

BIOLOGICAL CORRELATION BETWEEN TNF- α 238 G/A GENE POLYMORPHISMS AND GALLSTONE FORMATION AMONG IRAQI PATIENTS

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ABSTRACT

Gallstone is one of most biliary disorder affected by several risk factors such as Immune factors like inflammatory cytokines as well as ethnic variants which are important in most disease. Several studies estimated some cytokines can be detected in bile fluid. Previous findings demonstrated the correlation between cytokines and gall stone formation provided the unexpected result that cytokines deficiency markedly increased the formation of gallstones as well as reported that several specific cytokines have been implicated in cholesterol and lipoprotein metabolism. So in this research, tumor necrosis factor alpha (TNF- α) gene polymorphisms at position 238 G to A were studied, and its correlation with gallstone formation were estimated. On 85 gallstone Iraqi patients and 86 normal subjects, by amplification

refractory mutation system polymerase chain reaction (ARMS –PCR) technique; TNF- α 238 G/A gene polymorphism were evaluated. Present findings showed no significant differences in distribution of TNF- α GG and GA genotypes between patient and normal control group ($P < 0.05$). Whereas the correlation between AA mutant genotypes and gallstone formation was significant statistically ($P > 0.05$) and ($OR = 0.38, 95\% CI = 0.12 - 1.11$). These results suggested that AA mutant homogenous genotypes played a protective role against gallstone formation among Iraqi patients.

KEYWORDS: TNF- α 238, polymorphism, gallstone, Iraqi, patients.

INTRODUCTION

Gallstone is a result of precise biological and physiological biliary disorder lead to complicated treatment or surgery. There are many risk factors included; the most important

are genetics and environmental factors which are contributed towards susceptibility to the disease.^[1,2,3] Nutrition, obesity, rapid weight gain or loss, and exercise are additional factors.^[4,5]

Some of studies revealed that some genetic factors related to the risk of gallstone formation have been result of ethnic differences as well as same studies reported that gallstone formation associated with some of genes like, Lith 1 and Lith 2.^[6,7] Human gene polymorphisms such as LDL receptor-associated protein, apo B, apo A1, LDL receptor, and apo E genes are related to gallstone formation.^[1,8] In 1997 other researchers have been reported another risk factors having role in gallstone formation which are cytokines.^[9] Cytokines are proteins with low molecular weight produced by several immune cells, hepatocytes and gallbladder cells. Confirmatory studies demonstrated that biliary tract and gallbladder epithelial cells produce some cytokines such as interleukin-6 (IL-6), interleukine-8 (IL-8), TNF- α , Monocyte chemoattractant protein-1 (MCP-1), and express IL-6 and TNF- α receptors.^[10,11] Some investigation indicated that gallbladder can be modified by some cytokines.^[11] As well as found that TNF- α can be modified the intercellular signal transduction, ionic channel activities and absorption/secretions.^[12,13] Other cytokines also play some roles in the gallbladder and its epithelial cells.^[13,14]

Many types of mutations and polymorphisms in genes codifying the cytokines like IL-6, IL-8, and TNF- α . these mutations or polymorphisms have been related to the increase or decrease the secretion of cytokines in healthy population^[16,17] as well as in several diseases.^[15,16] It has been described that some cytokine genotypes influence the prognosis of several inflammatory and neoplastic diseases.^[17,18,19]

So the aim of the present study were to detect the association of the pro-inflammatory cytokine TNF-238 gene polymorphism and gallstone formation among Iraqi patients sample.

MATERIALS AND METHODS

Patients groups

Eighty six (86) Iraqi gallstone patients were enrolled in our study and Eighty five (85) health control. Demographic characteristics of all individuals are listed in table 1. All cases in this study were pathologically confirmed by ultrasound and X ray techniques as well as all patients underwent surgery.

Table 1: Characteristics of cases & control included in this study.

Total No.	Male	Female	Mean age	± SD (years)
Control =86	40	46	38.06	9.33
Cases = 85	41	44	37.12	6.75

DNA analysis

Isolation of DNA from blood for all cases and control groups performed by using of Bioneer company DNA isolation kit. All genotypes of TNF- α at position 238 G/A have been mapped to chromosome 6p21.3 for all individuals by using of (ARMS- PCR) technique. PCR products obtained with primers for internal control [5'-GC CC CT CC AG TT CT AG TT CTA TC-3' and 5'-CC GG AT CA TG CT TT CA GT GC-3'], allele A [5'-GC CC CT CC CA GT TC TA GT TC TA TC-3' and 5'-CA CA CT CC CA TC CT CC CT GG TC T-3'] and allele G [5'-AG AC CC CC CT CG GA AT CG-3' and 5'-CC GG AT CA TG CT TT CA GT GC-3'], observed a fragment of 608, 209 and 447 (bps), respectively (20). PCR protocol was used : 94 °C for 3 minutes; 35 cycles of 94 °C for 45 seconds, 60 °C for 45 seconds; 72 °C for 5 minutes using reagents from Bioneer company. Ethidium bromide staining showed TNF- α genotypes.

