International Journal of Celiac Disease, 2018, Vol. 6, No. 2, 42-46
Available online at http://pubs.sciepub.com/ijcd/6/2/6

DOI:10.12691/ijcd-6-2-6

Serum Level of Tumor Necrosis Factor-α and Its Gene Polymorphisms Has No Association with Susceptibility to Celiac Disease in Iranian Population

Zohreh Nasiri[1], Abdolrahim Nikzamir[1], Mohammad Rostami-Nejad[2,*], Majid Sirati -Sabet[1], Elham Aghamohammadi[3], Vahid Chaleshi[3], Mohammad Reza Zali[2]

[1]Department of clinical Biochemistry, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
*Corresponding author: m.rostamii@gmail.com

Abstract **Background:** TNFα is an important cytokine in celiac disease which causes the destruction of the intestinal tissue. It has been shown that polymorphisms in the promoter region of the TNFα gene increase its expression. In this study we investigated the -1031 C/T and -376 G/A gene polymorphisms of TNFα gene and also its serum level in Iranian celiac patients compared to healthy controls. **Methods:** In this cross sectional study, 104 newly diagnosed celiac disease and 102 healthy control were recruited during 2016. The DNA was extracted from peripheral blood. Specific primer pairs were designed and TNFα polymorphisms was determined by polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism (RFLP). For confirmation of finding some samples were randomly sequenced. Also to determine the serum concentration of TNFα the ELISA method was used. **Result:** The mean age of cases and control was 32, 33 years respectively. No significant difference was observed between CD patients and healthy controls regarding -1031 C / T and -376 G / A gene polymorphisms (for -376 G/A; p= 0.8 and for -1031 C/T; p=0.35 respectively). Also there was no significant difference in serum level of TNFα between celiac patients and healthy subjects (p= 0.18). **Conclusion:** According to our results, it seems that the TNF-α (-1031 C/T, -376 G/A) gene polymorphisms in combination with serum level may not be associated with the risk of celiac disease.

Keywords: *celiac disease, PCR-RFLP, TNFα gene polymorphism*

Cite This Article: Zohreh Nasiri, Abdolrahim Nikzamir, Mohammad Rostami-Nejad, Majid Sirati-Sabet, Elham Aghamohammadi, Vahid Chaleshi, and Mohammad Reza Zali, "Serum Level of Tumor Necrosis Factor-α and Its Gene Polymorphisms Has No Association with Susceptibility to Celiac Disease in Iranian Population." *Journal Name*, vol. 6, no. 2 (2018): 42-46. doi: 10.12691/ijcd-6-2-6.

1. Introduction

Celiac disease as an autoimmune disease caused by the use of special protein called gluten, that is commonly found in wheat, rye and barley, in genetically susceptible individuals [1]. Exposure to gluten leads to several autoantibodies that cause small bowel mucosa abnormality [2]. Following strict gluten-free diet, intestinal injuries include villous atrophy and crypt hyperplasia, can be improved [3]. Although the HLADQ2 and HLADQ8 molecules are known to be the most important genetic factor in celiac disease, but the role of other genetic factors that contribute to the onset of CD disease is highlighted [4]. TNFα is known as an important cytokine in the pathogenesis of celiac disease [5]. Investigation showed that, exposure of the mucosa to gluten causes the expression of TNF-α in CD patients [6]. TNFα plays an important role in damaging the intestinal tissue of patients with celiac disease [7]. Gliadin, which is derived from gluten digestion in the intestinal wall, is metabolized by the tissue transglutaminase enzyme. In this process, the glutamine roots are replaced by glutamic acid. These parts are attached to the HLADQ2 and HLADQ8 molecules of the APC surface and are provided to CD4+ T cells [8]. By activating Th1 with CD4+ T cells and secreting TNFα from TH1, the fibroblasts of the intestine is affected by this cytokine, which results increased metalloproteinase secretion and destruction of the intestinal tissue [9]. Also the TNFα gene is located between the HLA class I and class II genes [10] and its expression is adjusted at the transcriptional level [11]. It has been reported that sequence diversity in the regulatory region of this gene is associated with autoimmune diseases [12].

