ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576664

Evaluation of the Immunodot Technique Compared to the Elisa Technique for the Detection of IgA Antibodies to Tissue Transglutaminase

Oussama Aazzane[1,2*]; Hasnaa Bazhar [2]; Hassan Fellah [1,2]
[1]Laboratory of Cellular and Molecular Pathology, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Morocco.
[2]Immunology Laboratory, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Morocco.

***Corresponding Author:**
Oussama Aazzane
Immunology Laboratory, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Tarik Ibnou Ziad Street Casablanca, 20250, Morocco.

Abstract:- Celiac disease (CD) is a chronic autoimmune enteropathy characterized by intestinal villous atrophy secondary to gluten ingestion. In the biological diagnosis of CD, serology includes, among other tests, the search for IgA anti-tissue transglutaminase antibodies (IgA anti-tTG). Various techniques are employed, including ELISA and Immunodot. The objective of this work is to evaluate the diagnostic performance of the Immunodot test compared to the ELISA test for detecting IgA anti-tTG antibodies in the serum of patients with CD and controls. This is a comparative study of the Immunodot test against the ELISA test for the detection of IgA anti-tTG antibodies. The antibody dosage was performed on 96 samples belonging to 49 patients with CD and 47 healthy subjects. All samples were tested using the Immunodot test (DotDiver CeliAK IgA) and the ELISA test (Anti-tissue Transglutaminase Elisa (IgA) Test instruction). The comparison of the Immunodot test to the ELISA test revealed a sensitivity of 59% and a negative predictive value (NPV) of 73%.

Keywords:- *Celiac Disease, Anti-TG Antibodies, Immunodot, Elisa, IgA.*

I. INTRODUCTION

Celiac disease (CD) is an autoimmune disorder primarily affecting the small intestine, caused by the ingestion of gluten, a protein found in wheat, rye, and barley. It occurs in genetically predisposed individuals expressing a class II HLA molecule of type DQ2 or DQ8 [1,2,3,4,5]. Histologically, it manifests as intestinal villous atrophy with an increase in CD3+ and CD8+ intraepithelial lymphocytes. The diagnosis relies on clinical, biological, and histopathological evidence (small intestine biopsies), which constitute the "Gold Standard" diagnostic tool to date, despite their invasive nature [2,3,6,7].The screening for IgA anti-transglutaminase antibodies (IgA anti-tTG) is a crucial diagnostic tool for CD and is currently recommended as a screening test by NASPGHAN (North American Society For Pediatric Gastroenterology, Hepatology & Nutrition) and ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology and Nutrition) [1]. However, the tests used to detect these antibodies, such as enzyme-linked immunosorbent assay (ELISA) and Immunodot, have controversial diagnostic values concerning the diagnosis and monitoring of patients with CD [5]. In this context, the objective of this work is to evaluate the diagnostic performance of the Immunodot test compared to the ELISA test for detecting IgA anti-tTG antibodies in the serum of patients with celiac disease and controls.

II. PATIENTS AND METHODS

- *Patients.*

This is a comparative study of the Immunodot test (DotDiver CeliAK IgA) versus the ELISA test (Anti-tissue Transglutaminase Elisa (IgA) Test instruction) for the screening of autoantibodies IgA anti-tissue transglutaminase (IgA anti-tTG). We analyzed 96 serum samples from 49 confirmed celiac disease patients and 47 healthy subjects (controls). These sera are part of the serum bank of the Immunology Laboratory at the Faculty of Medicine and Pharmacy in Casablanca.

- *Methods.*

The characteristics of the two tests, Immunodot and ELISA, are shown in Table 1.

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576664

Table 1 Characteristics of the Immunodot and Elisa Tests Used.

