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The Relationship of Celiac Disease and Abortion in Females at Basra, Iraq

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ABSTRACT

Infertility can be a sign of the condition, and celiac disease, in particular, can lead to repeated abortions. The primary goal of this study was to look into the link between celiac disease and abortion, and in particular to evaluate immunological markers and genetic allele in miscarriage female. This study included 45 patients and 34 healthy adults ranging in age from 24 to 55 years. Five milliliters of blood was obtained from each participant and tested for tTg-IgA, IL-17A and HLA-DQ2/DQ8 using ELISA and RT PCR, respectively. Females with celiac disease had higher rates of abortion than controls (35.56% vs. 8.82%; P = 0.007, OD = 5.70, CI = 1.63 to 19.6), in addition significant difference (P< 0.001) between patients (mean±SD) 94.1±101, 369±638 and control 4.50±1.91, 15.0±3.41 were recorded in serum tTg-IgA and IL_17A, respectively. Furthermore, a high genetic propensity to CD was shown to be connected with the HLA class-II allele. The findings indicated that 7(15.56%) of 45 patients were positive for DQ8, with no significant difference between patients and controls, but 37 (82.22%) of 45 patients were DQ2 positive, with a significant difference between patients and controls. The results confirmed a marked association among HLA-DQ2 and history of abortion in woman ciliac patients. Increased Ttg-IgA and IL-17A tiers in female patients may play a crucial position inside the severity of the circumstance.

Key words: Celiac disease, real time PCR, IL_17A, IgA-tTg, HLA-DQ2/8 alleles

INTRODUCTION

Celiac disease is caused by an aberrant T cell response in the intestine to cereal gluten proteins. The illness has a substantial HLA linkage, and CD4+T lymphocytes that recognize gluten epitopes provided by disease-associated HLA-DQ allotypes are thought to be disease drivers (Khudher *et al.*, 2020; Sollid *et al.*, 2020). Gluten is a broad word for alcohol-soluble proteins found in grains such as wheat, rye, barley, spelt and kamut (Tye-Din *et al.*, 2018). The altered immune response to wheat gluten causes inflammation and villous atrophy in the small intestinal mucosa, which results in an increase in the number of infiltrating lymphocytes in the epithelium and lamina propria (Villanacci *et al.*, 2020). Along with overt symptoms and signs of mal-absorption, many cases of the disease go undiagnosed because they are subclinical, atypical, or even symptomless. In adults, the disease can cause infertility; celiac disease, in particular, has been linked to multiple abortions. These manifestations, the cause of which is unknown, are unrelated to the severity of the disease; the gluten-free diet strongly ameliorates the fertility (Caio *et al.*, 2019). According to several publications, celiac disease patients had increased rates of infertility, repeated abortions, intra-uterine growth restriction (IUGR) and stillbirths. Gynecological difficulties were observed in women with celiac disease, including delayed menarche, early menopause, infertility, recurrent abortion and fetal intrauterine growth retardation (Casella *et al.*, 2016; Di Simone *et al.*, 2021). In this research, the link between celiac disease and abortion was looked with emphasis on the hereditary aspect of the condition. The major purpose of this research was to investigate the relationship between celiac disease and abortion.

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MATERIALS AND METHODS

Forty-five patients with CD of Al–Faiha hospital\ GIT center, Basra were recruited in this study during Aug. 2019-Jan. 2020. Other 34 normal persons without any sign or symptoms of bowel disease were included as a control group with negative tTg. Informed consent was obtained from all participants. Five ml blood from each individual was collected in sterile gel tube and EDTA tube. Two hundreds µl of EDTA blood sample was used for DNA extraction. The remainder of blood sample in gel tube was left to clot at room temperature, centrifuged at 3000 rpm for 5 min. The serum of each sample was divided into several 300 µl aliquots and kept at -20 °C until used for further investigations including: interleukin 17a (Il-17a), tissue transglutaminase tTG-IgA and estimation of serum tTG_IgA antibodies level.

