Detection of Anti-GAD65 Antibodies in Sera of Diabetic Patients Using a Home-Made Latex Agglutination Kit

Alaa S. Alattabi[1], Dhamiaa M. Hamza[1], Khalid Khalil Alaaraji[1], Sawsan M. Jabbar[1], Abeer Thaher Naji AL-Hasnawi[1], Ali Mansoor Jasim[1]

[1]*Assist. Prof., Kerbala University, College of Medicine*

Abstract

Objectives: Compare sensitivity of anti-GAD65 result detected by ELISA with that one detected by home-made latex agglutination kit.

Patients and Method: Thirty seven patients were enrolled in this study from January, 2019 to March, 2019. The sample had been taken from patients attended diabetic unit in Imam Hussain medical city in kerbala. Informed consent was taken from each participant in the study. Serum samples were tested by ELISA technique (GAD65 ELISA Kit, CUSABIO BIOTECH CO., LTD. USA) and by home-made latex agglutination for anti-GAD 65.

Results: Data from the current study showed that readings of the home-made latex test are consistent with those detected by ELISA as a confirmatory test. When analyzed, results showed sensitivity 94.44%specificity 78.95%, positive predictive value 80.95,negative predictive value 3.75%, positive likelihood 4.49, negative likelihood 0.07.

Conclusion: This home-made latex agglutination kit sensitive as ELISA could be used as a reliable test to detect anti GAD65 in diabetic patients.

Keywords: *Homemade latex kit, Anti-GAD56 antibody, LADA.*

Introduction

Diabetes mellitus is a long standing metabolic disorder due to insufficient insulin secretion or no response for insulin result in increased blood glucose level leading to multiple signs and symptoms[1,2]. From etiological aspect Diabetes mellitus divide into four classes, class 1 diabetes mellitus,class 2 diabetes mellitus,gestational diabetes and other class of diabetes. The most common classes of diabetes are class 1 and class 2[3,4]. Regarding class latent autoimmune diabetes in adult (LADA), clinically similar to class 2 diabetes but still has shared character with class 1 diabetes which has most autoantibodies present in class 1 diabetes[5,6]. Age of onset is commonly start after age of 35 years, at the beginning there is no need for insulin treatment[7]. The latent autoimmune diabetes in adult constitute around ten to thirty percent of classes 2 diabetes also refer as one and half class of diabetes or class 1.5 diabetes[5,8]. One of these antibody is **Glutamic acid decarboxylase autoantibodies (GADA)** which is neither beta cell nor islet specific, GADA firstly discovered in the serum of patients complaining from stiff man syndrome which is a rare neurological disorder[9]. In the neurons and pancreatic beta cells the glutamic acid convert to g-aminobutyric acid (GABA) by enzyme Glutamic Acid Decarboxylase[10]. Which can be measured to evaluate human disease[11] by different method like ELISA . Using latex particles coated with IgG is well known test currently applied for immunoassay investigation. In this study we compare sensitivity of GAD 65 result detected by ELISA with that one detected by home made latex agglutination kit.

Corresponding Author:
Alaa S. Alattabi
Assist. Prof., Kerbala University, College of Medicine
e-mail: alattabi486@gmail.com

Patients and Method:

Thirty seven patient were enrolled in this study from January, 2019 to March,2019 all patients were attended diabetic unit in Imam Hussain medical city Ethically, all consents from patients and kerbala health director had been taken .

Homemade latex agglutination test kit preparation:

A homemade latex agglutination test kit for the detection of anti-GAD 65 antibodies in serum was developed as follows:

1. **Latex beads preparation:** These were purified from a latex kit for testing C-reactive protein (CRP). Simply, the beads were separated from the CRP antibody by elevating the pH (up to 9) by adding 1 M NaOH solution to the latex-antibody complex of the CRP kit content. Thereafter, incubation for 1 hour at 37 ^{0} C was done. This was shown to be enough to unbind the antibody from the latex beads. In order to purify the beads, mixture was centrifuged 10000 Xg for 30 minutes. Then, we got a clear supernatant and a pellet of beads which were harvested by discarding the supernatant. This was followed by three times wash-spin step with PBS and the beads were suspended in PBS to be read ready to bind the new antibody.A test was done for CRP using the positive control of CRP kit content and the reading was negative. This step was done to ensure that no anti-CRP antibody remnant was left behind.
2. **Preparation of latex-antibody solution:** The detection antibody of anti-GAD 65 was ordered from (Abcam). Beads were mixed with the antibody (1 microgram/ml final conc.), pH was optimized at 7, and the mixture was incubated at 37^{0}C for 20 hours. After that, the mixture was ready for use as latex agglutination test reagent for the detection of anti-GAD 65 antibodies and stored at 4^{0}C. **[(**Hideki et al. 2000), with some modifications]$^{(12)}$.

Homemade latex agglutination test kit evaluation: A homemade latex agglutination test kit for serum detection of anti-GAD 65 antibodies was evaluated together with a commercially available enzyme-linked immunosorbent assay (ELISA) kit for their use for detection of anti-GAD 65. All patient serum samples were tested by ELISA technique (GAD65 ELISA Kit, CUSABIO BIOTECH CO.,LTD.USA) and by home-made latex agglutination for anti-GAD 65.