Statistical Analysis

The association between TNF -238 G/A polymorphism and gallstone susceptibility was estimated by OR with the corresponding 95% confidence interval (CI). The significance of the pooled OR was determined by Z test. All studied groups were in Hardy-Weinberg equilibrium.

RESULTS**Patients and control groups**

There was no statistically significant correlation between cases and control regarding age.

Table 2. Gender distributions between cases and control in this study

Total No.	Male	Female	P value
Control =86	40	46	8.2
Cases = 85	41	44	

TNF- α 238 G/A genotypes distribution

Comparison of TNF- α 238 G/A genotypes between cases and control in present study demonstrated that A allele played as a protective factor. It was found that the frequency of

AA genotypes was 15.2 in cases while 29.4 % in control with statistically significant P value = 0.03. On the other hand GG and GA genotypes statistically there was no significant correlation with disease it was found as described in table 3.

Table 3: TNF- α 238 G/A genotypes distribution for cases and control in this study.

Gene	Cases	control	OR(95% CI)	P Value
TNF- α 238				
G/G	18(20.9)	13(15.3)	1.0	reference
G/A	55(63.9)	47(55.3)	0.85(0.35-2.05)	0.68
A/A	13(15.2)	25(29.4)	0.38 (0.12-1.11)	0.03*
		Allele frequency		
G allele	91(53.0)	73(43.4)	1.0	
A allele	81(47.0)	97(56.6)	0.67-1.05	0.06

*Indicate significant p value

DISCUSSION

As it well known that Deoxyribonucleic acid (DNA) molecule contaminating all genetic instructions that used in the development and functioning of all known living organisms. DNA molecule consisted of a long chain of repeating nucleotides (A,C,G and T) roughly 3 billion base pairs for humans.^[21] Normal nucleotides sequencing within DNA molecule represents precise normal genetics instructions then normal function of genes.^[21,22] So any changing or variation of that normal nucleotides sequencing result in abnormal genes function. Single nucleotides polymorphisms(SNPs) are the most common type of DNA sequence variations, Accounted about 90% of all DNA sequence variations. SNPs occur on average about every 100 to 300 bases and so are the major source of heterogeneity.^[22,23] It is found that DNA sequence variations included SNPs account for some of diseases, and therefore detecting them in human genome allows for examining genetic variation and risk for many common diseases. For example, a single base mutation in the Apolipoprotein E gene is associated with a higher risk for Alzheimer's disease.^[24] It is believed that the SNPs profile of a variety of diseases will be eventually characterized, and then it will only be a matter of time before physicians can screen individuals for susceptibility to a disease just by analyzing their DNA samples for specific SNP patterns.^[25]

Previous studies demonstrated the correlation between cytokines & gall stone formation provided the unexpected result that IL-4 deficiency markedly increased the formation of gallstones as well as reported that Several specific cytokines have been implicated in cholesterol and lipoprotein metabolism. One of these cytokines is interleukin-4 (IL-4).^[26,27]

Chemically reactive oxidants, radicals, and electrophilic mediators, such as hydrogen peroxide and oxyradicals, nitric oxide, malondialdehyde, 4-hydroxynonenal, or eicosanoids, are produced during inflammation, and these chemical mediators are known to induce a variety of biological reactions.^[28]

Therefore we aimed in this study to evaluate the correlation between TNF- α 238 G/A polymorphism and gallstone formation among Iraqi patients although there is very few studies argued the relation between gallstone formation and TNF- polymorphism. Present findings revealed that AA allele mutant genotype played as a protective role with disease. The most realistic interpretation for such protection it can be powered by the effort of previous researchers. firstly; Vaidyanathapuram et al. in 2004 reported that individuals with A allele have been high serum level of TNF- than individuals with G allele genotypes.^[29] Secondly; Reynoso et al in 1999 and Savard in 2002 shown that biliary tract and gallbladder epithelial cells produce cytokines, including IL-6, IL-8, TNF- α , MCP-1, and express IL-6 and TNF- α receptors. Some investigations indicated that cytokines can modify gallbladder epithelial cells functions.^[30,31] For instance, TNF- α can modify intracellular signal transduction, ionic channel activities or absorption/secretion functions.^[32,33] Inference for all above explanations; may be the influence of TNF- as a protective factor was indirectly out of its effect to regulate some biological processes within cells and tissues of gall bladder. As some studies have shown that cytokine included TNF- α stimulate and regulate inducible nitric oxide synthase and nitric oxide in normal gallbladder epithelial cells.^[33,34] So in-depth studies will be necessary to understand the real role of cytokines including TNF- α and its effect as protection or risk factor with gall stone formation.

CONCLUSION

The present study demonstrated that AA mutant homogenous genotypes played a protective role against gallstone formation, whereas no significant association between GA and GG polymorphism genotypes with disease.

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