Taisir Abdulelah Kadhim et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3153-3156

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

The role of IFN-ɣ gene polymorphism in *Campylobacter. jejuni* acquisition and disease severity

Taisir Abdulelah Kadhim[1], Maysa S. AL-Shukri[2] and Ahmed A. AL-Zubadi[2]

[1]Al-Muthana University/Iraq ; [2]Babylon University/Iraq

Abstract

Background: *Campylobacter jejuni* have been known as one of main causes of bacterial gastroenteritis (*Campylobacteriosis*) in human. *Campylobacter jejuni* acquisition, diseases severity and post infection complications might possibly associated with genetic susceptibility of the host. A single nucleotide polymorphism placed in the first intron of human IFN- ɣ gene (+874 T⁄ A) can putatively stimulate the secretion of cytokine through an influence on infection product. The aim of current study was to estimate the role of the SNP in the first intronic region of Interferon gamma gene in the acquisition of *C . jejuni* and disease susceptibility and severity. Methods: Samples were taken from 100 patients who were suffering from gastrointestinal disease and 41 healthy individuals with no history of gastrointestinal disease as controls were included in this study. The ages of patients and controls ranged from 0-10/ years. Stool samples were collected from each patients for direct detection. Blood samples were collected from all participants for DNA direct detection and gene susceptibility study as well as for estimation of IFN-ɣ by ELISA technique. Results: Direct molecular identification was positive in 41%. analyzed genetic association among IFN-ɣ +874 A/T polymorphisms and *C . jejuni* by the use of ARMS-PCR technique. The results also revealed at the site of IFN-ɣ +874 A/T SNP, there were no significant differences among patients and controls for all genotypes (p value 0.05). Thus, no association of both heterozygous AT genotype and the homozygous AA and TT genotype as risk factors for progress of *C. jejuni* gastroenteritis (OR=0.905, 1.129 and 1, respectively, 95% CI). Conclusions: There is no significant risk related among IFN-ɣ +874 A/T gene polymorphism and gastroenteritis disease caused by *C. jejuni*. The mean of IFN-ɣ serum level is significantly higher in patients with *C. jejuni* gastroenteritis disease compared with control groups.

Key words: *Campylobacter jejuni*, Polymorphism, IFN-ɣ, ELISA, Gastroenteritis.

INTRODUCTION

Campylobacter jejuni have been known as one of main causes of bacterial gastroenteritis (*Campylobacteriosis*) in human. It is the most common reason for gastroenteritis and enterocolitis, particularly in children. It causes systemic infection (rarely) in children and debilitated adults [1].

The infections in infants, young children and patients with compronmised immune system are considerably severe and happen not only with inflammation of stomach and intestines, but also with bacteraemia, responsive arthritis, meningitis, thrombophlebitis, sepsis (infection in in patients with HLA B27), endocarditis, Guillain- Barre syndrome or Miller– Fisher syndrome hemolytic -uremic syndrome, inflammatory bowel disease [2].

Campylobacter jejuni are difficult and require nutrient-rich medium and aerobic atmosphere. Strains were isolated and presumptively diagnosis as *Campylobacter* species via typical microbiological, biochemical methods and confirmed by molecular methods [3].

Large number of immuno-genetic studies estimated the role of genetic variations of immunologically important host genes as bases of the susceptibility, progress and outcome of infectious diseases. Among these important variations are single nucleotide polymorphisms (SNPs) [4].

Campylobacter jejuni acquisition, diseases severity and post infection complications might possibly associated with genetic susceptibility of the host [5]. A single nucleotide polymorphism placed in the first intron of human IFN- ɣ gene (+874 T⁄ A) can putatively stimulate the secretion of cytokine through an influence on infection product.

The aim of current study was to estimate the role of the SNP in the first intronic region of Interferon gamma gene in the acquisition of *C . jejuni* and disease susceptibility and severity.

METHODS

This case control study included 100 samples from patients suffering from gastrointestinal symptoms aged **from 0-10/ years and** from both sexes. Clinical samples (stool and blood) were collected from patients admitted to the Paediatrics out-patient clinics at Babylon Province: Babylon Maternity and Paediatrics Hospital and Al-Noor Paediatrics Hospital, during the period from February to September 2017. **On the other hand,** a total of 41 blood samples were collected from healthy individuals of both sexes, with no history of gastrointestinal or other diseases with age range approximately matched that of the patients, for analysis of genetic association between various SNPs and *C . jejuni.* Direct isolation of bacteria from stool sample achieved by use of specific primers 16S rRNA and 23S rRNA (Table 1).

