Meta Gene 19 (2019) 98–103

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Cytokine gene variations and their impact on serum levels of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs

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ARTICLE INFO

Keywords:
Cytokines
Single nucleotide polymorphism
Serum level
Iraq

ABSTRACT

The impact of ten cytokine gene single nucleotide polymorphisms [SNPs: *IFNG*$_{+874}$ (rs2430561), *IL2*$_{+166}$ (rs2069763), *IL2*$_{-330}$ (rs2069762), *IL4*$_{-33}$ (rs2070874), *IL4*$_{-590}$ (rs2243250), *IL4*$_{-1098}$ (rs2243248), *IL10*$_{-592}$ (rs1800872), *IL10*$_{-819}$ (rs1800871), *IL10*$_{-1082}$ (rs1800896), and *IL12B*$_{-1188}$ (rs3212227)] on serum level of IFN-γ, IL-2, IL-4, IL-10, and IL-12 was determined among 127 Iraqi Arabs (60 males and 67 females). Their age mean ± SD was 24.1 ± 15.5 years. The results revealed that IFN-γ, IL-2, IL-4, and IL-10 levels showed no gender-associated variation, while IL-12 level was significantly increased in females compared to males. Alleles of the ten SNPs showed polymorphic frequencies, and *C* allele of *IL12B*$_{-1188}$ SNP recorded the lowest frequency (27.2%). With respect to the impact of SNP genotype on cytokine level, only *IL2*$_{+166}$ (total sample and males), *IL4*$_{-33}$, and *IL4*$_{-590}$ (total sample and females) SNPs showed a significant impact on the level of their corresponding cytokines (IL-2 and IL-4). In conclusion, a positive influence of *IL2*$_{+166}$, *IL4*$_{-590}$, and *IL4*$_{-33}$ SNP genotypes on IL-2 and IL-4 levels is suggested, and such influence was gender-dependent.

1. Introduction

Cytokines are important low molecular weight glycoprotein molecules produced by different cells in response to a variety of immune stimuli. They regulate most aspects of innate and adaptive immune responses; including, inflammation and activation, migration, proliferation of cells, as well as apoptosis and hematopoiesis (Steinke and Borish, 2006). Their action has synergistic or antagonistic effects, and can function in autocrine, paracrine or endocrine fashion. By attaching to their cognate receptors on certain cell, cytokines promote cell signalling for subsequent biochemical changes that lead to express or suppress cytokine genes and their transcription factors (Turner et al., 2014). However, cytokines are under a genetic control, and with the recent knowledge on cytokine gene polymorphisms, differences between individuals have been discovered that influence susceptibility to diseases, their progression, severity, and clinical outcomes (Zabaleta et al., 2008). In addition, ethnic differences in patterns of cytokine gene polymorphisms at the population level have also been observed. They are mainly a consequence of natural selection imposed by environmental factors and complex host-pathogen interactions. These features are correlated with population-based variations in the ability to mount an immune response (Van Dyke et al., 2009).

Cytokine gene polymorphisms are naturally occurring DNA sequence variations, which differ from gene mutations in that they occur in normal healthy populations and have a frequency of at least 1%. Approximately 90% of DNA polymorphisms are single nucleotide polymorphisms (SNPs) due to single base substitutions (Fareed and Afzal, 2013). Although most SNPs are functionally neutral, some have effects on regulation of gene expression or on function of coded protein. These functional polymorphisms, despite being of low penetrance, could contribute to the differences between individuals in susceptibility to and severity of disease (Ramírez-Bello et al., 2013). Data generated over the years has identified several important SNPs in various cytokine genes that are important markers not only for a better understanding of etiology and pathogenesis of a disease, but also as potential biomarkers of disease susceptibility and severity (Bhushan and Perumal, 2012). A

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https://doi.org/10.1016/j.mgene.2018.11.005
Received 26 September 2018; Received in revised form 16 October 2018; Accepted 8 November 2018
Available online 09 November 2018

Table 1
Serum level of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs (total, males and females).

Cytokine	Level (pg/ml)						Mann-Whitney *U* test *p*-value
	Total Sample		Males		Females		
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	
IFN-γ	41.80 (39.7)	39.4 (0.8–199.3)	35. 9 (22.4)	39.7 (5.4–75.5)	45.3 (46.9)	39.2 (0.8–199.3)	0.857
IL-2	26.6 (19.0)	27.2 (0.7–78)	29.2 (18.4)	28.8 (0.7–78.0)	24.3 (19.4)	26.5 (0.8–72.8)	0.176
IL-4	46.0 (34.7)	32.9 (4.0–158.9)	45.2 (33.7)	35.4 (4.0–158.9)	46.4 (35.6)	32.7 (4.4–128.8)	0.787
IL-10	28.2 (16.4)	25.0 (3.8–124.1)	26.9 (11.3)	25.3 (5.8–57.2)	29.3 (20.0)	25.0 (3.8–124.1)	0.900
IL-12	13.3 (11.4)	14.2 (0.2–61.2)	10.3 (9.2)	10.1 (0.2–39.5)	15.2 (12.3)	16.5 (0.2–61.2)	0.020

SD: Standard deviation, *p*: Probability.

further focus of investigations has disclosed that cytokine gene SNPs can impact the cytokine serum levels. Therefore, such subject has attracted many researchers to investigate the association between cytokine SNPs and disease susceptibility in addition to their impact on cytokine production (Vandenbroeck, 2012).

