

Levels of IgG-ADV and Tnf α in Serum of Celiac Disease Patients

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Abstract

Original Research Article

Celiac disease is one of the commonest autoimmune diseases in digestive system, in which genetic predisposition play a key role in initiation and development of this disease. Also, viruses and cytokines lead to trigger and precipitate development of CD. The results of this disease is intestinal damage when gluten digesting. Its prevalence is one out of 100 person worldwide. The current study aims to estimation the levels of IgG-Adenovirus and tumor necrosis factor- α antibodies in serum of fifty eight unknown celiac disease patients etiology and twenty seven healthy cases as control group. The results showed significant increase the levels of IgG-ADV and TNF α in CD patients when comparing with control group. As well as, the same results showed the patients males and females have significantly higher levels of IgG-ADV and TNF α than control group. In conclusion, our results indicate that IgG-ADV and TNF- α are play an important roles in trigger and development of celiac disease, however there are other genes are associated with CD trigger and development specially in children..

Keywords: Celiac Disease, ADV, TNF α , autoimmune Disease, inflammation.

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INTRODUCTION

Celiac disease (CD) is associate inflammatory condition of the small intestine, triggered by the bodily function of gluten. It's the one most typical genetically food-based intolerance, the foremost common chronic diseases in childhood [1, 2]. Gluten may be a water insoluble protein complex that is found in wheat, barley and rye [3]. Tumor necrosis factor (TNF α) is one amongst the cytokines family that is concerned within the regulation of the immunologic response. TNF α gene set at intervals category III of Major Histocompatibility Complex (MHC) on chromosome 6p21.3. The foremost extensively investigated polymorphism was that within the promoter region at -308 positions. TNF α -308 G/A polymorphism have higher activity with A allele than G allele. Variety of studies incontestable a major association of this polymorphism with CD in numerous populations [4]. Celiac disease or gluten intolerance is commonly triggered by viral infections, caused by and Adenovirus 12 virus. There are many authors say that the sickness becomes active once such infections. Viral agent acting through different pathogenic mechanisms, such as molecular mimicry that ends in modulation of immune tolerance of the host organism. Our study aims to estimation the levels of IgG-Adenovirus and TNF α antibodies in serum of unknown CD patients etiology.

Aim and objectives

Study the relationship between Tissue transglutaminase and anti-gliadin antibodies and TNF α and Adenovirus antibodies in CD patients normal individuals.

MATERIALS AND METHODS

Five ml of celiac disease serum were collected from 58 patients who attending Al Kut Hospital in Wasit Province, Iraq. All patients were diagnosed and confirmed as celiac disease patients by specialized physicians and pathologists. Celiac disease patients were screening and diagnosed according to standard criteria, then divided according to gender.

Human Adenovirus (ADV-IgG) test

This test was done according to the instructions of manufacture company (QAYEE-BIO)

Principle

This test depends on a double antibodies sandwich enzyme-linked immunosorbent assay (ELISA) in one-step process to determine the level of Anti Adenovirus IgG (ADV-IgG) in serum samples.

Procedure

All reagents were pre-warmed at room temperature. Amount of fifty μ L of blanks, reference

sera, negative control and diluted patient sera (1:5) were pipetted into the wells. 50 µL of conjugate (HRP + Ab) were added into each well, except blank well. Then the plate sealed, and gently shaken, then incubated for hour at 37 °C.. The excess liquid was discarded and therefore the wells were filled with diluted washing liquid then mixed and shake for 30 seconds, after that washing liquid was discarded and the plate taped into absorbent papers to dry. This washing procedure was repeated 5 times. 50 µL of chromogen solution A were added, and then fifty µL of chromogen solution B were added to each well and shaken gently and incubated for ten minutes at 37°C away from light. Amount of 50 µL of stop solution was pipetted into the plate wells and shaken gently and incubated for 5 minutes. Read by ELISA reader at 450 nm wavelength.

Human Tumor Necrosis Factor Alpha (TNF- α) test

This test was done according to the instructions of manufacture company (Elabscience).