Genomic DNA extraction from stool specimens

This was done according to manufacturer's instructions (Geneaid/ Korea).

Genomic DNA extraction from fresh blood

(gSYNC ™ DNA Mini Kit Blood Protocol/ Geneaid/ Korea) was used according to the manufacturer's instructions.

Detection of IFN-ɣ gene polymorphism

The polymorphism in IFN_ɣ (+874T/A) gene was typed via using Amplification Refractory Mutation System-polymerase chain reaction method (ARMS-PcR).The typical ARMS test, which can discover a recognized SNP polymorphism involves two complementary reactions: one containing an ARMS primer specific for the normal DNA sequence and can not amplify mutant DNA at a given locus and the other containing a mutant- specific primer and does not amplify normal DNA . So, the precision of this technique is about 99.9% (9).

Genomic DNA strain was amplified by using specific kit of Taq DNA polymerase (PreMix-Bioneer). The reaction was achieved by using a general reverse primer and one from the two alleles (T/A) forward primer of 261bp.

To measure an effective PCR amplification, one internal control of 426 bp was amplified by using a primer designed from the nucleotide sequence of the human growth hormone [10]. All primers were diluted by (TE buffer) to be used with the concentration of 10 pmoles/ µl.

ELISA assay for IFN-ɣ

ELISA assay was achieved according to the method described by the manufacturing company (Elabscience / china).

Statistics

All data were analyzed using computer-based software, the Statistical Package for Social Sciences (SPSS) version 21 for windows 7. Comparisons were made using ANOVA test, independent sample *t*- test and odds ratio. P value<0.05 with 95% confidence interval were used to determine level of significance.

Differences in genotypes and alleles frequency were used to determine the IFN-ɣ serum levels for each genotypes and type of patients [11].

RESULTS AND DISCUSSION

Molecular identification of *Campylobactor jejuni*

In current study, the overall recovery of *C . jejuni* by using PCR is (41% and 40 %) based on specific primers16S rRNA and 23S rRNA with molecular length about 402 and 650 bp, respectively, as in Figure 1. This overall recovery rate of *C .jejuni* was compatible with that seen in a study conducted by [6] who found that 40% were positive for PCR detection by using specific primer for detection of *C .jejuni*.

The advances in molecular biology methods have facilitated PCR revealing of DNA from numerous fastidious microorganisms. For instance *C . jejuni* has been highly recommended. The development of particular primers depending on genes such as 16S rRNA, and 23S rRNA is useful. In this study, both genes used are diagnostic genes, however, the amplification of the 23S rRNA gene (40%) was lower than that of the 16S rRNA primer (41%). This result may be due to the sequence likeness between *Campylobacter* species, the 16S rRNA gene sequence can not be used to distinguish among very thoroughly associated species, such as *C . jejuni* and *C .coli*. The 23S rRNA gene can be used to distinguish among *C ampylobacter* species and strains [12]

Detection of genetic susceptibility to *C. jejuni* in association with variable gene polymorphisms

IFN-ɣ +874 A/T gene polymorphism

Blood samples from 41 patients with *C. jejuni* and 41 controls were subjected for DNA extraction and detection of +874 SNP in the intronic region of IFN-ɣ gene by using ARMS PCR technique. The product of the results of ARMS were identified by gel electrophoresis as the alleles AT were appeared in 261pb, whereas the internal control appeared in 426bp as shown in the Figure 4.

The genotypes and allele frequencies of IFN -ɣ +874 A/T polymorphisms were compared between patients with *C. jejuni* and control groups. Risks of getting *C. jejuni* among different genotypes of IFN-ɣ +874 A/T polymorphisms were estimated.

The results in Table 2 showed the genotype frequency revealing that the homozygous AA genotype frequency was found 12 (29%) in patients group and 11(27%) in the healthy group, whereas homozygous TT genotype frequency was found 12 (29%) in patients group and 12 (29%) in the healthy group and heterozygous AT genotype frequency was found to be 17 (42%) in patients' group and 18 (44%) in the healthy group.

Moreover, A allele frequency was 50% in patients group and 50% in the healthy group; whereas T allele frequency was 51% in patients group and 49% in the healthy group (Table 3).