Accordingly, the present investigation aimed to determine the impact of ten cytokine gene SNPs [$IFNG_{+874}$ (rs2430561), $IL2_{+166}$ (rs2069763), $IL2_{-330}$ (rs2069762), $IL4_{-33}$ (rs2070874), $IL4_{-590}$ (rs2243250), $IL4_{-1098}$ (rs2243248), $IL10_{-592}$ (rs1800872), $IL10_{-819}$ (rs1800871), $IL10_{-1082}$ (rs1800896), and $IL12B_{-1188}$ (rs3212227)] on serum level of five important cytokines (IFN-γ, IL-2, IL-4, IL-10 and IL-12) in a sample of healthy Iraqi Arabs. These cytokines are involved in humoral and cell-mediated mediated immunities, as well as in regulation of immune response, and assessing the correlation between cytokine genotypes and their serum phenotypic expression under healthy conditions can make a better understanding of cytokine SNP functional role than in diseased populations.

2. Subjects and Methods

2.1. Subjects

The study was approved by the Ethics Committee at the Iraqi Ministry of Health, in which a sample of 127 unrelated healthy Iraqi Arabs (60 males and 67 females) were recruited during the period January 2013–December 2015. Age mean ± SD in total sample was 24.1 ± 15.5 years (males: 21.7 ± 13.9 and females: 26.3 ± 16.7 years). The participants were carefully inspected for ethnicity before blood sample collection. Arabic (Ethnologue three-letter language code, *arb*) was the mother-tongue language for them and their parents (father and mother). In addition, there were no marriages of mixed ethnicities in their families up to grandfathers and grandmothers, as participants declared. They were mostly blood donors (Central Blood Bank, Baghdad) and their blood profile of laboratory tests carried out by the bank were negative. Some participants were younger than 16 years old, and they were referred to the Healthcare Units in Baghdad for routine health check. None of the subjects was smoker or under any type of therapy, and had no chronic diseases.

2.2. Blood sample collection

From each participant, 5 ml of venous blood were collected and distributed into two types of tubes: 2 ml in a plain tube for serum collection and 3 ml in EDTA tube for DNA isolation. The sera and isolated DNA was stored at −20 °C until laboratory manipulations.

2.3. Assessment of cytokine serum level

Sandwich enzyme linked immunosorbent assay (ELISA) was employed to measure serum levels of five cytokines (IFN-γ, IL-2, IL-4, IL-10, and IL-12) using commercially available kits, and instructions of manufacturer were followed (PeproTech Company, UK).

2.4. Determination of cytokine gene SNPs

The DNA was isolated from whole EDTA blood using a ready kit (ReliaPrep™ Blood gDNA Miniprep System; Promega Corporation, USA). The isolated DNA was subjected for genotyping of ten cytokine SNPs in the promoter regions of *IFNG*, *IL2*, *IL4*, *IL10* and *IL12B* genes: $IFNG_{+874}$ (rs2430561), $IL2_{+166}$ (rs2069763), $IL2_{-330}$ (rs2069762), $IL4_{-33}$ (rs2070874), $IL4_{-590}$ (rs2243250), $IL4_{-1098}$ (rs2243248), $IL10_{-592}$ (rs1800872), $IL10_{-819}$ (rs1800871), $IL10_{-1082}$ (rs1800896), and $IL12B_{-1188}$ (rs3212227). The Cytokine CTS-PCR-SSP (Collaborative Transplant Study-Polymerase Chain Reaction-Sequence Specific Primer) Tray Kit (University Clinic, Heidelberg, Germany) was used to determine SNP genotypes according to instructions of manufacturer.

2.5. Statistical analysis

Data of cytokine serum levels were given as mean, standard deviation (SD), median, and range. After a normality test of original data, significant differences were assessed by the non-parametric Mann–Whitney test or Kruskal-Wallis test. Allele frequency of SNPs was estimated by direct gene counting method, while the departure from Hardy Weinberg equilibrium (HWE) was assessed using Pearson chi-squared goodness of fit test. Odds ratio (OR) together with its 95% CI (confidence interval) was also estimated for males versus females. The difference was considered significant when the probability (two-tailed Fisher's exact *p*) value was ≤ 0.05. The online HWE calculator for two alleles, WinPepi software version 11.65, and SPSS statistical package version 13.0 were employed to carry out these analyses.

3. Results

3.1. Cytokine serum level

IFN-γ showed the highest level median (39.4 pg/ml), while IL-12 was observed to have the lowest level (14.2 pg/ml). There were no significant gender-associated variations in the distribution of the five cytokine levels, with the exception of IL-12, which showed a significant increased median in females compared to males (16.5 vs. 10.1 pg/ml) (Table 1).

Table 2
Allele and genotype frequencies of *IFNG*, *IL2*, *IL4*, *IL10* and *IL12B* gene SNPs among Iraqi Arabs (total, males and females).