Characteristics	**Immunodot test** DotDiver CeliAk IgA (GENERIC ASSAYS, Germany) Test instruction	**Elisa test** Anti-tissue Transglutaminase Elisa (IgA) Test instruction
Technique	Automated	Manual
Principle	Immunodot on sensitized strips	Immunoenzymatic on sensitized plates
Methods	Qualitative	Qualitative Semi-quantitative
Antigens used	Sensitized strips by: - Deamidated gliadin. - Transglutaminase.	96-well microplates sensitized with recombinant transglutaminase.
Conjugated	Goat anti-human IgA antibodies conjugated to alkaline phosphatase	Rabbit anti-human IgA antibodies conjugated to peroxidase
Sample	Human Serum	Human Serum
Sample volume	10 µl of the serum diluted at 1:141	100 µl of the serum diluted to 1:201
Interpretation of the results	Positive: if the test coloration is more intense than that of the negative control.	Negatif : <20 ul/ml Positif : ≥ 20 ul/ml
Duration of the analysis	90 minutes	95 Minutes
Number of Tests/ Kit	24	96
Sensitivity/specificity (%)	100/ 98	100/ 97

➢ *Statistical Analysis*

Table 2 shows the statistical formulas used to calculate sensitivity, specificity, diagnostic accuracy, positive and negative predictive values (PPV and NPV), Youden's index, likelihood ratio, kappa coefficient, and Matthews correlation coefficient of the Immunodot test compared to those of the ELISA test.

Table 2 Statistical calculation formulas [8, 9].

Symbols	**Formulas**
Sensitivity « Se » (%)	TP / (TP+ FN)
Specificity « Sp » (%)	TN / (TN+ FP)
Diagnostic precision (%)	(TP + TN) / T
Positive predictive value « PPV » (%)	TP / (TP + FP)
Negative predictive value « NPV » (%)	TN / (TN+ FN)
Youden's index (Y)	Se +Sp – 1
Likelihood Ratio « LR » (%)	Se / (1-Sp)
kappa coefficient (K)	(P0 – Pe) / (1- Pe)
Matthews correlation coefficient (MCC)	$(TP \times TN - FP \times FN) / \sqrt{[(TP + FP)(TP + FN)(TN + FP)(TN + FN)]}$

TP: True positive; FP: False positive; TN: True negative; FN: False negative; T: Total number of samples; P0: Observed agreement between raters; Pe: Expected agreement by chance.

III. RESULTS

➢ *Characteristics of Patients and Controls.*

The distribution of patients by age and sex shows that 35% (N=17) are adults and 63% (N=31) are female (sex ratio = 0.58). Regarding the controls, 59% (N=28) are adults and 49% (N=23) are female (sex ratio = 1.04) (Table 3).

Table 3 Distribution of Patients and Controls by Age and Sex.

Variables	**Patients (N=49)**	**Controls (N=47)**
Age, n (%)		
Adults	**17 (35)**	**28 (59)**
Children	**32 (65)**	**19 (41)**
Gender, n (%)		
Women	**31 (63)**	**23 (49)**
Men	**18 (37)**	**24 (51)**
Sex-ratio	**0.58**	**1.04**

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576664

➢ *Evaluation of the Diagnostic Performance of the Immunodot Test versus the ELISA Test for the Detection of IgA Anti-tTG Antibodies.*

- *Results Obtained by the Immunodot Technique.*

Among the 49 patient sera tested for IgA anti-tTG, 29 were positive and 20 were negative. In contrast, all controls were negative for IgA anti-tTG (Table 4).

- *Results Obtained by the ELISA Technique.*

All patient sera tested positive for IgA anti-tTG, and all control sera tested negative for IgA anti-tTG (Table 4).

Table 4 Results of Immunodot vs. ELISA for the Detection of IgA Anti-tTG Antibodies.

Variables	Patients (N=49)		Controls (N=47)		Total
	IgA anti-tTG (+)	IgA anti-tTG (-)	IgA anti-tTG (+)	IgA anti-tTG (-)	
Immunodot test	**29**	**20**	**0**	**47**	**96**
Elisa test	**49**	**0**	**0**	**47**	**96**

tTG : Tissue transglutaminase

Table 5 shows the results of the Immunodot compared to those of the ELISA.