In addition, it has been shown that there are several polymorphisms in the promoter region of this cytokine they are effective to increasing its secretion [7]. A large number of single nucleotide polymorphisms in the upstream region of the TNFα gene are located at -1031

851, -419, -376, -308, -28, -863, -857, -49 and -49 in relation to the initiator region of the translation [13]. Considering the effect of promoter polymorphisms on the expression of TNFα gene and the role of this cytokine in the pathogenesis of celiac disease, the aim of this study is to determine the association between polymorphisms -376 G>A(rs1800750) and -1031 C>T (rs1799964) in the promoter region of TNFα gene and CD and also its serum level in Iranian population.

2. Material and Methods

2.1. Patients

In this case- control study, proven celiac disease were enrolled as a patient group using following inclusion criteria: seropositive patients whom were confirmed by histopathological examination. Patients with other autoimmune diseases such as IBD and type 1 diabetes were excluded from study. Also negative celiac disease during endoscopy and serology and without family history of celiac disease were included as control.

Both groups were selected at the age range of 18-70 years without any sexual limitation. Patients have signed the consent form and study was approved by the Ethics Committee of Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Blood Collection and Genotyping

Blood samples (10 ml) were collected from both groups, DNA was extracted from PBMC cells using the commercial kit (Invitrogen, USA) according to manufacture instructions. For the two promoter regions -376 G/A (rs1800750) and -1031C/T (rs1799964), the appropriate primer pairs were designed using Gene Runner software. -376 G/A polymorphism was amplified using following primer pairs: forward: ACACAGCTTTTCCCT CCAAC and revers: GGACACACAAGCATCAAGGA and forward: TCTGTCTGGCTGAGGATTTC and reverse: TCTCCTACCCATTGCTGTG primer pairs were used for amplification of -1031 C/T (rs1799964). Proliferation of DNA was performed using the PCR technique (Table 1).

Table 1. Materials used for PCR reaction Polymerization rs1799964 C/T and rs1800750 G/A

materials	Volume in reaction	Ultimate concentration
10x PCR Buffer*	2.5 µl	1x
50mM $MgCl_2$	0.75 µl	1.5mM
10mM dNTP Mix	0.5 µl	0.5mM
Primer F	0.5 µl	10 pmol
Primer R	0.5 µl	10 pmol
Taq DNA polymerase	0.5 µl	1.25 Unit*
Template DNA	1µl	100ng
Sterile dd water	25 µl	

The length of the PCR products for rs1799964 and rs1800750 was 763bp and 292 bp, respectively. The accuracy of the PCR product was confirmed by electrophoresis 1. % agarose gel. 10 µl of The PCR product of -376 G/A (rs1800750) was digested at 37°C for 2 hours with mluc I restriction enzyme (New England Biolabs). 10 µl of The PCR polymorphism-1031C/T product under the action of bbsI enzyme (New England Biolabs) was digested at 37 ° C for 1 hour. The products of the RFLP was analyzed by electrophoresis 3. % agarose gel. The TNFα serum level was also measured using the ELISA kit (Invitrogen by Thermo Fisher Scientific).

2.3. Statistical Analysis

We used the SPSS version 18.0 in order to statistical analysis of results. The chi-square (χ^2) test was used for the calculation of genotypic and allelic frequency. If $P \leq 0.05$ the differences was considered significant.

3. Result

Out of 104 celiac disease patients (mean age 32 years old and mean BMI: 23), 38 (36.5%) were male and 66 (63.5%) were female. Of the 102 healthy individuals (mean age= 33 year old; and mean BMI: 24), 56 (54.9%) were female and 46 (45.1%) were male. Most predominant symptom in case group was anemia (68.3%) followed by bloating (35.6%) and abdominal pain (29.8%) and between control group anemia (84.3%), neurological problems (20.6%) and bloating (20.6%) were more frequent. Demographic and clinical presentation were illustrated in Table 2.