Cytokine SNP	Allele/ genotype	Total sample		Males		Females		*p*-value
		N	%	N	%	N	%	
$IFNG_{+874}$	*T*	97	57.7	34	54.8	63	59.4	0.628[a]
(rs2430561)	*A*	71	42.3	28	45.2	43	40.6	
N = 84	TT	30	35.7	9	29.0	21	39.6	0.535[b]
	TA	37	44.0	16	51.6	21	39.6	
	AA	17	20.2	6	19.4	11	20.8	
HWE *p*-value		0.371		0.815		0.194		
$IL2_{+166}$	*G*	166	65.4	76	63.3	90	67.2	0.598[a]
(rs2069763)	*T*	88	34.6	44	36.7	44	32.8	
N = 127	GG	54	42.5	25	41.7	29	43.3	0.593[b]
	GT	58	45.7	26	43.3	32	47.8	
	TT	15	11.8	9	15.0	6	8.9	
HWE *p*-value		0.923		0.603		0.497		
$IL2_{-330}$	*G*	105	41.3	47	39.2	58	43.3	0.526[a]
(rs2069762)	*T*	149	58.7	73	60.8	76	56.7	
N = 127	GG	24	18.9	10	16.7	14	20.9	0.821[b]
	GT	57	44.9	27	45.0	30	44.8	
	TT	46	36.2	23	38.3	23	34.3	
HWE *p*-value		0.400		0.666		0.471		
$IL4_{-33}$	*C*	110	63.2	42	61.8	68	64.2	0.750[a]
(rs2070874)	*T*	64	36.8	26	38.2	38	35.8	
N = 87	CC	38	43.7	13	38.2	25	47.2	0.497[b]
	CT	34	39.1	16	47.1	18	34.0	
	TT	15	17.2	5	14.7	10	18.8	
HWE *p*-value		0.136		0.983		0.057		
$IL4_{-590}$	*C*	101	58.0	38	55.9	63	59.4	0.753[a]
(rs2243250)	*T*	73	42.0	30	44.1	43	40.6	
N = 87	CC	32	36.8	11	32.3	21	39.6	0.718[b]
	CT	37	42.5	16	47.1	21	39.6	
	TT	18	20.7	7	20.6	11	20.8	
HWE *p*-value		0.236		0.790		0.194		
$IL4_{-1098}$	*T*	111	63.8	43	63.2	68	64.2	1.000[a]
(rs2243248)	*G*	63	36.2	25	36.8	38	35.8	
N = 87	TT	40	46.0	16	47.1	24	45.3	0.878[b]
	TG	31	35.6	11	32.4	20	37.7	
	GG	16	18.4	7	20.6	9	17.0	
HWE *p*-value		**0.032**		0.076		0.191		
$IL10_{-592}$	*C*	167	65.8	77	64.2	90	67.2	0.691[a]
(rs1800872)	*A*	87	34.2	43	35.8	44	32.8	
N = 127	CC	58	45.7	26	43.3	32	47.8	0.884[b]
	CA	51	40.1	25	41.7	26	38.8	
	AA	18	14.2	9	15.0	9	13.4	
HWE *p*-value		0.221		0.466		0.325		
$IL10_{-819}$	*C*	161	63.9	79	65.8	82	62.1	0.600[a]
(rs1800871)	*T*	91	36.1	41	34.2	50	37.8	
N = 126	CC	59	46.8	30	50.0	29	43.9	0.819[b]
	CT	43	34.1	19	31.7	24	36.4	
	TT	24	19.1	11	18.3	13	19.7	
HWE *p*-value		**0.004**		**0.022**		0.065		
$IL10_{-1082}$	*A*	171	67.3	82	68.3	89	66.4	0.789[a]
(rs1800896)	*G*	83	32.7	38	31.7	45	33.6	
N = 127	AA	59	46.5	29	48.3	30	44.8	0.937[b]
	AG	53	41.7	24	40.0	29	43.3	
	GG	15	11.8	7	11.7	8	11.9	
HWE *p*-value		0.561		0.557		0.807		
$IL12B_{-1188}$	*A*	150	72.8	61	74.4	89	71.8	0.750[a]
(rs3212227)	*C*	56	27.2	21	25.6	35	28.2	
N = 103	AA	59	57.3	24	58.5	35	56.5	0.916[b]
	AC	32	31.1	13	31.7	19	30.6	
	CC	12	11.7	4	9.8	8	12.9	
HWE *p*-value		**0.029**		0.282		0.055		

SNP: Single nucleotide polymorphism, N: Absolute number, HWE: Hardy-Weinberg equilibrium, *p*: probability. Bold indicates HWE p-value (assess the significant departure from the equilibrium).

[a] Comparison between allele frequencies.

[b] Comparison between genotype frequencies.

3.2. HWE and allele and genotype frequencies

A HWE test was performed for the ten cytokine SNPs. There were no significant variations between the observed and expected genotype frequencies; therefore a good agreement with the equilibrium was observed. Three exceptions were encountered; $IL4_{-1098}$ in total sample, $IL10_{-819}$ in total sample and males, and $IL12B_{-1188}$ in total sample, in which a significant departure from the equilibrium was recorded.

All SNP alleles showed polymorphic frequencies and the lowest frequency was recorded for *C* allele of $IL12B_{-1188}$ SNP (27.2%). In addition, there were no significant variations between males and females in the distribution of allele and genotype frequencies of the ten SNPs (Table 2). The SNPs were also presented in terms of allele carriers, and again and no significant gender-associated variation was observed (Table 3).

3.3. Impact of SNPs on cytokine serum level

Only three SNPs ($IL2_{+166}$, $IL4_{-33}$, and $IL4_{-590}$) significantly influenced IL-2 and IL-4 serum levels. The GT genotype of $IL2_{+166}$ SNP showed the highest median of IL-2 in total sample and males (30.5 and 32.4 pg/ml, respectively) compared to GG (12.8 and 24.2 pg/ml, respectively) or TT genotype (25.4 and 28.7 pg/ml, respectively). $IL4_{-33}$ and $IL4_{-590}$ were the further two SNPs, in which the CC genotype demonstrated a significant increased median of IL-4 in total sample (77.0 and 82.2 pg/ml, respectively) compared to the corresponding CT (25.0 and 32.9 pg/ml, respectively) or TT (32.9 and 20.8 pg/ml, respectively) genotype. Such impact of $IL4_{-33}$ and $IL4_{-590}$ was mainly confined to females rather than males (CC: 81.3 and 83.2; CT: 21.8 and 32.7; TT: 30.0 and 14.3 pg/ml, respectively) (Table 4). Such profile was almost similar when the medians of cytokines were inspected in the participants distributed according to their allele carrier genotypes of SNPs (Table 5).