Table 5 Comparison of Serological Results (IgA Anti-tTG) of the Immunodot Compared to Those of the ELISA.

		Elisa test		Total
		Positive	Negative	
Immunodot test	**IgA anti-tTG (+)**	**29**	**00**	**29**
	IgA anti-tTG (-)	**20**	**47**	**67**
Total		**49**	**47**	**96**

tTG: Tissue transglutaminase ; (+) : Positive; (-) : Negative

Among the characteristics found for the Immunodot test, the sensitivity and specificity were 59% and 100%, respectively, and the positive predictive value (PPV) and negative predictive value (NPV) were 100% and 73.13%, respectively (Table 6).

Table 6 Characteristics of the Immunodot Test.

Characteristics of the test	Valeurs
Sensitivity (%)	**59**
Specificity (%)	**100**
Diagnostic precision (%)	**79**
Positive predictive value (%)	**100**
Negative predictive value (%)	**73.13**
Youden's index (Y)	**0.59**
Likelihood Ratio (%)	**41**
kappa coefficient (K)	**0.80**
Matthews correlation coefficient (MCC)	**0.315**

IV. DISCUSSION

Serological screening for celiac disease (CD) relies on the detection of specific serum antibodies, namely IgA/IgG anti-tissue transglutaminase antibodies. Various methods are available, including the ELISA test, which is still considered the gold standard. This test uses a recombinant antigen, is accessible to all laboratories, and is characterized by good sensitivity (100%) and specificity (97.8%). The Immunodot is a qualitative serological screening test for CD that detects IgA anti-TG and anti-GD antibodies. It is characterized by better reproducibility (Table 1).

In this study, we evaluated the characteristics of the Immunodot test compared to the ELISA test for the screening of IgA anti-tTG antibodies in a cohort of 96 sera from patients with CD and 47 controls. Table 5 shows that 20 sera positive for IgA anti-tTG by the ELISA test (true positives) were found to be negative by the Immunodot test (false negatives). Among the causes of this discrepancy, we can mention:

- The delay between sample collection and analysis: between 1 day and 7 days for the ELISA test and a delay of 3 months for the Immunodot.
- Storage conditions (+4°C for the ELISA test and -20°C for the Immunodot).

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576664

Other reasons may include the sample sizes (patients and controls), which could explain this discrepancy between the two tests used. It is imperative, when comparing two methods, to assess both the intrinsic and extrinsic validity of the tests involved. According to Table 6, which presents the characteristics of the Immunodot test, we obtained a sensitivity of 59% and a specificity of 100%. The low sensitivity of the Immunodot may be explained by the zone effect (i.e., too many antibodies or too much antigen). It should be noted that the Immunodot test, being qualitative, only yields a positive result when the IgA anti-tTG antibody titer exceeds 41.02 uL/ml, whereas the threshold titer for ELISA is ≥ 20 uL/ml, knowing that it is a semi-quantitative test. The diagnostic accuracy of the Immunodot is 0.79, indicating that this test has reasonable accuracy in identifying positive and negative samples. Furthermore, predictive values are considered among the indicators that assist in interpreting and validating extrinsically the test being evaluated, namely the Immunodot.

The positive predictive value (PPV) of the Immunodot is 100%, meaning that when the result is positive, there is a 100% chance that the patient has the disease. We also observe that the negative predictive value (NPV) is 73.13%, which means that more than 70% of patients with negative IgA anti-tTG have a chance of not being ill.

The discussion on predictive values (PPV and NPV) is delicate because their values always depend on disease prevalence and characteristics of the study population.

The Youden index is 0.59, indicating that our technique (Immunodot) has relatively good performance. The likelihood ratio of 41% means that a positive result from the Immunodot has a probability of being truly positive 41 times over. This value is relatively low, which could limit the clinical utility of the Immunodot. There is satisfactory reliability since we found a kappa coefficient of 0.80 and a weak to moderate correlation with ELISA, as indicated by Matthew's correlation coefficient of 0.315.