Table 2. Demographic data and clinical presentation in the patient and control group

Clinical presentation	Case n=104 (%)	Control n=102 (%)
BMI	23	24
age	32	33
sex		
male	38 (36.5)	46 (45.1)
female	66 (63.5)	56 (54.9)
Diarrhea	18 (17.3)	9(8.8)
Bloating	37(35.6)	21(20.6)
Abdominal pain	31(29.8)	18(17.6)
Anemia	71(68.3)	86(84.3)
Bone diseases	8(7.7)	0
Neurological defects	24(23.1)	21(20.6)
Menstrual problems	2(1.9)	1(1)
Infertility	4(3.8)	4(3.9)
Abortion	4(3.8)	3(2.9)

Allele and genotype frequency of rs17799964 and rs1800750 were studied in patients with celiac disease in compared to control group. For rs1800750 polymorphism, three different bands derived from MLUCI digestion including GG (292bp), TG (292bp, 101bp, and 191bp), TT (101bp, 191bp) were observed on electrophoresis gel, but only GG and TG genotypes were detected (Figure 1). There was no a significant correlation between the allele

and genotype frequency of the rs1800750 polymorphism and CD compared with the control group (p = 0.82, p=0.8 respectively).

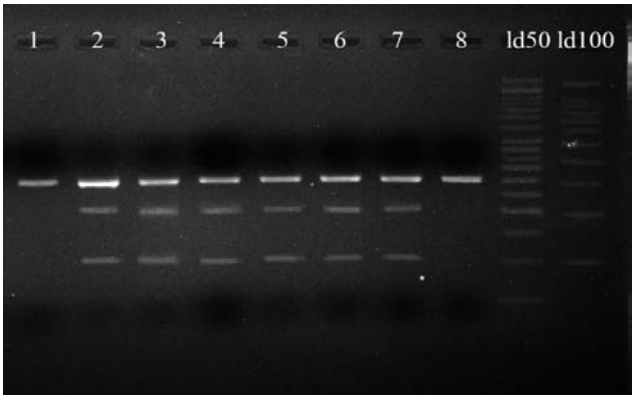

Figure 1. Gel electrophoresis of enzymatic digestion on agarose gel 3% for rs1800750. Sample 1 and 8 were not cleavage by restriction enzyme. Samples 2-7 were cleavage with restriction enzyme

For rs1799964 polymorphism, three different bands including TT (763bp), CC (634bp- 129 bp), CT (763bp-634bp-129bp) were observed on electrophoresis gel using BBSI digestion enzyme. The digested products were separated on 3% gel electrophoresis (Figure 2). Also, no significant correlation was observed between allele and genotype frequency of the rs1799964 polymorphism and CD compared with the control group (p = 0.07, p=0.35 respectively) (Table 3, Table 4).

The association between rs1799964 and rs1800750 SNPs and age, sex, BMI of celiac disease was studied. The results showed no significant difference between rs1800750 and rs1799964 polymorphisms and investigated variables (for rs1800750: p=0.83, p = 0.83 and p = 0.24 respectively and for rs1799964: p = 0.33, p = 0.25 and p = 0.62, respectively).

On the other hands the serum cytokine level of TNFα in both groups was investigated and the results showed that there was no significant difference between the mean serum level of TNFα in patients group (mean= 0.07) compare with the healthy population (mean= 0.07), (p-value= 0.1) (Figure 3).