Table 1 The primer sequences and PCR conditions with their amplicon size (Base pair (BP)

Gene's Name	Primer Sequence (5´- 3´)	Size (BP)	conditions	Reference
Identification Genes				
16S rRNA	F5'CGCACGGGTGAGTAAGGTAT 3' R-5' TAAACACATGCTCCACC GC T 3'	402	94°c 5min 1x 94°c 1min 58°c 30s 30x 72°c 1min 72°c 5min 1x	[6]
23S rRNA	F-5' TATACCGGTAAGGAGTGCTGGAG 3' R-5' ATCAATTAACCTTCGAGCACCG 3'	650	95°c 6min 1x 95°c 30s 59°c 30s 30x 72°c 30s 72°c 5min 1x	[7]
SNPs-coding Genes				
IFN-ɣ	R 5-TCAACAAAGCTGATACTCCA-3	261	95°c 1min 1x 95°c 15 s 62°c 50s 10x 72°c 40s	[8]
IFN- ɣ -A	F 5-TTCTTAC AACACAAAATCAAATCA-3			
IFN- ɣ -T	F 5-TTCTTACAACAC AAAATCAAATCT-3			
Internal control	F 5-GCCTTCC AACCATTCCCTTA-3	426	95°c 20s 56°c 50s 20x 72°c 50s	
	R 5-TCAC GGATTTCTGTTGTGTTTC -3			

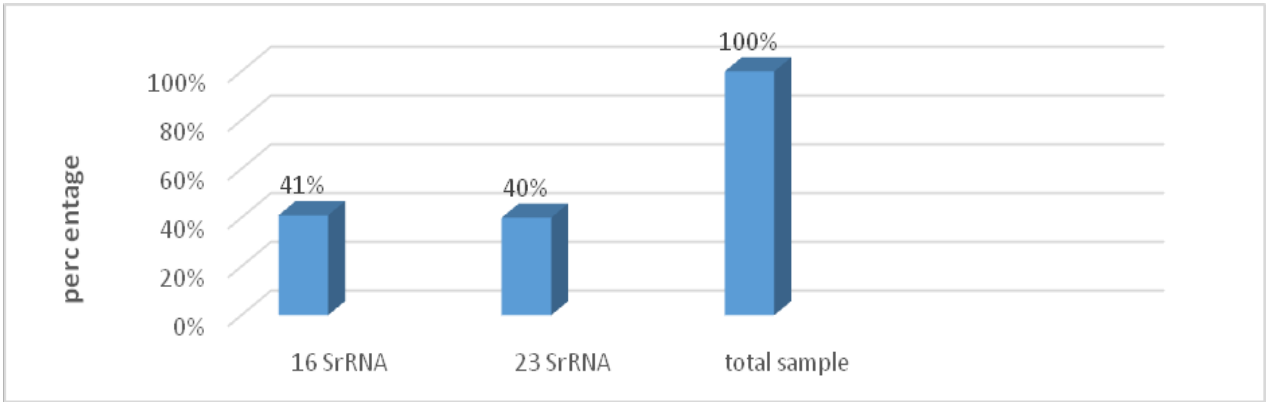

Figure 1 Percentage of molecular detection for *C . jejuni* by 16S rRNA and 23S rRNA