Statistical Analysis: To confirm the home-made latex kit results with those obtained by ELISA. Indicators like specificity, sensitivity, predictive values and likelihood determination were obtained when readings of both kits were analyzed using the biostatistic software Graph Pad Prism and Bayes Theorem Online Calculator.

Results

Data from the current study showed that readings of the home-made latex test are consistent with those detected by ELISA as a confirmatory test. Number of patients that showed positive anti-GAD65 result for latex technique were 21 of 37 patients while those positive GAD65 tested by ELISA technique were 18 of 37 patients as showed in table (1). Number of patients that showed positive serum for anti-GAD65 antibody by both technique latex and ELISA were 17 patients while those showed positive serum for anti-GAD 65 antibody by latex technique only and negative for ELISA were 4 patients and only one patients positive serum for anti-GAD 65antibody by ELISA technique only as showed in table (2) . Sensitivity 94.44%, specificity 78.95%, positive predictive value 80.95%, negative predictive value 93.75%, positive likelihood 4.49, negative likelihood 0.07, accuracy 86.49%.

Table (1): Seropositivity for Anti- GAD 65 antibody

Anti-GAD65 +ve by ELISA	Anti-GAD 65 -ve by ELISA	Anti-GAD65 +ve by latex	Anti-GAD65 -ve by latex
18	19	21	16

Table (2): Seropositivity for anti-GAD 65 antibody by latex and ELISA technique

	Latex+ve anti-GAD 65	Latex-ve GAD 65	P value
ELISA+ve GAD65	17	1	0.00001
ELISA-ve GAD 65	4	15	

	Latex+ve anti-GAD 65	Latex-ve GAD 65	P value
Sensitivity of latex/ELISA	94.44%		
Specificity	78.95%		
Positive likelihood ratio	4.49		
Negative likelihood ratio	0.07		
Positive predictive value	80.95		
Negative predictive value	93.75%		
Accuracy	86.49%		

Discussion

This homemade rapid latex test provide fast results, less expensive than ELISA and can easily be applied in routine laboratories in order to detect anti-GAD 65 antibodies thus facilitating the diagnosis of diabetes mellitus with a relatively high level of reliability

Conclusion

This home-made latex agglutination kit sensitive as ELISA could be used as a reliable test to detect anti GAD65 in diabetic patients.

Recommendation: It is highly recommended to follow this technique in order to prepare homemade latex agglutination test kits for the detection of other important diagnostic and prognostic markers in diabetes and even other clinical conditions.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1. Jayakumar A. Greenspan's Basic and Clinical Endocrinology. The Yale Journal of Biology and Medicine. 2012 Dec;85(4):559.
2. American Diabetes Association. Standards of medical care in diabetes--2014. Diabetes care. 2014 Jan;37:S14.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2009 Jan 1;32(Supplement 1):S62-7.
4. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee (2013). Definition, diagnosis and classification of diabetes mellitus prediabetes and metabolic syndrome 2013; 37: S8-S11.
5. Stenström G, Gottsäter A, Bakhtadze E, Berger B, Sundkvist G. Latent autoimmune diabetes in adults: definition, prevalence, β-cell function, and treatment. Diabetes. 2005 Dec 1;54(suppl 2):S68-72.
6. Krause S, Landherr U, Agardh CD, Hausmann S, Link K, Hansen JM, Lynch KF, Powell M, Furmaniak J, Rees-Smith B, Bonifacio E. GAD autoantibody affinity in adult patients with latent autoimmune diabetes, the study participants of a GAD65 vaccination trial. Diabetes Care. 2014 Jun 1;37(6):1675-80.
7. Goel A, Chiu H, Felton J, Palmer JP, Brooks-Worrell B. T-cell responses to islet antigens improves detection of autoimmune diabetes and identifies patients with more severe β-cell lesions in phenotypic type 2 diabetes. Diabetes. 2007 Aug 1;56(8):2110-5.
8. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM. Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age?. Diabetes. 2005 Dec 1;54(suppl 2):S62-7.
9. De Aizpurua HJ, Wilson YM, Harrison LC. Glutamic acid decarboxylase autoantibodies in preclinical insulin-dependent diabetes. Proceedings of the National Academy of Sciences. 1992 Oct 15;89(20):9841-5.
10. Sorenson RL, Garry DG, Brelje TC. Structural and functional considerations of GABA in islets of Langerhans: β-cells and nerves. Diabetes. 1991 Nov 1;40(11):1365-74.

11. Pihoker C, Gilliam LK, Hampe CS, Lernmark Å. Autoantibodies in diabetes. Diabetes. 2005 Dec 1;54 (suppl 2):S52-61.

12. Kohno H, Akihara S, Nishio O, Ushihima H. Development of a simple and rapid latex test for rotavirus in stool samples. Pediatrics International. 2000 Aug 16;42(4):395-400.