Principle

This test depends on double antibodies Sandwich-ELISA in one-step process to determine the level of TNF- α protein in serum samples. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm. The OD value is proportional to the concentration of Human TNF- α . You can calculate the concentration of Human TNF- α in the samples by comparing the OD of the samples to the standard curve.

Procedure

All reagents were pre-warmed at room temperature. 100µL standard or samples were pipetted to each well. The plate was incubated 90 minutes at 37°C. The liquid removed. 100µL Biotinylated Detection Ab pipetted to each well. The plate was incubated for 1 hour at 37°C. The plate was washed 3 times. 100µL HRP Conjugate pipetted to each well. The plate was incubated then for 30 minutes at 37°C. The plate was washed 5 times. 90µL Substrate Reagent pipetted to each well. The plate was incubated for 15 minutes at

37°C. 50µL Stop Solution pipetted to each well. Read at 450nm by ELISA reader.

RESULTS AND DISCUSSION

Human Adenovirus (IgG-ADV)

Although table (1) showed no significant difference between patients and control group regarding the number of the individuals which infected with virus, table (2) displayed the significant difference between both groups regarding the concentration level of IgG-ADV antibody.

Table (1) showed 17 (29.3%) of CD patients were infected with ADV and 7 (25.9%) of control group has ADV.

It's well known, CD is an autoimmune disease caused by genetic default and some environmental factors, however, viruses play important role in trigger of some diseases. In later decade many researchers study the possible relationship between CD and viruses. Several studies achieved about the role of viral infections, mainly intestinal adenovirus and enterovirus in CD, one study was revealed that viral infections lead to induce type 1 interferons [5], which lead to break tolerance and precipitate in initiation of CD in mice [6].

In study achieved on 80 CD Romanian children, the result found 25 patients (31.2%) of them, were with IgA-ADV [7]. On the other hand, indicated in their study, it unlikely that Ad 12 is implicated in the coeliac disease pathogenesis [8].

Another study reported that ADV was persistence in the 31% of GI tract of identified [9]. New study using RT-PCR technique was found that adenovirus not associated with celiac disease [10].

As shown in table (2), the mean value of IgG-ADV antibody in celiac disease patients was 45.32 pg / ml, while the mean value of IgG-ADV in control group was 0.28 pg / ml. Statistical analysis of data by using t-test between the two groups showed a significant increase value of IgG-ADV in celiac disease patients compared to control group, (P \leq 0.004).

Table-1: The number of CD patients and control group infected with IgG-ADV

Cases	Patients	Control group
Total	58 (100%)	27 (100%)
Positive No. %	17 (29.3%)	7 (25.9%)
Negative No. %	41 (70.7%)	20 (74.1%)

This increase may be due to the effect of ADV, that can induce adaptive and innate immune responses in infected tissues [11], as well as, due to homologous sequence between a 12 amino acid from the adenovirus 12 E1B protein and protein A-gliadin.

Moreover, we showed the same significantly results (p<0.05) between patient males and control group, patient females and control group. The same

results are reported by other study done by Gabriel *et al.* who they showed the most CD patients in their study with IgA-ADV were females [7].

Recently several studies carried out about the relation between IgG-ADV and celiac disease patients, and showed no significant association between CD patients and ADV infection. No significant relationship between patients with CD and healthy group [12].

Another study also showed no significant difference between celiac patients and control group in relation

IgG-ADV [13].

Table-2: The level of IgG-ADV antibody concentration in total, females and males in CD patients and control group

Cases	N.	Mean pg/ml	Std.Deviation	Std.Error	P value
Patients	58	45.32	111.05	14.84	P<0.004 (S)
Control group	27	0.28	0.23	0.04	
Patient males	27	67.89	136.58	27.31	P<0.02 (S)
control group	12	0.31	0.25	0.07	
Patient females	31	27.12	83.18	14.93	P<0.08 (N.S)
control group	15	0.25	0.22	0.05	
Males	27	67.89	136.58	27.31	P<0.1 (N.S)
Females	31	27.12	83.18	14.93	

Tumor Necrosis Factor Alpha (TNF- α)

Table (3) refers to the expression of TNF- α in 58 CD patients with mean 84.75 and 27 individuals as

control group with mean 6.10. There was a significant increase of TNF- α mean in celiac patients compared to control group, with significantly difference (P \leq 0.001).