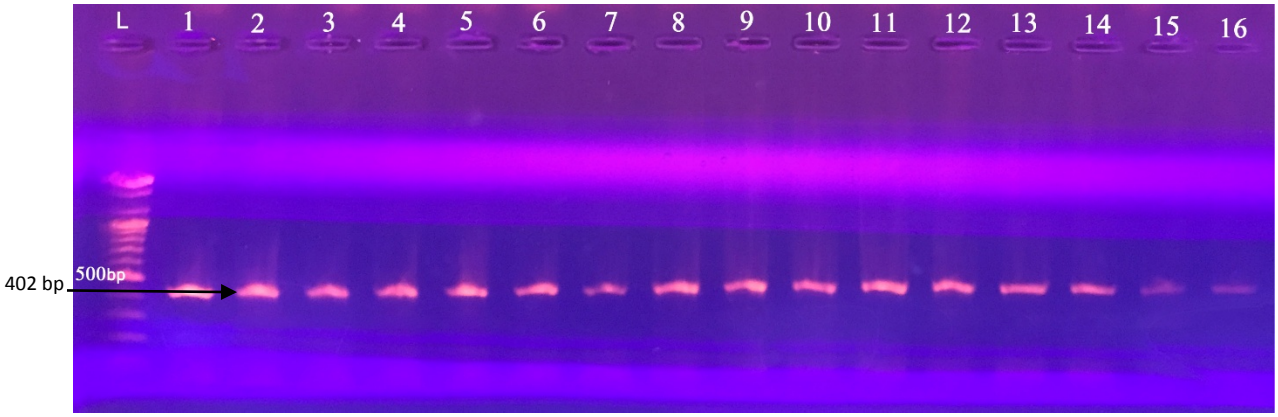

Figure 2 Gel electrophoresis of 1.5% agarose for *16S rRNA* PCR products visualized below Ultra-Violet light after staining via Ethidium Bromide. Gel was electrophoresed for 1h at 70 Volt. The purified DNA concentration was 10µg/µl at OD 260/280 ≥ 1.8, and the size of PCR product was 402 bp. All lanes show positive results.

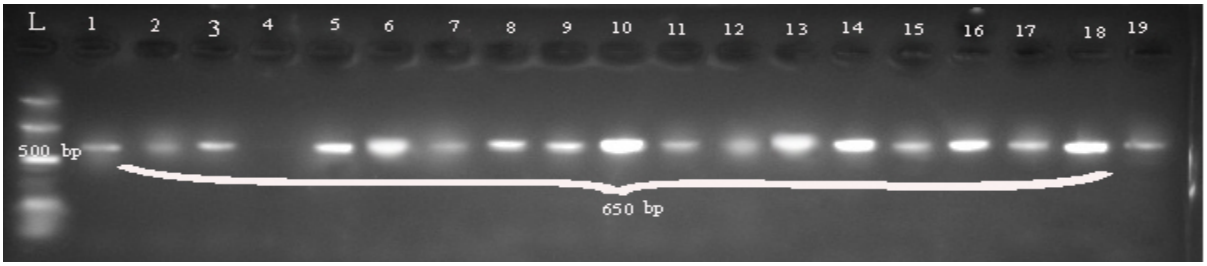

Figure 3 Gel electrophoresis of 1.5% agarose for *23S rRNA* PCR products visualized below Ultra-Violet light after staining via Ethidium Bormide. Gel was electrophoresed for 1h at 70 Volt. The purified DNA concentration 10 µg/µl at OD 260/280 ≥ 1.8, and the size of PCR product is 650 bp. All lanes show positive result except lane 4 show negative results.

Table 2 Genotype distribution of +874 A/T SNP of patients and control.

Genotype	Patients	Controls	P value	OR
AA	12 (29%)	11 (27%)	0.05	1.129
AT	17 (42%)	18 (44%)	0.05	0.905
TT	12 (29%)	12 (29%)	0.05	1
Total	41	41		

Table 3 Allele frequencies "A/T" for the genotypes of +874 A/T for patients and control

Allele frequency	Patients	Controls	P value	OR
A	50%	50%	0.05	1.04
T	49%	51%		

Table (5) Mean IFN-ɣ serum level in patients with *C . jejuni* gastroenteritis compared with control groups according to genotypes

Study subjects	Mean±SE. of IFN-ɣ concentration in subjects having AA genotype	Mean±SE of IFN-ɣ concentration in subjects having AT genotype	Mean±SE of IFN-ɣ concentration in subjects having TT genotype	P-value
Patients	1034.8±278 *	516.9±94 *	600±139 *	0.05
Controls	266±121	13.5±45	107.5±62	0.05

Patients

$\frac{1}{AA}$ $\frac{2}{AA}$ $\frac{3}{TT}$ $\frac{4}{TT}$ $\frac{5}{AT}$ $\frac{6}{AT}$

Amplification Refractory Mutation System -polymerase chain reaction (ARMS) of agarose gel electrophoresis resulted in finding of A/T +874 polymorphism of IFN- ɣ gene. Every patient was presented by 2 lanes for A and T alleles, respectively, to amplify a product of (261bp). A couple of primers were used as inner control to augment a PCR amplicon of (426 bp) to estimate a good PCR augmentation.

The genotypes of six patients, 1 and 2 with the homozygous genotype AA, 3 and 4 with TT homozygous genotype, 5 and 6 patients with heterozygous Genotype (AT).

