Frequency distribution of *IL-17A* G197A (rs2275913) and *IL-17F* A7488G (rs763780) polymorphisms among healthy Sudanese population

Nouh Saad Mohamed (✉ nouh_saad@outlook.com)
Ahfad University for Women School of Pharmacy  https://orcid.org/0000-0001-6843-3361

Emmanuel E. Siddig
University of Khartoum

Abdallah E. Ahmed
Alfarrabi College for Science and Technology

Musab MA. Albsheer
Sinnar University Faculty of Medicine and Health Sciences

Hanadi Abdelbagi
Ahfad University for Women School of Pharmacy

Eman T. Ali
University of Khartoum

Anadel A. Alsubki
Nile University

Sabah A. Abdalaziz
Nile University

Madinna Mustafa
Nile University

Mohamed S. Muneer
University of Khartoum

Hussam A. Osman
Ahfad University for Women School of Pharmacy

Maha M. Osman
Alfarrabi College for Science and Technology

Mohamed S. Ali
Al-Neelain University Faculty of Medicine

Ali MM. Edris
University of Bisha

Ayman Ahmed
University of Khartoum
Abstract

Objectives: IL-17A G197A and IL-17F A7488G polymorphisms has been identified to be associated with the susceptibility to many diseases. This study aimed to investigate the frequency distribution of IL-17A G197A and IL-17F A7488G polymorphisms among healthy Sudanese population. A descriptive cross-sectional hospital-based molecular study conducted in different sites throughout Sudan. Two ml blood samples were collected from 717 healthy participants. Demographic data and the medical history of the participants were collected.

Results: Of the 717 participants, 355 (49.5%) were males and 362 (50.5%) were females, their mean age was 30.2±17.2 and 32.2±16.5, respectively. For IL-17A, the most frequent genotype detected among males and females was IL-17A heterozygote allele (AG); 215 (60.6%) and 194 (53.6%), respectively. Whereas, for IL-17F, the most frequent allele among males and females was the homozygote allele (AA); 298 (83.9%) for males and 322 (89.0%) for females. HWE for genotype distributions of IL-17A was showing statistical insignificance for IL-17A among males and females, P value 0.614. While HWE for IL-17F reached the equilibrium level, P value 0.048. The most frequent age group was those aged between 21 to 40 years; 281 (39.2%). Arab constituted the major ethnicity of the study participants; 418 (58.3%), P value 0.034.

Introduction

T helper 17 (Th17) cells, is one of the CD4 T helper cells lineages that been defined as a unique effector subset of cells [1], in particular through the production of Interleukins (ILs) mainly IL-17A and IL-17F [1, 2]. The IL-17 family of cytokines contains other 4 members including; IL-17B, IL-17C, IL-17D, and IL-17E [2]. Both IL-17A and IL-17F are considered as inflammation-related genes [3]. Although little is known about most of the IL-17 family members, IL-17F was discovered to share the strongest homology to IL-17A. The previous report on IL-17A and IL-17F in inducing the expression of other various adhesion molecules, cytokines, and chemokines was reported [4]. Previously, polymorphisms of IL-17A G197A (rs2275913) and IL-17F A7488G (rs763780) were found to be associated with the increased susceptibility to rheumatoid arthritis and ulcerative colitis, respectively [5, 6]. Also, IL-17A and IL-17F were investigated in gastric cancer risks and the association of each single nucleotide polymorphism (SNP) with subtypes of gastric cancer according to its clinicopathological features and their roles in prognosis [7]. IL-17A and IL-17F have also been associated with the pathogenesis of a growing list of autoimmune and inflammatory diseases, such as inflammatory bowel diseases and psoriasis [8, 9]. Several studies have found excess expression of IL-17A in various tumor tissues, including prostate cancer, colorectal cancer, breast cancer, and gastric cancer [10-13]. Moreover, increasing evidence suggested the role of IL-17A in Helicobacter pylori-related gastric diseases [14, 15].

In Sudan, no study has ever investigated the frequency distribution of IL-17A G197A (rs2275913) and IL-17F A7488G (rs763780) polymorphisms among the Sudanese population. In a previous study conducted by Wu et al., 2010 provided the first evidence that the IL-17F A7488G coding variant increases gastric
cancer risks in a low-risk Chinese population, and revealed its association with subtypes of clinicopathologic features of the gastric cancer patients [7]. Studies are needed to investigate the distribution of *IL-17A G197A* (rs2275913) and *IL-17F A7488G* (rs763780) polymorphisms. Also, the result of the known population structure of this gene has implications for understanding the epidemiology not only of cancer, but also the increased susceptibility towards gastric inflammations in Sudan, and the potentials for more effective treatment therapy. In this study, we aimed to determine the frequency of *IL-17A G197A* (rs2275913) and *IL-17F A7488G* (rs763780) polymorphisms among a healthy Sudanese population.