4. Discussion

The representative profile of the current Iraqi Arabs was inspected by comparing the investigated cytokine SNP genotype frequencies with their correspondent frequencies in a recent study that documented genotype frequencies of 22 cytokine SNPs (including the present SNPs) in Iraqi Arabs (Ad'hiah et al., 2018). No significant variations between the genotype frequencies of the two studies were observed, and accordingly the enrolled population can be validated and representative. However, the aim of present study was beyond determining allele and genotype frequencies, and instead it focused on the impact of some cytokine SNP genotypes on their phenotypic profile in sera of a healthy rather than a diseased population (i.e. examining a SNP effect under healthy conditions without the interference of disease).

In diseased populations, it is well presented that cytokines mediate prominent actions during immunological reactions, and changes in their serum levels may consequence in abnormal or ineffective immune responses (Turner et al., 2014). In this context, a number of cytokine SNPs have been suggested to be associated with alternations in cytokine serum levels (Vandenbroeck, 2012). The investigated cytokines are prominent signature of subsets of T helper (Th) lymphocytes; Th1 (IFN-γ and IL-2) and Th2 (IL-4) cells, as well as T regulatory (IL-10) cells, which mediate and regulate most aspects of immunological reactions. IL-12 is also critical cytokine that regulates the balance between Th1 and Th2 lymphocytes, and involved in inducing naïve CD4+ T cell differentiation to become IFN-γ-producing Th1 effector cells in cell-mediated immune responses (Gagliani and Huber, 2017). Therefore, a correlation between genetic polymorphisms and phenotypic expression

Table 3
Allele carrier frequency of cytokine SNP genotypes among Iraqi Arabs (total, males and females).

Cytokine SNP	Allele carrier genotype	Total sample		Males		Females		OR (95% CI)	Reverse OR	*p*-value
		N	%	N	%	N	%			
IFNG$_{+874}$	TT	30	35.7	9	29.0	21	39.6	0.62 (0.24–1.59)	1.60	0.356
	TA + AA	54	64.3	22	71.0	32	60.4			
IL2$_{+166}$	GG	54	42.5	25	41.7	29	43.3	0.94 (0.47–1.88)	1.07	0.860
	GT + TT	73	57.5	35	58.3	38	56.7			
IL2$_{-330}$	GG	24	18.9	10	16.7	14	20.9	0.76 (0.31–1.85)	1.32	0.652
	GT + TT	103	81.1	50	83.3	53	79.1			
IL4$_{-33}$	CC	38	43.7	13	38.2	25	47.2	0.69 (0.29–1.65)	1.44	0.508
	CT + TT	49	56.3	21	61.8	28	52.8			
IL4$_{-590}$	CC	32	36.8	11	32.4	21	39.6	0.73 (0.30–1.78)	1.37	0.649
	CT + TT	55	63.2	23	67.6	32	60.4			
IL4$_{-1098}$	TT	40	46.0	16	47.1	24	45.3	1.07 (0.46–2.52)	0.93	1.000
	TG + GG	47	54.0	18	52.9	29	54.7			
IL10$_{-592}$	CC	58	45.7	26	43.3	32	47.8	0.84 (0.42–1.68)	1.20	0.722
	CA + AA	69	54.3	34	56.7	35	52.2			
IL10$_{-819}$	CC	59	46.8	30	50.0	29	43.9	1.28 (0.64–2.56)	0.78	0.592
	CT + TT	67	53.2	30	50.0	37	56.1			
IL10$_{-1082}$	AA	59	46.5	29	48.3	30	44.8	1.15 (0.58–2.31)	0.87	0.724
	AG + GG	68	53.5	31	51.7	37	55.2			
IL12B$_{-1188}$	AA	59	57.3	24	58.5	35	56.5	1.09 (0.49–2.40)	0.92	1.000
	AC + CC	44	42.7	17	41.5	27	43.5			

SNP: Single nucleotide polymorphism, N: Absolute number, OR: Odds ratio, CI: Confidence interval, *p*: Two-tailed Fisher's exact probability.

may make a more understanding of the cellular production of cytokines.

Out of the ten investigated SNPs, only three (*IL2*$_{+166}$, *IL4*$_{-33}$, and *IL4*$_{-590}$) influenced the serum level of their corresponding cytokines (IL-2 and IL-4). Studies in this context have not been well-elaborated, and some scattered results were reported in the control groups of case-control studies. For IFN-γ, the homozygous TT genotype of *IFNG*$_{+874}$ SNP was associated with the highest level compared to the other two genotypes (AA and AT), but the difference was no significant. Peresi and colleagues were also in favor of no significant influence of *IFNG*$_{+874}$ and *IFNG*$_{+2109}$ SNPs on IFN-γ plasma level in a healthy group of a Brazilian control (Peresi et al., 2013). A similar finding was also reported by Hussein et al. but in atopic Egyptian patients rather than control, in which the data were not given (Hussein et al., 2009). Regarding *IL2* SNPs (*IL2*$_{-330}$ and *IL2*$_{+166}$), the present findings indicate the absence of any correlation between *IL2*$_{-330}$ SNP genotypes and IL-2 serum level, and such observation is consistent with the findings of Wei et al., who reported no significant differences between the three genotypes of *IL2*$_{-330}$ SNP (GG, GT and TT) in their relation to IL-2 serum level (Wei et al., 2010). However in an Egyptian study, a contradictory result was reported, and the TT genotype was associated with the highest level of IL-2 (Mohamed et al., 2017). For *IL2*$_{+166}$ SNP, the current results demonstrated that carriers of GT genotypes (total sample and males) showed the highest level of IL-2 compared to those of GG or TT genotype. In Iranians, such findings are partially supported, and *IL2*$_{+166}$ SNP also influenced IL-2 level but through TT genotype rather than GT genotype (Sayad and Movafagh, 2014).