Table -3: The level of TNF- α in total and subclasses of CD patients and control group

TNF- α	N	Mean	Std. Deviation	Std. Error Mean	P value
Patients	58	84.75	79.35	16.91	P \leq 0.005 (S)
Control group	27	6.10	1.78	1.19	
Patient males	27	111.88	105.41	31.78	P \leq 0.006 (S)
control group	12	0.11	0.07	0.01	
Patient females	31	57.61	22.19	6.69	P \leq 0.001 (S)
control group	15	0.10	0.06	0.01	
Males	27	0.11	0.071	0.013	P \leq 0.5 (N.S)
Females	31	0.10	0.067	0.012	

As shown in table (3), the results showed increase the level of TNF- α with highly significant differences between total patients and control group (P \leq 0.005), patient males and control group (P \leq 0.006) and between patient females and control group (P \leq 0.001). It is well known, TNF α play an important role in inflammation, and their genes associated with susceptibility to several autoimmune diseases [14]. CD as autoimmune disease is genetic susceptible, and there is a region located on TNF α was associated with celiac disease.

Marafini *et al.* reported in their study both TNF- α and IFN- γ -producing ILCs were significantly increased in patients when compared with control group [15]. De Albuquerque *et al.* suggested in their study that TNF- α (-308 G>A) polymorphism lead to development of CD in Italian patients [16]. Another study concluded that the TNF- α -308 G>A gene polymorphism significantly contributes to CD susceptibility [17]. In the opposite direction, Klemenak *et al.* Observed TNF- α level was significantly decreased in children with CD [18]. On the other hand, some studies recorded there was no association between level of TNF- α and its gene polymorphisms with celiac disease susceptibility in Iranian population [19]. Al Obeed *et al.* study the gene expression of TNF- α in colorectal cancer and showed overexpression of TNF- α in colorectal cancer

than seen in adjacent normal colorectal tissue ,and suggesting that TNF- α is promising prognostic tool for colorectal cancer [20]. In USA researchers concluded that celiac disease patients have an increased risk for developing small intestinal malignancies [21].

In conclusion, our results indicate that ADV and TNF- α are play an important roles in trigger and development of celiac disease, however there are other genes are associated with CD trigger and development specially in children.

CONCLUSIONS

- There is an association between TNF- α cytokine aids in development of CD.
- There is an association between adenovirus infection and celiac disease development.

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REFERENCES

1. Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, Danielsson L, Halvarsson B, Hammaroth S, Hernell O, Karlsson E. Prevalence