**Materials And Methods**

**Study design, study sites, samples and data collection**

This is a descriptive cross-sectional hospital-based molecular study conducted in different sites throughout Sudan including; Khartoum and Madani (central region); New Halfa, Port Sudan, and Gedaref (eastern region); River Nile (North region); and Ad Damazin and Kosti (southern region). Two ml blood samples were collected from 717 healthy participants recruited at the health facilities of each site. Blood samples were preserved in sodium citrate blood containers. Demographic data and the medical history of the participants were collected. Participation in this study was fully voluntary, and only individuals who expressed interest willingly to participate in this study by signing a written informed consent form were included in the study. Pregnant women, children aged less than one year, participants with a history of ulcerative colitis and rheumatoid arthritis, beside immune-compromised patients were excluded from the study to reduce hemoglobin loss in case of pregnant women and infants and to avoid bias in the results of SNPs frequency distribution in case of those with history of ulcerative colitis and rheumatoid arthritis.

**PCR-RFLP for *IL-17F A7488G* and *IL-17A G197A* genotyping**

The genomic DNA was extracted from blood samples using QIAamp DNA blood Mini Kit (Qiagen Inc., Germany). DNA was re-suspended in 200 µl of 1X TE-buffer and stored at -20°C until molecular investigations. *IL-17A G197A* and *IL-17F A7488G* genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers used for *IL-17A G197A* and *IL-17F A7488G* were as follows: sense 5-AACAAGTAAGAATGAAAAGAGGACATGGT-3 and anti-sense 5-CCCCCAATGAGGTCATAGAAGAATC-3 for *IL-17A*; sense 5-ACCAAGGCTGCTCTGTTTCT-3 and anti-sense 5-GGTAAGGAGTGGCATTTCTA-3 for *IL-17F* as described previously [7]. The PCR amplification was performed in a total volume of 25µl mixture containing using single tube PCR i-Taq premix (iNtRON Biotechnology, Korea), mixed with 1 µl genomic DNA, and 1 µM of each primer and incubated in MJ research thermocycler (USA) using the previously described amplification condition [7]. PCR products were digested overnight at 37°C with *XagI* and *NlaIII* (New England BioLabs, England) to determine the genotypes of *IL-17A G197A* and *IL-17F A7488G*, respectively. Digested amplicons were separated using 3% agarose gel electrophoresis. To confirm the genotyping results, randomly selected PCR products were
sequenced using Sanger deoxy ribonucleic acid sequencing method using ABI 3730 sequencing system provided by BGI company (BGI, China).

**Statistical analysis:**

Data were analyzed using the Statistical Package for the Social Sciences (SPSS v20). Hardy-Weinberg equilibrium (HWE) was performed using Pearson’s $\chi^2$ test. Differences in allele frequency were analyzed using Fisher’s exact test, a P value < 0.05 was considered significant. The sequences of IL-17A and IL-17F products were analyzed using BioEdit v7 software for the confirmation of sequences polymorphisms.

**Results**

**PCR-RFLP and sequencing results:**

Amplified PCR products of each of IL-17A G197A and IL-17F A7488G bands sizes were 102 and 143 base pairs (bp), respectively. The results of enzyme digestion using XagI and NlalII produced several fragments grouped into 3 types of fragments (Figure 1).

The selected samples were accurately confirming the cutting sites of each enzyme, and showing the correct SNP (Figure 2).

**Frequency of IL-17A G197A and IL-17F A7488G genotypes**

In this study a total of 717 healthy participants were included. 355 (49.5%) were males and 362 (50.5%) were females, their mean age was 30.2±17.2 and 32.2±16.5, respectively.

For IL-17A, the most frequent genotype detected among males and females was IL-17A heterozygote allele (AG); 215 (60.6%) and 194 (53.6%), respectively. Whereas, for IL-17F, the most frequent allele among males and females was the homozygote allele (AA); 298 (83.9%) for males and 322 (89.0%) for females. No statistical significance association for frequency distribution of the different IL-17A and IL-17F genotypes based on gender, P values were 0.113 and 0.136, respectively. HWE for genotype distributions of IL-17A was showing statistical insignificance for IL-17A among males and females, HWE (Fisher exact test = 0.614). While HWE for IL-17F reached the equilibrium level, HWE (Fisher exact test = 0.048).

Based on age groups, the most frequent age group was those aged between 21 to 40 years; 281 (39.2%), followed by participants aged between 1 to 21 years; 188 (26.2%). Concerning the ethnicity, Arab constituted the major ethnicity of the study participants; 418 (58.3%). Frequency distribution across the different ethnic groups was statistically significant for IL-17A genotypes, P value 0.034 (Table 1).

The subgroup analysis of IL-17A and IL-17F genotypes distribution across the different ethnic groups revealed a statistically significant difference of IL-17A genotypes among Arabs compared to the different
ethic groups. (See additional file 1). Whereas, no statistically significant difference obtained for IL-17F genotypes distribution across the different Sudanese ethnic groups (additional file 2).

