0.614
Total Positive PRA	4 (8.16%)	7 (13.72%)	0.374

While there were numerical differences in the PRA class II and both the PRA class I and PRA class II groups when comparing the BD patient group to the control group, no statistically significant changes were found (Table 5).

Table 5: Comparison of PRA screening results between the control group and the BD group.

	BD Groups (n=100)	Control Groups (n=100)	p
Positive PRA Class I	1 (%1)	4 (%4)	1.00
Positive PRA Class II	7 (%7)	3 (%3)	0.197
PRA Class I and II Positive	3 (%3)	1 (%1)	0.851
Total Positive PRA	11 (%11)	8 (%8)	0.570

The research included 60 individuals with type 1 diabetes, among whom 33 were females and 27 were males. The average age of female patients was 30.7 ± 12.2 years, while that of males was 28.8 ± 11.5 years. Screening of PRA findings revealed that among female patients, one tested positive for PRA class I, one for PRA class II, and one for both PRA class I and II. In male patients, one tested positive for PRA class I, two for PRA class II, and one for both PRA class I and II. The results of the PRA screening test showed no significant variation among males and females across all categories according to statistical analysis (Table 6).

Table 6: PRA screening findings, mean age, and number of T1D patients.

	Female (n=33)	Male (n=27)	p
Number of participants	33	27	-
Mean Age (Years)	30.7±12.2	28.8±11.5	-
Positive PRA Class I	1 (3.03%)	1 (3.7%)	1.00
Positive PRA Class II	1 (3.0%3)	2 (7.4%)	0.860
PRA Class I and II Positive	1 (3.03%)	1 (3.7%)	1.00
Total Positive PRA	3 (9.09%)	4 (14.8%)	0.690

Table 7: Comparison between the T1D patient group and the control group's PRA screening findings.

	T1D Groups (n=100)	Control Groups (n=100)	p
Positive PRA Class I	2 (%3.33)	4 (%4)	1.00
Positive PRA Class II	3 (%5)	3 (%3)	1.00
PRA Class I and II Positive	2 (%3.33)	1(%1)	0.851
Total Positive PRA	7 (%11.66)	8 (%8)	1.00

There was no statistically significant difference seen between the three groups when compared to the control group (Table 7).

Every patient with an AS, BD, or T1D diagnosis had data gathered about them, such as when they were first diagnosed, the drugs they were taking to treat the condition, any pregnancy histories for female volunteer patients, any prior organ transplant history, and whether or not they had ever received blood transfusions. It was concluded, therefore, that the data gathered produced no variations that would have an impact on the PRA screening outcomes.

The mean PRA class II MFI values of all three patient groups were found to be greater than those of the control group when the MFI values of the patient groups were compared with the group under study. Moreover, the MFI values of the AS and BD groups were found to be greater than the control group, whilst the MFI values of the T1D patient group were found to be lower, when the PRA class II MFI values of the three patient groups were compared with those of the control group. Nevertheless, the statistical analysis revealed no discernible variation (Table 8).

Table 8: Comparison of the control group's PRA Positive MFI values with those of AS, BD, and T1D patients.

	PRA Class I MFI (min-max)	PRA Class I MFI Means	p	PRA Class II MFI (min-max)	PRA Class II MFI Means	p
Control	1148-1722 (n=4)	1468.67	-	1009-2163 (n=4)	1601	-
AS	1216-6818 (n=8)	3636.47	0.197	1204-9142 (n=12)	3223.99	0.521
BH	1081-5095 (n=4)	2774.52	0.718	1126-5948 (n=10)	2711.51	0.862
T1D	1159-1920 (n=4)	1585.75	1.00	1248-1717 (n=5)	1459.67	1.00

4. Discussion

The cause of AS, a persistent inflammatory illness, is not entirely known. In a 1973 study, the correlation between AS and HLA-B27 was initially discovered. A total of 407 HLA-B27 subtypes have been identified as a result of further studies that sought to investigate the link between AS and HLA. Of these, subtypes B27:03, B27:07, B27:06, B27:27, B27:29, and B27:47 were determined to be protective factors, while subtypes B27:04 and B27:15 to be risk factors. Further research revealed no correlation between AS susceptibility and subtypes B27:01, B27:02, B27:05, B27:08-15, B27:17-20, B27:23-24, B27:33, B27:35, B27:40, B27:46, B27:49, and B27:67 [10–14].