When examining our study results, it is important to consider existing limitations. First, it should be noted that the studied sample was relatively small, which could limit the generalizability of our conclusions. Additionally, we did not have access to all clinical data from patients with celiac disease, such as symptoms and medical history. This may impact result interpretation and underscores the importance of having complete access to all relevant information when analyzing a complex disease like celiac disease. Despite these limitations, our study could contribute to advancing research on this condition and help inform future medical decisions.

V. CONCLUSION

This study highlighted a significant discrepancy between the results obtained by the Immunodot test and the ELISA for measuring IgA anti-tTG antibodies. Nevertheless, the Immunodot demonstrated a negative predictive value (NPV) of 73%, suggesting that it may be used as an alternative screening test for celiac disease. However, its low sensitivity indicates that results should be interpreted with caution. Further studies are needed to confirm these findings and evaluate the performance of these tests on larger and more diverse population samples.

ACKNOWLEDGEMENTS

➢ *Conflict of Interest.*

We declare that there are no conflicts of interest to disclose.

➢ *Authors Contribution.*

The authors of this article have made significant contributions to the design, data collection, analysis, and manuscript writing. Their individual contributions are as follows:

Aazzane Oussama: Writing - original version, Conceptualization, Methodology, Data collection. **Bazhar Hasnaa:** Formal analysis, Visualization. **Fellah Hassan:** Investigation, Supervision, Conceptualization, Methodology, Validation, Revision and Editing.

➢ *Availability of Data*

The data used in this research is available upon request from the authors (Oussama Aazzane, and Hassan Fellah).

REFERENCES

[1]. Tasuku T,Kenji I. Celiac Disease Genetics, Pathogenesis, and Standard Therapy for Japanese Patients. International Journal of Molecular Sciences. 2023 Jan 20 ;24(3) :2075. doi: 10.3390/ijms24032075.

[2]. KHATER S, CELLIER C. Celiac disease: diagnosis and treatment. Journal of Medical Biology. 2020.

[3]. National Institutes of Health consensus development conference statement on celiac disease, June 28–30, 2004. Gastroenterology. 2005; 128(4 Suppl 1):S1–9. doi: 10.1053/j.gastro.2005.02.007.

[4]. Fasano A, Catassi C. Clinical practice. Celiac disease. The New England Journal of Medicine. 2012 ; 367(25) :2419–26. doi.org/10.1056/NEJMcp1113994.

[5]. Kelly CP, Bai JC, Liu E, Leffler DA. Advances in diagnosis and management of celiac disease. Gastroenterology. 2015; 148(6):1175–86. doi: 10.1053/j.gastro.2015.01.044.

[6]. Malamut G, Cellier C. Celiac disease. EMC. 2016. doi: 10.1016/S1634-6939(16)60111-4.

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576664

[7]. Husby S, Koletzko S, Korponay-Szabó I, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. Journal of Pediatric Gastroenterology and Nutrition. 2020 Jan;70(1):141-156. doi: 10.1097/MPG.0000000000002497.

[8]. Khadiry B, Fellah H, et al. Evaluation of the ELISA technique compared to Immunodot in the screening of extractable nuclear antigens antibodies Sm, SSA and SSB. International Journal of Innovation and Applied Studies. 2022, May, pp. 493-501.

[9]. Ousmane S, El Fakir S, El Rhazi K, et al. Estimating the roc curve. Moroccan Journal of Public Health. 2015, vol 2, n° 2.

[10]. Schaefer L, Dyke J , Meglio F, et al. Evaluation of microparticle enzyme immunoassays for immunoglobulins G and M to rubella virus and Toxoplasma gondii on the Abbott IMx automated analyzer. Journal of Clinical Microbiology.1989 Nov; 27(11):2410-3. doi: 10.1128/jcm.27.11.2410-2413.1989.