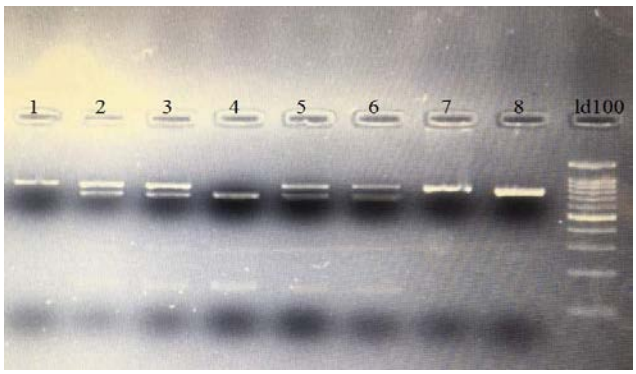

Figure 2. Gel electrophoresis of enzyme digestion on 3% for rs1799964. Sample1 and 7 and 8 were not cleavage by restriction enzyme. Samples 2-6 were cleavage with restriction enzyme

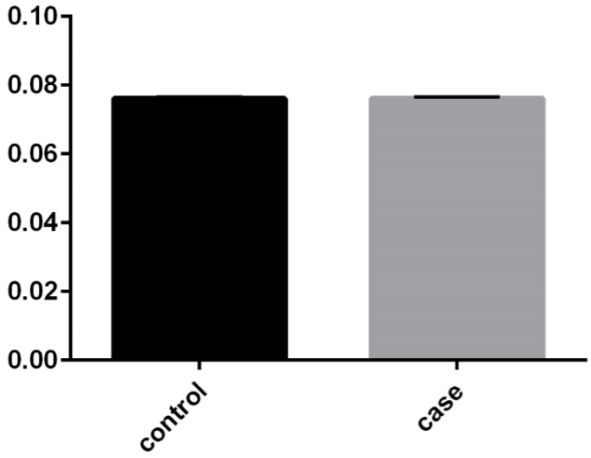

Figure 3. Relationship between serum levels of TNFα in CD patients and healthy subjects

Table 3. The frequency of genotypes and alleles in the patient and control group for

SNP Rs1800750	Case n=104 (%)	Control n=102(%)	OR	CI	p-value
GG	71(68.3)	68(66.7)	reference	reference	reference
TG	33(31.7)	34(33.3)	1.07	0.6-1.92	0.80
allele					
G	175(84)	170(83)	reference	reference	reference
A	33(16)	34(17)	1.06	0.6-1.7	0.82

Table 4. The frequency of genotypes and alleles in the patient and control group for rs 1799964

Rs1799964	Case n=104(%)	Control n=102(%)	OR	CI	p-value
CC	9(8.7)	4(3.9)	reference	reference	reference
CT	28(26.9)	23(22.5)	2.519	0.741-8.557	0.139
TT	67(64.4)	75(73.5)	1.848	0.503-6.7	0.355
Allele					
C	46(22.1)	31(15.2)	reference	reference	reference
T	162 (77.9)	173(84.8)	1.585	0.958-2.621	0.073

4. Discussion

Genetic factors play an important role in celiac disease [14]. CD has a strong genetic link with the HLA molecule, but it has been shown that the role of this molecule in the development of the CD is about 40%. Consequently, the function of other genetic compounds is considered in the pathogenesis of CD [4]. Studies have shown that the HLA gene contains several genetic predisposing factors for CD disease independent of the DQ gene [15]. On of them is TNFα gene that is located between the HLA Class I and Class II region [16] and as an inflammatory cytokine plays important roles in celiac disease [17]. It has been shown that some polymorphisms in the promoter region of TNFα cause the transcription of the TNFα gene and consequently, of increasing its production [7]. For these reasons we investigated the allelic and genotype frequencies of -1031 and -376 polymorphisms among healthy and CD patients. Significant differences were not observed between TNFα -1031 and -376 allele and genotype and susceptibility to CD. Also we have not found a relationship between these polymorphisms and age, sex and BMI. These results proposed that TNFα gene does not have relation to celiac disease in our population.