The levels of anti Ttg antibody and IL_17a were assessed in all samples using anti_tissue transglutaminase antibody (tTg) IgA kit (AESKULISA, GERMANY), IL_17a kit (MyBiosource USA, Cat No.RDEEH 3267), in accordance with the manufacturer's recommendations. All samples were subjected to real-time PCR DNA extraction using a DNA extraction tool (PROMIGA, USA) following the manufacturer's instructions. Amplifications using sequence-specific primers for DQA1 × 05 (DQ2), DQB1 × 02 (DQ2), and DQA1 × 03 (DQ8) were done in separate - reactions using the real time PCR technique (Fast real time pcr, Applied Biosystems, USA). As an internal control, human growth hormone (HGH) primers were utilized. RT PCR procedure for primers DQA1 × 05 (DQ2), DQB1 × 02 (DQ2), and DQA1 × 03 (DQ8) was done following Selleski *et al.* (2015). The human growth hormone gene (HGH) was amplified as an internal check . Table 1 shows the primer sequences for DQA1 × 05 (DQ2), DQB1 × 02 (DQ2), DQA1 × 03 (DQ8) and HGH.

Table 1. PCR primers utilized in this investigation

	Primers	Sequence
HLADQ2DQA1 × 05	Forward	5'-ACGGTCCCTCTGGCCAGTA-3'
	Reverse	3'-AGTTGGAGCGTTTAATCAGAC-5'
HLADQ2 DQB1 × 02	Forward	5'-GTGCGTCTTGTGAGCAGAAG-3'
	Reverse	3'-GCAAGGTCGTGCGGAGCT-5'
HLA DQA1 × 03	Forward	5'-TTCACTCGTCAGCTGACCAT-3'
	Reverse	3'-CAAATTGCGGGTCAAATCTTCT-5'
HGH	Forward	5'- GCC TTC CCA ACC ATT CCC TTA-3'
	Reverse	3-TCA CGG ATT TCT GTT GTG TTT-5'

Statistical Package for Social Sciences (SPSS) version 25 was used for Chi squared Mann Whitney test. The significance of differences, if any, was considered at $P<0.05$; for significance.

RESULTS AND DISCUSSION

There was marked significant difference between patients 94.1±101, 369±638 and control 4.50±1.91, 15.0±3.41 in serum tTg-IgA and IL_17A, respectively (Table 2). Patients having positive history of abortion were more susptible to abortion than control having negative history of abortion (Table 3).

Table 2. Levels of tTg-IgA, IL-17A in patients and control group ($P<0.001$)

Groups		Mean±SD
tTg-IgA U/ml	Patients	94.1±101
	Control	4.50±1.91
IL-17A pg/ml	Patients	369±638
	Control	15.0±3.41

The present study indicated highly significant differences between patients (82.22%) and control (23.35%) for DQ2 positive. However, there were no significant differences between patients 7 (15.56%) and controls 5 (14.71%) in DQ8 positive (Table 4).

The mean *Tm* for the three analyzed alleles *DQA1 × 05, DQB1 × 02 and DQA1 × 03* were 82.1±0.1°C, 88.20±0.13°C and 82.8±0.11°C, recpectivily (Fig. 1). For the HGH gene, the evidenced mean Tm was 86.3±0.1°C.