In the case of *IL4* gene, *IL4*$_{-590}$ and *IL4*$_{-33}$ SNPs showed a positive influence on IL-4 production through their CC genotypes, which were markedly associated with an increased level of IL-4, especially in females, in whom the difference was highly significant. In contrast, Malutan et al. observed no significant difference in IL-4 serum levels between *IL4*$_{-590}$ genotypes of Romanians (Malutan et al., 2016), and the same observation was also made in Chinese children (Shang et al., 2016). The exact mechanism for this change in IL-4 level is not well-defined, but it may be possible that such alterations (polymorphisms) influenced the transcription rate of *IL4* gene. However, the influence of both SNPs was restricted to females, and some gender effect is suggested. It is difficult to explain such finding as probably there has been no evidence that confirm or contradict such outcome, but Babula et al. reported a positive association between *IL4*$_{-589}T$ allele homozygosity and vaginal IL-4 levels in women with recurrent vulvovaginal candidiasis (Babula et al., 2005).

With respect to *IL10* gene, the three investigated SNPs (*IL10*$_{-592}$, *IL10*$_{-819}$, and *IL10*$_{-1082}$) showed no impact on IL-10 level. Similar findings were reported in Egyptians (Talaat et al., 2014) and Brazilians (Peresi et al., 2013), but not in Polish. The *IL10*$_{-1082}$ SNP influenced IL-10 production in the latter population, and a statistically higher level of IL-10 among carriers of *G* allele was noticed (Lesiak et al., 2014). In addition, it has also been recently reported that IL-10 level in carriers of *IL10*$_{-819}$ TT genotype was significantly higher than those of CC genotype, but in a total Brazilian sample of type 2 diabetes mellitus patients and controls (Rodrigues et al., 2017).

The final SNP that showed no impact on serum level of IL-12 is *IL12B*$_{-1188}$. In agreement with such finding, Paradowska-Gorycka and co-workers examined the relationship between IL-12 levels in IL-12-positive/negative rheumatoid arthritis and control group in relation to *IL12B*$_{-1188}$ genotypes and found no significant association, neither among patients nor in healthy subjects (Paradowska-Gorycka et al., 2017). However, in a further study, carriers for *IL12B*$_{+1188}C$ allele were reported to have significantly higher plasma IL-12 levels than individuals with the AA genotype in a Brazilian control (Peresi et al., 2013). In present study, it was also observed that individuals with CC and AC genotype had an increased level of IL-12, especially females but the difference was not significant. The females also showed a significant increased level of IL-12 compared to males. Similarly, Attallah et al. also reported an increased serum level of IL-12 in psoriasis Egyptian female patients compared to males, but the difference was not significant, and no data for control was given (Attallah et al., 2016).

As presented, discrepancies were observed between studies regarding the influence of cytokine SNP genotypes on their phenotypic expression in sera of healthy individuals, and this might be due to variations in host

Table 4
Relation of cytokine gene SNPs to serum level of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs (total, males and females).