The role of -1031 and -376 TNFα polymorphisms in CD has not yet clearly stated, and because of that it is essential to investigate these polymorphisms in groups of more populations with different ethnic background. -1031 and -376 SNPs have been studied in few celiac papulations studies. In a recent study in 2015 by Rossi et al. they found that C allele of polymorphism -1031 and allele A of polymorphism -376 were associated with celiac disease [18]. The question is why we were not found the same result like what they reported. It could be explained by difference in the prevalence of celiac disease in different parts of the world and due to the fact that different populations have different genotypes. Also our result is may due to low sample size.

These SNPs have been studied in other diseases especially Non-immune diseases and different studies did not find any association between these SNPs and Non-immune diseases. Danfort et al. (2008) did not find association between -1031 C/T polymorphism and risk of prostate cancer [19]. Yang et al. in 2009 in the Korean population showed that TCT and CCC haplotypes of -1031 C / T are significantly associated with the risk of gastric cancer in smokers [20]. According to the result from Kuroda et al. no significant correlation was seen between -1031 C/T polymorphism and sarcoidosis in a Japandosis in Japan [21].

Th1 secreted cytokines have been shown to be associated with autoimmune diseases [22]. TNFα is one of the most important cytokine that releases from Th1 [23]. It has been shown that TNFα, by increasing the activity of metalloproteinases in CD, causes the destruction of the intestinal tissue [17]. In this study also, the serum level of TNFα was investigated among patients and control groups. According to the results, no significant difference was observed between serum TNFα concentration between two groups (P = 0.1). our reports on TNFα level serum was similar to Romaldini et al. that showed no significant difference in serum TNFα concentration in untreated CD, patients under treatment and control group [24]. In 2014, Björck et al. also did not find significant difference in serum TNFα concentration of children with CD in comparison with control group [25]. Street and colleagues in 2007, reported that concentration of serum TNFα in CD subjects before GFD treatment showed an increase compared with the control group, whereas after treatment for 6 months, the serum TNFα concentration was decreased [26].

In conclusion, we showed that TNFα -1031C/T and -376G/A SNPs are not associated with celiac disease in Iranian population. However, a wider population is needed to confirm these findings.

Acknowledgements

We thank all people who helped us. Research supported by Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Science (Tehran, Iran).

References

[1] Azodi MZ, Peyvandi H, Rostami-Nejad M, Safaei A, Rostami K, Vafaee R, et al. Protein-protein interaction network of celiac disease. Gastroenterology and Hepatology from bed to bench. 2016; 9(4): 268.

[2] Pourhoseingholi MA, Rostami-Nejad M, Barzegar F, Rostami K, Volta U, Sadeghi A, et al. Economic burden made celiac disease an expensive and challenging condition for Iranian patients. Gastroenterology and Hepatology from bed to bench. 2017.

[3] Rostami-Nejad M, Haldane T, AlDulaimi D, Alavian SM, Zali MR, Rostami K. The role of celiac disease in severity of liver disorders and effect of a gluten free diet on diseases improvement. Hepatitis monthly. 2013; 13(10).

[4] Greco L, Corazza G, Babron M-C, Clot F, Fulchignoni-Lataud M-C, Percopo S, et al. Genome search in celiac disease. The American Journal of Human Genetics. 1998; 62(3): 669-75.

[5] de la Concha EG, Fernández-Arquero M, Vigil P, Rubio A, Maluenda C, Polanco I, et al. Celiac disease and TNF promoter polymorphisms. Human Immunology. 2000; 61(5): 513-7.

[6] Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, et al. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology. 1998; 115(3): 551-63.

[7] Louka AS, Lie BA, Talseth B, Ascher H, Ek J, Gudjónsdóttir AH, et al. Coeliac disease patients carry conserved HLA-DR3-DQ2 haplotypes revealed by association of TNF alleles. Immunogenetics. 2003; 55(5): 339-43.