The level of antitissue transglutaminase antibody (tTg) IgA in patient group of DQ2 +Ve (100±99.1 U/ml) was more than DQ2 –Ve (64.8±111 U/ml). But the level of interluckin-17A did not show any significance between DQ2 +Ve and DQ2 –Ve in patient group. However, in control group, the level of tTg-IgA in patient group of DQ2 -Ve (4.73±1.61 U/ml) was more than DQ2+Ve (3.75±2.66 U/ml). The level of interluckin-17A did not show any significance

Table 3. History of abortion between female in patients and control

Hs of abortions	Control	Patients	OD	95% CI	P-value
Negative N (%)	31 (91.18%)	29 (64.44%)	5.70	1.63 to 19.6	0.007
Positive N (%)	3 (8.82%)	16 (35.56%)			
Total	34 (100%)	45 (100%)			

Table 4. The prevalence (%) of CD predisposing HLA-DQ genotypes in patients and control

Parameter		Positive No. (%)	Negative No. (%)	Total No. (%)	P-value	
DQ2	Patients N (%)	37 (82.22%)	8 (17.78%)	45 (100%)	<0.001	S
	Control N (%)	8 (23.35%)	26 (76.65%)	34 (100%)		
DQ8	Patients N (%)	7 (15.56%)	38 (84.44%)	45 (100%)	0.917	NS
	Control N (%)	5 (14.71%)	29 (85.29%)	34 (100%)		

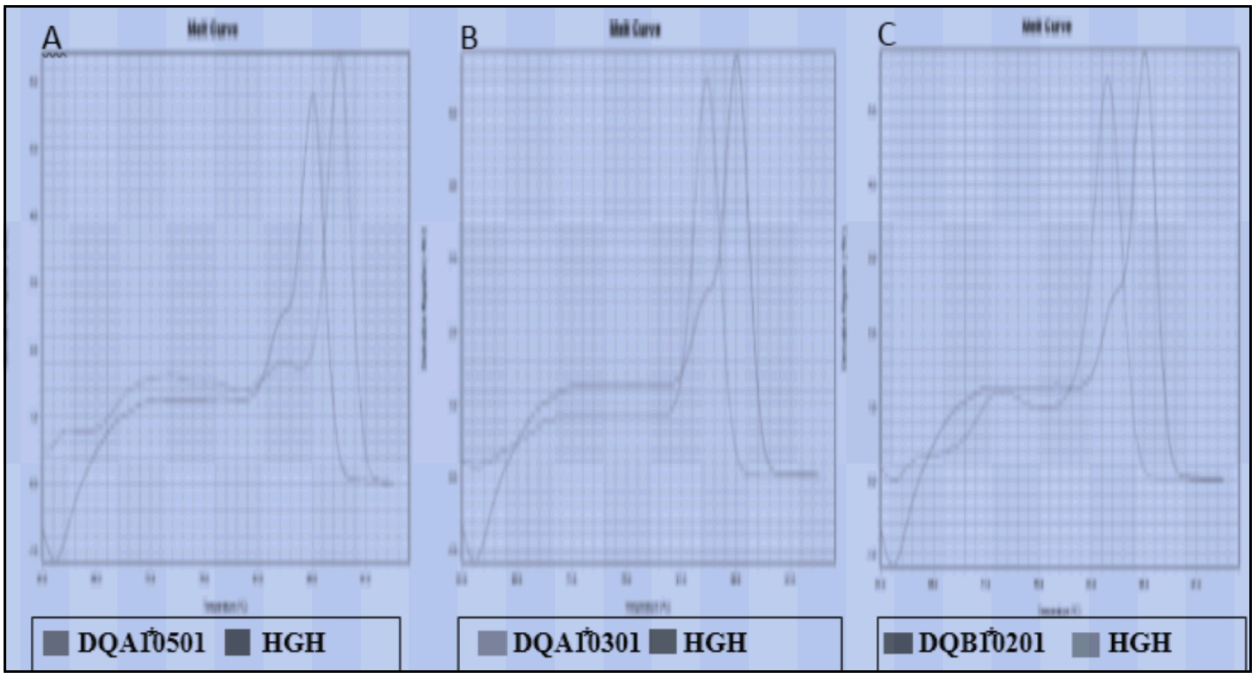

Fig. 1. Melting curves of the analyzed alleles. A: DQA1 × 05, B: DQB1 × 02 and C: DQA1 × 03.

between DQ2+Ve and DQ2–Ve in control group (Table 5). Further, there was no significant difference in the level of tTg _ IgA and IL-17A between DQ8 +Ve and DQ8 -Ve in patient and control groups except the level of IL-17A increased more in patients group of DQ8 -Ve (426±679 Pg/ml) than DQ8+Ve (60.3±85.2 Pg/ml).