Behçet's disease is a systemic vasculitis that is located at the nexus of autoimmune and autoinflammatory disorders. It is typified by recurrent aphthous ulcers and ocular inflammation, and it can also occasionally affect the central nervous system, large vessels, joints, lungs, kidneys, and gastrointestinal tract [15, 16]. Mizuki as al. (2000) and Gül et al. (2001) have conducted studies on Behçet's disease and found that patients who test positive for HLA-B51 and HLA-B57 had higher rates and more severe cases of vaginal ulcers, cutaneous symptoms, positive pathergy tests, and ocular diseases. Moreover, research has shown that HLA-B35 functions as a protective factor. The phenotypic frequency of HLA-B51 has been reported to be increased by the MICA-A6 allele from non-HLA genes [17]. Additionally, mutations in IL-23 and IL-10 have been linked to an increased risk of developing Behçet's disease [18]. Furthermore, ERAP1 and HLA-B51 have been discovered to positively correlate in recent research [19].

T-cell-mediated death of pancreatic beta cells is the cause of type 1 diabetes (T1D), an autoimmune and complex hereditary illness [20]. According to Al-Terehi et al. (2016), there is also a belief that the disease develops as a result of the interaction between hereditary and environmental variables. Research examining the relationship between HLA and type 1 diabetes has revealed that certain genotypes and alleles, including HLA-DR09/DQ09, HLA-DR17/DQ02 [21], HLA-DQB103, and HLA-DQB102 [22], may be associated with a higher risk of type 1 diabetes, whereas other genotypes and alleles, including HLA-DR07/DQ09, DR15/DQ06 [21], HLA-DQB106, and HLA-DQB103/HLA-DQB1*06 [22], may offer protection against type 1 diabetes.

Looking over these three papers, it is clear that their primary focus is on the relationship between HLA and diseases; however, our research has not come across any studies that address the development of anti-HLA antibodies. Each of the three disease categories that were identified for our study was compared to the control group independently after PRA screening tests were administered to patients who met the internationally recognized diagnostic criteria. Compared to the control group, none of the disease groups showed any statistically significant differences in the comparison. Strong infections that occurred before blood collection, allergic reactions, or unidentified illnesses could all be causes of positive PRA screening test results in the control group.

The recipient's HLA antibodies are the largest barrier to organ transplantation. These antibodies are detected through the PRA screening test, which determines the suitability of the organ transplant candidate. The body's defensive mechanism creates antibodies against its own antigens in autoimmune disorders. If a person with an autoimmune condition is a candidate for an organ transplant, the creation of HLA autoantibodies may cause organ rejection. In order to avoid this, it's critical to find out if the patient has any autoimmune illnesses when gathering epicrisis data for organ transplantation. To avoid potential organ rejection, an autoimmune condition should be examined more thoroughly and its risks taken into account if it is present.

5. Conclusion

Autoimmune illnesses have been shown to produce autoantibodies in numerous investigations; however, it remains unclear if these autoantibodies are related to the human leukemia antigen (HLA). In this context, a prior study found positive in PRA class II screening tests in patients with RA after examining the literature. We created a study to ascertain the presence of PRA in three distinct autoimmune disorders in an effort to respond to the question, "Are there developments of anti-HLA antibodies in common autoimmune diseases?" We are the first to look into the relationship between AS, BH, and T1D illnesses and anti-HLA antibodies. Results were not statistically significant, despite a numerical difference between the control group and the illnesses. However, given the significant numerical disparities found, more care should be taken to find out if these illnesses are present while gathering a patient's medical history when they are about to have an organ transplant. A PRA screening test was employed as the analysis approach in this investigation. More precise PRA identification tests, such as LSA or SAB, which are more precise than the Luminex PRA screening test, can be employed to overcome these problems. We were unable to add LSA or SAB tests in our study due to financial restrictions. In the future, PRA identification techniques like LSA or SAB may be used to increase the sensitivity of the study. Furthermore, data from multiple centers' repeated research with higher sample sizes might be examined.

Limitations of the Study

Due to insufficient budget in our study, we were unable to include tests such as SAB and LSA. In future studies, it may be possible to plan more advanced research by incorporating highly sensitive PRA identification tests like SAB and LSA, and by increasing the sample size.

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None.

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, H.D. and M.K.; methodology, H.D. and M.K.; data curation, M.K and H.D.; Formal analysis, M.K. and H.D.; funding acquisition, H.D. and M.K.; writing-review and editing, H.D. and M.K.

All authors have read and agreed to the published version of the manuscript.

Ethical Approval

All participants gave informed consent and the local institutional ethics committee approved the study methods (Date: 30.06.2022 no: B.30.2.ATA.0.01.00/549)" 'Declarations Section'-'Ethics approval and consent to participate' sub-section.

We declare that written consent has been obtained from all participants.

Data sharing statement

The data presented in this study are available on request from the corresponding author.

Consent to participate

Consent was obtained from the patients participating in the study.

Informed Statement

Informed consent was obtained from all subjects involved in the study.

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