These results showed that most patients and controls were carrying the heterozygous AT genotype (dominant genotype), so it is considered as the wild type and with no significant difference between patients and controls regarding both heterozygous wild genotype AT and the homozygous AA and TT genotype (p values 0.05). Also, with no association of both heterozygous AT genotype and the homozygous AA and TT genotype as risk factors for progress of *C. jejuni* gastroenteritis (OR=0.905, 1.129 and 1, respectively, 95% CI).

Regarding allele frequency, allele T was higher than allele A among controls with no significant difference between patients and controls regarding both alleles (p value 0.05, OR=1.04, 95% CI). Since allele A occurs in low frequency, the possibility of appearance of the risky genotype AA is reduced.

Current study revealed that there is no significant risky association among IFN-ɣ +874 A/T gene polymorphism and *C. jejuni* gastroenteritis disease. This finding is in disagreement that reported in another study [5] who found that the risk of acquiring clinical gastroenteritis with *Campylobacter jejuni/ coli* is related to the IFN-ɣ +874 A/T of intron 1. This could be due to limited sample size or the effect of other environmental factors or geographic variation between patients. Also, Forte *et al*., (13) suggested that the +874 TT genotype was, connected with an increased production of IFN- ɣ, was found to be significantly less frequent in Mediterranean Spotted Fever patients than in the control group.

Cell-mediated immune response comprising Th1 is functioning in the proneness to *C.jejuni* acquisition and disease development since it raised the threatening of HIN. Additionally, mucosal immne response and the severity of inflammation are weakened when T cell is well regulated [14].

Influence of the genotypes on IFN_ɣ concentrations

Statistical analysis showed that there is no difference in the IFN-γ serum level of *C. jejuni* gastroenteritis-diseased individuals with genotype AA from those with AT and TT which is in disagreement with previous studies in different regions, similar study [15] had concluded that IFN-γ levels increased in the TT genotype in Egyptian atopic patients and haemodialysis patients; whereas study by [16] Chinese people established that all IFN-γ gene variants of the first intronic region (+874T/A) play no role in serum level of Interferon gamma. However, the levels of IFN-γ in serum were greater in diseased individuals with the genotypes AA, AT, and TT when compared with those in control individuals (1034.8±278 vs 266±121, 516.9±94 vs 13.5±45 and 600±139 vs107.5±62, p= 0.05) (Table 5).

C . jejuni was considered to be only extracellular bacteria signifying that humoral immune responses are essential in the control of *C . jejuni* infections. This is dependable with results that *C . jejuni* isolates are commonly susceptible to the bactericidal action in normal human serum of antibody and complement [17]. Though, new data confirm its capability to invade several host cells specifically mononuclear phagocytes and even enterocytes.

On the other hand, previous data recommended that interferon γ (IFN-γ) might be connected with defence. For example, Tribble et al. [18] who established that *Campylobacter*-specific immunoglobulin A (IgA) and interferon γ (IFN-γ) are linked with resistance to most clinical diseases signifying these components might be significant markers of protective immunity. The mechanisms of changed gene expression connected through polymorphisms may be attributed to that a sequence alterations in the intronic region in a gene can effect the synthesis of the peptide of that gene .

SNPs that are not in protein-coding regions might still affect gene splicing, transcription factor binding, messenger RNA degradation or the sequence of non-coding RNA. Gene expression affected via this type of SNP is referred to as an eSNP (expression SNP) and may be upstream or downstream from the gene [18].

CONCLUSIONS

There is no significant risk related among IFN-ɣ +874 A/T gene polymorphism and gastroenteritis disease caused by *C. jejuni*. The mean of IFN-ɣ serum level is significantly higher in patients with *C. jejuni* gastroenteritis disease compared with control groups.

Ethical Clearance: It was obtained from the Scientific Research Committee Babylon Maternity and Paediatrics Hospital and Al-Noor Paediatrics Hospital, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

REFERENCES

1- Grzybowska-Chlebowczyk U, Kalita B, Flak-Wancerz A, Jasielska M, Więcek S, Wojcieszyn M,Horowska-Ziaja S, Chlebowczyk W, Woś H. Clinical course of Campylobacter infections in children. Pediatriapolska. 2013; 88: 329 – 334.

2- van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome. Lancet Neurol. 2008; 7: 939–50. http://dx.doi.org/10.1016/S1474-4422(08)70215-1.