As gluten intolerance can grow throughout life at any time, there is no particular age in celiac disease (Tye-Din *et al.*, 2018). In general, female patients with celiac disease are higher than male patients (Tye-Din *et al.*, 2018). The current study showed 45 female true positive cases of CD and 34 healthy female (Kim *et al.*, 2017). The results showed that 35.56% from total patients had history of abortion and 8.82% females of control had history of abortion, in addition high levels of tTg-IgA among patient's female (74.00 U/ml) compared to the control (5.00 U/ml) with significant differences. IL-17A released by cell subset activated T-cell and T-helper17. Past studies have demonstrated elevation of this in the mucosa of untreated patients (Faghih *et al.*, 2018). This study found extremely significant differences between patients and controls, which was consistent with earlier investigations. The causes of the rise in spontaneous abortions are unknown, however, gluten in the diet may play a role (Garrido-Gimenez and Alijotas-Reig, 2015; Cartee and Murray, 2019). Eight females of total 15 female patients had previous abortion. Its high heritability and close association with

Table 5. The differences of IL-17A and tTg -IgA according to the presence or absence of DQ2 and DQ8 in 45 patients and 34 control

		DQ2 +Ve Mean±SD	DQ2 -Ve Mean±SD	P-value	
Patients group	Number	37	8		
	tTg _ IgA U/ml	100±99.1	64.8±111	0.035	Sig.
	IL-17A Pg/ml	424±691	114±112	0.525	Not Sig.
Control group	Number	8	26		
	tTg - IgAU/ml	3.75±2.66	4.73±1.61	0.037	Sig.
	IL-17A Pg/ml	14.9±1.25	15.0±3.86	0.735	Not Sig.
	DQ8 +Ve Mean±SD	DQ8 -Ve Mean±SD	P-value		
Patients group	Number	7	38		
	tTg _ IgA U/ml	76.7±118	97.3±99.0	0.276	Not Sig.
	IL-17A Pg/ml	60.3±85.2	426±679	0.018	Sig.
Control group	Number	5	29		
	tTg - IgAU/ml	4.20±2.28	4.55±1.88	0.550	Not Sig.
	IL-17A Pg/ml	13.8±2.05	15.2±3.58	0.604	Not Sig.

HLA was a notable feature of celiac disease (Kuja-Halkola *et al.*, 2016). In separate experiments, a rapid real time PCR technique to test for the presence of each allele (DQA1 05, DQB1 02 and DQA1 03) and HGH as an internal control was employed. This study discovered that the predominant HLA related with the vast majority of celiac disease patients was DQ2 (DQA1 05, DQB1 02), with a minority of individuals having HLA-DQ8. This was consistent with many earlier research investigations. The present study showed that the mean±SD = 76.7±118 of tTg-IgA in DQ2 positive was greater than DQ2 negative patients the mean±SD = 64.8±111 with significant differences (0.035). In addition to HLA grade 2 gene molecule related to immune system response contributed to CD development (Sciurti *et al.*, 2018; Espino and Núñez, 2021). The data revealed that 15 femal patients which had previous abortion were DQ2 positive and two of them were DQ8 positive. Based on the data presented, it was concluded that untreated celiac women with abnormal Ttg-IgA and IL-17A had difficulty in maintaining their pregnancy. IL_17A was compared according to DQ2, DQ8 positive or negative patients. IL_17A was the first member of IL_17 families which was considered as pro-inflammatory cytokine. IL_17A was released by activated T_cell and T_helper17 cell sub set.

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