[8] Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nature Reviews Immunology. 2013; 13(4): 294.

[9] Schuppan D. Current concepts of celiac disease pathogenesis. Gastroenterology. 2000; 119(1): 234-42.

[10] Nemec P, Pavkova-Goldbergova M, Stouracova M, Vasku A, Soucek M, Gatterova J. Polymorphism in the tumor necrosis factor-α gene promoter is associated with severity of rheumatoid arthritis in the Czech population. Clinical rheumatology. 2008; 27(1): 59-65.

[11] Tsytsykova AV, Goldfeld AE. Inducer-specific enhanceosome formation controls tumor necrosis factor alpha gene expression in T lymphocytes. Molecular and cellular biology. 2002; 22(8): 2620-31.

[12] van Heel DA, Udalova IA, De Silva AP, McGovern DP, Kinouchi Y, Hull J, et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF-κB transcription factors. Human molecular genetics. 2002; 11(11): 1281-9.

[13] Hajeer AH, Hutchinson IV. TNF - α gene polymorphism: Clinical and biological implications. Microscopy research and technique. 2000; 50(3): 216-28.

[14] Trynka G, Wijmenga C, van Heel DA. A genetic perspective on coeliac disease. Trends in molecular medicine. 2010; 16(11): 537-50.

[15] Woolley N, Mustalahti K, Mäki M, Partanen J. Cytokine gene polymorphisms and genetic association with coeliac disease in the Finnish population. Scandinavian journal of immunology. 2005; 61(1): 51-6.

[16] Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, et al. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic acids research. 1985; 13(17): 6361-73.

[17] Aziz I, Evans KE, Papageorgiou V, Sanders DS. Are patients with coeliac disease seeking alternative therapies to a gluten-free diet. J Gastrointestin Liver Dis. 2011; 20(1): 27-31.

[18] Rossi E, Basso D, Zambon C-F, Navaglia F, Greco E, Pelloso M, et al. TNFA Haplotype genetic testing improves HLA in estimating the risk of celiac disease in children. PloS one. 2015; 10(4): e0123244.

[19] WY, Yu K, et al. TNF polymorphisms and prostate cancer risk. The Prostate. 2008; 68(4): 400-7.

[20] Yang JJ, Ko K-P, Cho LY, Shin A, Gwack J, Chang S-H, et al. The role of TNF genetic variants and the interaction with cigarette smoking for gastric cancer risk: a nested case-control study. BMC cancer. 2009; 9(1): 238.

[21] Kuroda H, Saijo Y, Fujiuchi S, Takeda H, Ohsaki Y, Hasebe N. Relationship between cytokine single nucleotide polymorphisms and sarcoidosis among Japanese subjects. Sarcoidosis vasculitis and diffuse lung disease. 2013; 30(1): 36-42.

[22] Moudgil KD, Choubey D. Cytokines in autoimmunity: role in induction, regulation, and treatment. Journal of Interferon & Cytokine Research. 2011; 31(10): 695-703.

[23] Lionetti E, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. International reviews of immunology. 2011; 30(4): 219-31.

[24] Romaldini CC, Barbieri D, Okay TS, Raiz Jr R, Cançado EL. Serum soluble interleukin-2 receptor, interleukin-6, and tumor necrosis factor-α levels in children with celiac disease: response to treatment. Journal of pediatric gastroenterology and nutrition. 2002; 35(4): 513-7.

[25] Björck S, Lindehammer S, Fex M, Agardh D. Serum cytokine pattern in young children with screening detected coeliac disease. Clinical & Experimental Immunology. 2015; 179(2): 230-5.

[26] Street ME, Volta C, Ziveri MA, Zanacca C, Banchini G, Viani I, et al. Changes and relationships of IGFS and IGFBPS and cytokines in coeliac disease at diagnosis and on gluten‐free diet. Clinical endocrinology. 2008; 68(1): 22-8.