3- Talukder KA, Aslam M, Islam Z, et al. Prevalence of virulence genes and cytolethal distending toxin production in Campylobacter jejuni isolates from diarrheal patients in Bangladesh. J Clin Microbiol. 2008; 46: 1485-1488.

4- Hartog JE, Ouburg S, Land JA, Lyons JM, Ito JI, Peña AS. and Morré SA. Do host genetic traits in the bacterial sensing system play a role in the development of Chlamydia trachomatis-associated tubal pathology in subfertile women? BMC Infect. Dis. 2006; 6: 122.

5- Nielsen H, Steffensen R. snd Ejlertsen T. Risk and Prognosis of Campylobacteriosis in Relation to Polymorphisms of Host Inflammatory Cytokine Genes. Scandinavian Journal of Immunology. 2012; 75: 449–454.

6- Al-Nasrawi HAA. Phylogenetic analysis of Campylobacter jejuni from human and birds sources in Iraq. African Journal of Microbiology Research. 2016; 10(21): 752-758.

7- Willoughby PF, Nettleton M, Quirie MA, Maley G, Foster M, Toszeghy DG, Newell. A multiplex polymerase chain reaction to detect and differentiate Campylobacter fetus subspecies fetus and Campylobacter fetus -species venerealis: use on UK isolates of C. fetus and other Campylobacter spp. 2005; 99(4): 758–766.

8- Dolores LM, Arnalich F, Serantes R, Gonzalez A, Codoceo R, Madero R, Vazquez JJ and Montiel C. lnterferon-ɣ and lnterleukin-10 Gene Polymorphisms in Pulmonary Tuberculosis. 2003.

9- Duta- Cornesu G, Simon- Gruita A, Constantin N, Stanciu F, Dorina MD, Banica MDD, Tuduce R, Stoian CV. A comparative study of ARMS – PCR and RFLP – PCR as methods for rapid SNP identification, Romanian Biotechnological Letters. 2009; 14(6): 4845-4850.

10- Perrey C, Turner SJ, Pravica V,Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-a, TNF-(3 and TGF-(31 gene polymorphisms. Transpl Immunol. 1999; 7: 127-128.

11- Way NE. Daniel W. Biostatistics basic concept and methodology for the health science, 9th edition, 2010.

12- Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. (2015). Global epidemiology of Campylobacter infection. Clin Microbiol Rev. 2015. doi:10.1128/CMR.00006-15.

13- Forte GI, Scola L, Misiano G, Milano S, Mansueto P, Vitale G, Bellanca F, Sanacore M, Vaccarino L, Rini GB, Caruso C, Cillari E, Lio D. and Mansueto S. Relevance of Gamma Interferon, Tumor Necrosis Factor Alpha, and Interleukin-10 Gene Polymorphisms to Susceptibility to Mediterranean Spotted Fever. Clinical AND Vaccine Immunology. 2009; 16(6): 811–815.

14- Janssen R, Krogfelt KA, Cawthraw SA, Pelt WV, Wagenaar JA, Owen RJ. Host-pathogen interactions in Campylobacter infections: the host perspective. Clin Microbiol Rev. 2008; 21: 505–18.

15- Hussein YM, Ahmad AS, Ibrahem MM, El Tarhouny SA, Shalaby SM, Elshal AS, et al. Interferon Gamma Gene Polymorphism as a Biochemical Marker in Egyptian Atopic Patients. J Investig Allergol Clin Immunol. 2009; 19(4): 292-8.

16- Xu1 H, Li1 W, Fu H and Jiang G. Interferon-Gamma Gene Polymorphism +874 (A/T) in Chinese Children with Henoch-Schönlein Purpura. Iran J Allergy Asthma Immunol. 2014; 13(3): 184-189.

17- Abram M, VuÆckovic´D, Wraber B. and Doric´M. Plasma cytokine response in mice with bacterial infection. Mediators of Inflammation. 2000; 9: 229–234.

18- Tribble DR, Baqar S, Scott DA, et al. Assessment of the duration of protection in Campylobacter jejuni experimental infection in humans. Infect Immun. 2010; 8: 1750–9.

19- Puneetpal MS, Kumar JP, Surinder S, Taranpal K. "SNP–SNP interactions within APOE gene influence plasma lipids in postmenopausal osteoporosis". Rheumatology International. 2010; 31(3): 421–3.