Cytokine SNP Genotype	Level (pg/ml)								
	Total Sample			Males			Females		
	Mean (SD)	Median (Range)	*p*-value	Mean (SD)	Median (Range)	*p*-value	Mean (SD)	Median (Range)	*p*-value
IFNG $_{+874}$			0.384			0.938			0.294
TT	49.9 (49.8)	43.5 (29.6–199.3)		36.0 (22.3)	42.2 (6.7–63.7)		55.9 (57.2)	44.6 (6.8–199.3)	
TA	35.6 (34.5)	29.6 (0.9–137.2)		35.1 (23.0)	38.6 (5.4–70.7)		36.0 (41.8)	12.5 (0.9–137.2)	
AA	41.0 (28.4)	44.7 (0.8–116.0)		37.9 (25.0)	27.2 (13.4–75.5)		42.6 (31.1)	46.0 (0.8–116.0)	
IL2 $_{+166}$			**0.017**			**0.046**			0.308
GG	20.6 (18.4)	12.8 (0.8–72.8)		22.0 (16.4)	24.2 (0.7–52.8)		19.4 (20.2)	7.4 (0.8–72.8)	
GT	32.3 (18.4)	30.5 (1.1–78.0)		36.5 (19.2)	32.4 (1.8–78.0)		28.9 (17.3)	29.5 (1.1–66.5)	
TT	26.5 18.0	25.4 (3.5–61.0)		28.4 (14.3)	28.7 (6.6–48.0)		23.5 (23.6)	15.3 (3.5–61.0)	
IL2 $_{-330}$			0.605			0.209			0.476
GG	23.3 (19.6)	22.3 (0.8–78.0)		20.3 (15.0)	22.3 (1.4–41.9)		25.3 (22.7)	24.3 (0.8–72.8)	
GT	26.8 (18.6)	26.8 (0.7–78.0)		32.7 (18.6)	28.7 (0.7–78.0)		21.4 (17.1)	24.8 (0.8–62.5)	
TT	28.2 (19.4)	29.2 (1.7–73.8)		29.0 (18.8)	29.9 (1.8–73.8)		27.5 (20.4)	27.3 (1.7–66.5)	
IL4 $_{-33}$			**0.001**			0.749			< **0.001**
CC	65.8 (41.8)	77.0 (4.0–158.9)		58.1 (48.7)	36.1 (4.0–158.9)		69.8 (38.2)	81.3 (7.0–128.8)	
CT	29.8 (16.0)	25.0 (4.4–66.2)		36.1 (16.0)	32.8 (16.4–66.2)		24.1 (14.0)	21.8 (4.4–50.4)	
TT	32.5 (16.6)	32.9 (8.5–71.1)		41.1 (20.0)	34.6 (21.3–71.1)		28.2 (13.8)	30.0 (8.5–56.5)	
IL4 $_{-590}$			< **0.001**			0.203			< **0.001**
CC	70.9 (41.7)	82.2 (8.5–158.9)		68.0 (47.2)	52.5 (16.4–158.9)		72.4 (39.7)	83.2 (8.5–128.8)	
CT	34.5 (17.6)	32.9 (4.0–93.0)		34.0 (15.6)	35.4 (4.0–62.4)		35.0 (19.4)	32.7 (7.0–93.0)	
TT	25.1 18.6	20.8 (4.4–71.1)		35.2 (22.9)	22.6 (18.8–71.1)		18.7 (12.6)	14.3 (4.4–44.8)	
IL4 $_{-1098}$			0.101			0.670			0.115
TT	52.4 (34.3)	44.7 (4.0–128.8)		44.4 (26.4)	38.8 (4.0–101.0)		57.6 (38.3)	44.9 (8.5–128.8)	
TG	45.9 (40.0)	30.0 (4.4–158.9)		54.5 (48.6)	36.1 (6.3–158.9)		41.3 (35.0)	28.5 (4.4–124.4)	
GG	30.1 (16.2)	26.5 (8.5–66.2)		32.6 (15.3)	27.8 (22.3–66.2)		28.1 (17.5)	25.2 (8.5–56.5)	
IL10 $_{-592}$			0.107			0.262			0.308
CC	31.7 (19.8)	27.5 (5.8–124.1)		29.2 (13.1)	29.4 (5.8–51.8)		33.7 (24.0)	26.8 (5.8–124.1)	
CA	24.5 (12.9)	25.0 (3.8–71.1)		24.3 (8.3)	25.0 (10.5–36.2)		24.8 (16.2)	24.3 (3.8–71.1)	
AA	27.1 (10.8)	25.0 (9.7–57.2)		27.4 (12.7)	25.0 (9.7–57.2)		26.7 (9.4)	22.1 (14.6–40.6)	
IL10 $_{-819}$			0.348			0.298			0.648
CC	29.4 (15.6)	27.3 (5.8–65.7)		28.5 (12.6)	29.3 (5.8–51.8)		30.5 (18.4)	26.4 (5.8–65.7)	
CT	25.3 (13.3)	25.0 (3.8–71.1)		23.8 (7.9)	25.0 (9.7–37.2)		26.4 (16.5)	25.5 (3.8–71.1)	
TT	26.1 (10.7)	22.0 (13.7–57.2)		27.8 (12.4)	25.0 (13.7–57.2)		24.7 (9.3)	22.0 (14.6–50.1)	
IL10 $_{-1082}$			0.241			0.696			0.258
AA	26.8 (10.8)	25.0 (8.3–57.2)		27.5 (10.6)	26.8 (9.7–57.2)		26.2 (11.2)	24.4 (8.3–54.5)	
AG	28.5 (21.5)	25.0 (3.8–124.1)		26.0 (12.6)	25.0 (5.8–51.8)		30.6 (26.8)	20.8 (3.8–124.1)	
GG	32.1 (14.3)	31.6 (5.8–53.1)		27.3 (10.9)	30.3 (6.8–40.9)		36.4 (16.2)	40.7 (5.8–53.1)	
IL12B $_{-1188}$			0.393			0.804			0.307
AA	12.5 (12.2)	11.9 (0.2–61.2)		10.2 (7.9)	10.5 (0.2–23.1)		14.1 (14.3)	14.7 (0.2–61.2)	
AC	14.8 (10.9)	17.4 (0.3–39.5)		11.5 (12.2)	5.5 (0.3–39.5)		17.1 (9.6)	19.4 (1.6–35.7)	
CC	12.8 (8.6)	12.4 (0.8–61.2)		7.0 (5.7)	6.9 (0.8–13.2)		15.7 (8.6)	17.7 (2.5–27.2)	

SNP: Single nucleotide polymorphism, SD: Standard deviations; *p*: Kruskal Wallis test probability. Bold indicates the p-value assesses the significance of difference between medians of three genotypes at each locus.

genetic and non-genetic factors of various ethnic populations. Equally important, the environmental challenge in each population is also different and may have an impact on cytokine production. Such theme has been addressed in studies within the Human Functional Genomics Project, which assessed the effect of genetic and non-genetic host factors and intestinal microbiome on cytokine responses in humans. The association between these factors and circulating cytokines (IL-6, IL-18, IL-18-binding protein, IL-1β, and IL-1 receptor antagonist) were analyzed in 534 healthy subjects after stimulation with 19 different microbial (bacteria, fungi and viruses) and non-microbial metabolic stimuli. An important conclusion depicted that non-genetic host factors (age or gender) have a distinct effect on cytokine responses. Annual seasonality was also described to be an important environmental factor that can influence cytokine production (Ter Horst et al., 2016). Therefore, future studies in this regard should make a concern of these factors when assessing the cytokine SNP effects on their systemic profile.

5. Conclusion

A positive impact of *IL2* $_{+166}$, *IL4* $_{-33}$, and *IL4* $_{-590}$ SNP genotypes on serum level of IL-2 and IL-4 is suggested in Iraqi Arabs. Of notice in this context is *IL4* $_{-33}$ and *IL4* $_{-590}$ SNPs among females. However, the present study is limited by the sample size, and a larger number of a population can give a better profile of gene SNP impact on cytokine serum level. Equally important, the results could be more fruitful if SNP genotypes were correlated with gene expression of cytokines.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 5
Relation of cytokine gene SNP genotypes of allele carriers to serum level of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs (total, males and females).