- of childhood celiac disease and changes in infant feeding. *Pediatrics*. 2013 Mar 1;131(3):e687-94.
2. Guandalini S & Assiri A. Celiac Disease. *JAMA Pediatrics*. 2014; 168(3), 272.
 3. Fasano A, Catassi C. Clinical practice. Celiac disease. *N Engl J Med*. 2012; 367: 2419-2426.
 4. El-Akawi ZJ, & Mansour AS. The role of cytotoxic T-Lymphocyte associated antigen 4 (CTLA4) +49A/G and tumor necrosis factor alpha (TNF- α) -308G/A polymorphism in the development of Celiac Disease in Jordanian patients. *Journal of Health Science*. 2010; 56(1), 41-46.
 5. Devendra D and Eisenbarth GS. Interferon alpha β a potential link in the pathogenesis of viral-induced type1 diabetes and autoimmunity. *Clin Immunol*. 2004; 111(3):225 \pm 33.
 6. Brown JJ, Jabri B, & Dermody TS. A viral trigger for celiac disease. *PLOS Pathogens*. 2018; 14(9), e1007181.
 7. Gabriel S, Alexandru P, Nicolae M, Mariana A, Victor C, Doru D. Adenoviruses in celiac disease in children. *Revista ROMÂNĂ DE BOLI infecțioase*. 2011;14(1)
 8. Lawler M, Humphries P, O'Farrelly C, Hoey H, Sheils O, Jeffers M, O'Briain DS, Kelleher D. Adenovirus 12 E1A gene detection by polymerase chain reaction in both the normal and coeliac duodenum. *Gut*. 1994 Sep 1;35(9):1226-32.
 9. Kosulin K, Geiger E, Vécsei A, Huber WD, Rauch M, Brenner E, Wrba F, Hammer K, Innerhofer A, Pötschger U, Lawitschka A. Persistence and reactivation of human adenoviruses in the gastrointestinal tract. *Clinical Microbiology and Infection*. 2016 Apr 1;22(4):381-e1.
 10. Kahrs CR, Chuda K, Tapia G, Stene LC, Mårild K, Rasmussen T, Rønningen KS, Lundin KE, Kramna L, Cinek O, Størdal K. Enterovirus as trigger of coeliac disease: nested case-control study within prospective birth cohort. *bmj*. 2019 Feb 13;364:1231.
 11. Yamamoto Y, Nagasato M, Yoshida T, Aoki K. Recent advances in genetic modification of adenovirus vectors for cancer treatment. *Cancer science*. 2017 May;108(5):831-7.
 12. Tarish HR, Hameed WS, Abdul-Mehdi RJ & Alsherees HAA. (). Role of previous adenovirus infection and its association with IFN- α in occurrence of Celiac Disease in Iraqi patients. 2016; 4(4): 10326-10330.
 13. Abdul-Mehdi RJ. HLA-DQ genotyping and its relationship to some immunological and microbial factors in patients with celiac disease in Najef province. M.Sc. thesis Department of Med. Microbiology University of AL Kufa. 2016. Iraq
 14. Garrote JA, Arranz E, Tellería JJ, Castro J, Calvo C, Blanco-Quirós A. TNF α and LT α gene polymorphisms as additional markers of celiac disease susceptibility in a DQ2-positive population. *Immunogenetics*. 2002 Nov 1;54(8):551-5.
 15. Marafini I, Monteleone I, Di Fusco D, Cupi ML, Paoluzi OA, Colantoni A, Ortenzi A, Izzo R, Vita S, De Luca E, Sica G. TNF- α producing innate lymphoid cells (ILCs) are increased in active celiac disease and contribute to promote intestinal atrophy in mice. *PloS one*. 2015 May 7;10(5):e0126291.
 16. De Albuquerque Maranhão RM, Martins Esteves FA, Crovella S, Segat L, Eleuterio Souza PR. Tumor necrosis factor- α and interleukin-6 gene polymorphism association with susceptibility to celiac disease in Italian patients. *Genetics and Molecular Research*. 2015;14(4):16343-52.
 17. Khan S, Mandal RK, Jawed A, Dar SA, Wahid M, Panda AK, Areeshi MY, Khan ME, Haque S. TNF- α -308 G> A (rs1800629) Polymorphism is Associated with Celiac Disease: A Meta-analysis of 11 Case-Control Studies. *Scientific reports*. 2016 Sep 6;6:32677.
 18. Klemenak M, Dolinšek J, Langerholc T, Di Gioia D, Mičetić-Turk D. Administration of *Bifidobacterium breve* Decreases the Production of TNF- α in Children with Celiac Disease. *Digestive diseases and sciences*. 2015 Nov 1;60(11):3386-92.
 19. Barartabar Z, Nikzamir A, Sirati-Sabet M, Aghamohammadi E, Chaleshi V, Nejad MR, Asadzadeh-Aghdai H, Zali MR. The relationship between 174 G/C and-572 G/C of IL-6 gene polymorphisms and susceptibility of celiac disease in the Iranian population. *Przegląd gastroenterologiczny*. 2018;13(4):293.
 20. Al Obeed OA, Alkhalaf KA, Al Sheikh A, Zubaidi AM, Vaali-Mohammed MA, Boushey R, Mckerrow JH, Abdulla MH. Increased expression of tumor necrosis factor- α is associated with advanced colorectal cancer stages. *World Journal of Gastroenterology: WJG*. 2014 Dec 28;20(48):18390.
 21. Green PH, Stavropoulos SN, Panagi SG, Goldstein SL, McMahan DJ, Absan H, Neugut AI. Characteristics of adult celiac disease in the USA: results of a national survey. *The American journal of gastroenterology*. 2001 Jan 1;96(1):126-31.