Cytokine SNP	Allele carrier genotype	Level (pg/ml)								
		Total Sample			Males			Females		
		Median	Range	*p*-value	Median	Range	*p*-value	Median	Range	*p*-value
$IFNG_{+874}$	TT	43.5	6.7–199.3	0.346	42.2	6.7–63.7	0.983	44.6	6.8–199.3	0.230
	TA + AA	31.2	0.7–137.2		33.5	5.4–75.5		31.2	0.7–137.2	
$IL2_{+166}$	GG	12.8	0.7–72.8	0.006	24.2	0.7–52.8	0.018	7.4	0.7–72.8	0.149
	GT + TT	29.4	1.1–78.0		30.7	1.8–78.0		28.0	1.1–66.5	
$IL2_{-330}$	GG	22.3	0.8–72.8	0.404	22.3	1.4–41.9	0.088	24.3	0.8–72.8	0.622
	GT + TT	27.3	0.7–78.0		29.7	0.7–78.0		26.5	0.7–66.5	
$IL4_{-33}$	CC	77.0	4.0–158.9	< 0.001	36.1	4.0–158.9	0.506	81.3	7.0–128.8	< 0.001
	CT + TT	26.2	4.4–71.1		34.6	16.4–71.1		24.1	4.4–56.5	
$IL4_{-590}$	CC	82.2	8.5–158.9	< 0.001	52.5	16.4–158.9	0.098	83.2	8.5–128.8	0.001
	CT + TT	28.4	4.0–93.0		29.6	4.0–71.1		26.6	4.4–93.0	
$IL4_{-1098}$	TT	44.7	4.0–128.8	0.051	38.8	4.0–101.0	0.506	44.9	8.5–128.8	0.051
	TG + GG	27.8	4.4–158.9		28.1	6.3–158.9		27.1	4.4–124.4	
$IL10_{-592}$	CC	27.5	5.8–124.1	0.051	29.4	5.8–51.8	0.113	26.8	5.8–124.1	0.154
	CA + AA	25.0	3.8–71.1		25.0	9.7–57.2		23.6	3.8–71.1	
$IL10_{-819}$	CC	27.3	5.8–65.7	0.147	29.3	5.8–51.8	0.164	26.4	5.8–65.7	0.366
	CT + TT	25.0	3.8–71.1		25.0	9.7–57.2		23.8	3.8–71.1	
$IL10_{-1082}$	AA	25.0	8.3–57.2	0.983	26.8	9.7–57.2	0.539	24.4	8.3–54.5	0.719
	AG + GG	25.0	3.8–124.1		25.0	5.8–51.8		26.4	3.8–124.1	
$IL12B_{-1188}$	AA	11.9	0.1–61.2	0.182	10.5	0.1–23.1	0.781	14.7	0.1–61.2	0.127
	AC + CC	15.4	0.2–39.5		5.5	0.2–39.5		19.4	1.6–35.7	

SNP: Single nucleotide polymorphism, SD: Standard deviation; *p*: Mann-Whitney *U* test probability.

Acknowledgement

The cooperation of participants and their families is appreciated.

References

Ad'hiah, A.H., Ahmed, Z.A., Al-Naseri, M.A., Salman, E.D., Al-Saffar, O.B., Ahmed, H.S., Hussain, T.A., Idan, E.M., Hassan, I.B., Shihab, B.A., Mohammed, L.O., Al-Nasiri, F.S., 2018. Cytokine gene polymorphisms in Iraqi Arabs. Hum. Immunol. 79, 91–92. https://doi.org/10.1016/j.humimm.2017.12.009.

Attallah, D.A., Awad, S.M., Salama, R.H.M., Mahran, A.M., Ahmed, A.O., 2016. Serum levels of IL-12 and IL-23 in psoriatic patients with or without concomitant hepatitis C. J. Egypt. Wom. Dermatol. Soc. 13, 71–76. https://doi.org/10.1097/01.EWX.0000481338.75599.27.

Babula, O., Lazdane, G., Kroica, J., Linhares, I.M., Ledger, W.J., Witkin, S.S., 2005. Frequency of interleukin-4 (IL-4) -589 gene polymorphism and vaginal concentrations of IL-4, nitric oxide, and mannose-binding lectin in women with recurrent vulvovaginal candidiasis. Clin. Infect. Dis. 40, 1258–1262. https://doi.org/10.1086/429246.

Bhushan, S., Perumal, N.B., 2012. Disease associated cytokine SNPs database: an annotation and dissemination model. Cytokine 57, 107–112. https://doi.org/10.1016/j.cyto.2011.10.009.

Fareed, M., Afzal, M., 2013. Single nucleotide polymorphism in genome-wide association of human population: a tool for broad spectrum service. Egypt. J. Med. Hum. Genet. 14, 123–134. https://doi.org/10.1016/J.EJMHG.2012.08.001.

Gagliani, N., Huber, S., 2017. Basic aspects of T helper cell differentiation. Methods Mol. Biol. 1514, 19–30. https://doi.org/10.1007/978-1-4939-6548-9_2.

Hussein, Y.M., Ahmad, A.S., Ibrahem, M.M., El Tarhouny, S.A., Shalaby, S.M., Elshal, A.S., El Said, M., 2009. Interferon gamma gene polymorphism as a biochemical marker in Egyptian atopic patients. J Investig Allergol Clin Immunol 19, 292–298.

Lesiak, A., Zakrzewski, M., Przybyłowska, K., Rogowski-Tylman, M., Wozniacka, A., Narbutt, J., 2014. Atopic dermatitis patients carrying G allele in −1082 G/A IL-10 polymorphism are predisposed to higher serum concentration of IL-10. Arch. Med. Sci. 6, 1239–1243. https://doi.org/10.5114/aoms.2014.47833.

Malutan, A.M., Drugan, C., Drugan, T., Ciortea, R., Mihu, D., 2016. The association between interleukin-4 -590C/T genetic polymorphism, IL-4 serum level, and advanced endometriosis. Cent. J. Immunol. 41, 176–81. https://doi.org/10.5114/ceji.2016.60992

Mohamed, S., El, S., Mohamed, R., Ali, A., Elsayed, R., Abd, M., Hussien, G., Ewida, T., Abdel, A., Nemr, K., 2017. The association of interleukin-2 gene polymorphism with its plasma concentration in Egyptian multiple sclerosis patients. Am. J. Biochem. 7, 37–42. https://doi.org/10.5923/j.ajb.20170703.01.

Paradowska-Gorycka, A., Sowinska, A., Stypińska, B., Haladyj, E., Pawlik, A., Romanowska-Próchnicka, K., Olesinska, M., 2017. IL-12B gene polymorphisms and IL-12 p70 serum levels among patients with rheumatoid arthritis. Scand. J. Immunol. 85, 147–154. https://doi.org/10.1111/sji.12514.

Peresi, E., Ragozo, L., Oliveira, C., Laurentino, W., Alessandra, É., Nunes, P., Pessoa, J., Jr, A., Ayres, J.A., Rita, M., Fortes, P., Graviss, E.A., Pereira, A.C., Calvi, S.A., 2013. Cytokine polymorphisms, their influence and levels in Brazilian patients with pulmonary tuberculosis during antituberculosis treatment. Tuberc. Res. Treat. 2013, 285094. https://doi.org/10.1155/2013/285094.

Ramírez-Bello, J., Vargas-Alarcón, G., Tovilla-Zárate, C., Fragoso, J.M., 2013. Single nucleotide polymorphisms (SNPs): functional implications of regulatory-SNP (rSNP) and structural RNA (srSNPs) in complex diseases. Gac. Med. Mex. 149, 220–228.

Rodrigues, K.F., Pietrani, N.T., Bosco, A.A., Campos, F.M.F., Sandrim, V.C., Gomes, K.B., 2017. IL-6, TNF-α, and IL-10 levels/polymorphisms and their association with type 2 diabetes mellitus and obesity in Brazilian individuals. Arch. Endocrinol. Metab. 61, 438–446. https://doi.org/10.1590/2359-3997000000254.

Sayad, A., Movafagh, A., 2014. The association of −330 interleukin-2 gene polymorphism with its plasma concentration in Iranian multiple sclerosis patients. Scientifica 2014 (724653). https://doi.org/10.1155/2014/724653.

Shang, H., Cao, X.L., Wan, Y.J., Meng, J., Guo, L.H., 2016. IL-4 gene polymorphism may contribute to an increased risk of atopic dermatitis in children. Dis. Markers 2016, 1021942. https://doi.org/10.1155/2016/1021942.

Steinke, J., Borish, L., 2006. 3. Cytokines and chemokines. J. Allergy Clin. Immunol. 117, S441–S445. https://doi.org/10.1016/j.jaci.2005.07.001.

Talaat, R.M., Abdel-Aziz, A.M., El-Maadawy, E.A., Abdel-Bary, N., 2014. Interleukin 10 gene promoter polymorphism and risk of diffuse large B cell lymphoma (DLBCL). Egypt. J. Med. Hum. Genet. 15, 7–13. https://doi.org/10.1016/J.EJMHG.2013.09.001.

Ter Horst, R., Jaeger, M., Smeekens, S.P., Oosting, M., Swertz, M.A., Li, Y., Kumar, V., Diavatopoulos, D.A., Jansen, A.F.M., Lemmers, H., Toenhake-Dijkstra, H., van Herwaarden, A.E., Janssen, M., van der Molen, R.G., Joosten, I., Sweep, F.C.G.J., Smit, J.W., Netea-Maier, R.T., Koenders, M.M.J.F., Xavier, R.J., van der Meer, J.W.M., Dinarello, C.A., Pavelka, N., Wijmenga, C., Notebaart, R.A., Joosten, L.A.B., Netea, M.G., 2016. Host and environmental factors influencing individual human cytokine responses. Cell 167, 1111–1124.e13. https://doi.org/10.1016/j.cell.2016.10.018.

Turner, M.D., Nedjai, B., Hurst, T., Pennington, D.J., 2014. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim. Biophys. Acta, Mol. Cell Res. 1843, 2563–2582. https://doi.org/10.1016/j.bbamcr.2014.05.014.

Van Dyke, A.L., Cote, M.L., Wenzlaff, A.S., Land, S., Schwartz, A.G., 2009. Cytokine SNPs: comparison of allele frequencies by race and implications for future studies. In: Cytokine, https://doi.org/10.1016/j.cyto.2009.02.003.

Vandenbroeck, K., 2012. Cytokine gene polymorphisms and human autoimmune disease in the era of genome-wide association studies. J. Interf. Cytokine Res. 32, 139–151. https://doi.org/10.1089/jir.2011.0103.

Wei, Y.-S., Lan, Y., Zhang, L., Wang, J.-C., 2010. Association of the interleukin-2 polymorphisms with interleukin-2 serum levels and risk of nasopharyngeal carcinoma. DNA Cell Biol. 29, 363–368. https://doi.org/10.1089/dna.2010.1019.

Zabaleta, J., Schneider, B.G., Ryckman, K., Hooper, P.F., Camargo, M.C., Piazuelo, M.B., Sierra, R.A., Fontham, E.T.H., Correa, P., Williams, S.M., Ochoa, A.C., 2008. Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. Cancer Immunol. Immunother. 57, 107–114. https://doi.org/10.1007/s00262-007-0358-4.