Table 1: Frequency distribution of *IL-17A* and *IL-17F* genotypes among participants gender, age groups and participants ethnicity.

| Gender        | IL-17 A genotypes no. (%) | IL-17 F genotypes no. (%) | Total |
|---------------|----------------------------|----------------------------|-------|
|               | AA | AG | GG | P* | AA | GA | GG | P* |       |
| Male          |    |    |    |    |    |    |    |    | 355 (49.5) |
|               | 127 (35.8) | 215 (60.6) | 13 (3.7) | 0.113 | 298 (83.9) | 42 (11.8) | 15 (4.2) | 0.136 |
| Female        | 147 (40.6) | 194 (53.6) | 21 (5.8) |   | 322 (89.0) | 28 (7.7) | 12 (3.3) |   |
| Age group     |    |    |    |    |    |    |    |    | 362 (50.5) |
| 1 - 20 years  | 71 (37.8) | 108 (57.4) | 9 (4.8) | 0.977 | 166 (88.3) | 15 (8.0) | 7 (3.7) | 0.231 |
| 21 - 40 years | 110 (39.1) | 160 (56.9) | 11 (3.9) |   | 231 (82.2) | 37 (13.2) | 13 (4.6) |   |
| 41 - 60 years | 54 (40.0) | 74 (54.8) | 7 (5.2) |   | 118 (87.4) | 13 (9.6) | 4 (3.0) | 135 (18.8) |
| 61 - 80 years | 9 (30.0) | 19 (63.3) | 2 (6.7) |   | 29 (96.7) | 1 (3.3) | 0 (0.0) | 30 (4.2) |
| 81 - 100 years | 30 (36.1) | 48 (57.8) | 5 (6.0) |   | 76 (91.6) | 4 (4.8) | 3 (3.6) | 83 (11.6) |
| Ethnicity     |    |    |    |    | 0.034 | 0.992 | 418 (58.3) |
| Arab          | 142 (34.0) | 257 (61.5) | 19 (4.5) |   | 363 (86.8) | 40 (9.6) | 15 (3.6) |   |
| Beja          | 8 (30.8) | 16 (61.5) | 2 (7.7) | 23 (88.5) | 2 (7.7) | 1 (3.8) | 26 (3.6) |
| Fallata       | 8 (40.0) | 11 (55.0) | 1 (5.0) | 17 (85.0) | 3 (15.0) | 0 (0.0) | 20 (2.8) |
| Fur           | 30 (50.8) | 26 (44.1) | 3 (5.1) | 50 (84.7) | 6 (10.2) | 3 (5.1) | 59 (8.2) |
| Nuba          | 34 (35.1) | 59 (60.8) | 4 (4.1) | 82 (84.5) | 11 (11.3) | 4 (4.1) | 97 (13.5) |
| Nubian        | 52 (53.6) | 40 (41.2) | 5 (5.2) | 85 (87.6) | 8 (8.2) | 4 (4.1) | 97 (13.5) |

*P: P value.

**Discussion**

The polymorphisms of *IL-17A* G197A (rs2275913) and *IL-17F* A7488G (rs763780) has been associated with susceptibility to various types of proinflammatory diseases and gastric cancer [5-7]. Additionally, knowing the population structure of this gene was noted to be uniquely beneficial in means of treatment and understanding diseases prognosis [16]. In this study, we aimed to determine the frequency of *IL-17A* and *IL-17F* polymorphisms among healthy Sudanese populations. The results obtained in this study, revealed that the distribution of *IL-17A* and *IL-17F* was statistically insignificant among males and females. This agreeing with previously conducted studies [17, 18]. Although, the study participants were selectively healthy individuals this could provide a hint towards the chance of difference occurrence when
including unhealthy individuals diagnosed with gastric cancer, breast cancer or rheumatoid arthritis [7, 17, 19, 20].

Interestingly, this result also supports the fact that this gene could be significantly linked with susceptibility to certain diseases such as *H. pylori* infection [21]. Since *H. pylori* infections reported in Sudan are quietly increasing [22-25], and here, the high frequency of *IL-17A* and *IL-17F* genotypes can increase the susceptibility towards *H. pylori* infection. However, this assumption needs further investigations.

Regarding, the distribution among the different age groups obtained in this study, although, no association was found, *IL-17A* polymorphism has been associated with early TNM staging and poorly differentiated gastric cancers with aging [7]. Moreover, this result showed the degree of population structure for this gene which will help in cancer onset prediction especially among elderly. This was also seen by the small number of elderlies been included hence most elders were diagnosed previously with rheumatoid arthritis and gastric colitis and been excluded from the study. This was in line with the previous hypotheses that *IL-17A* polymorphism may influence the development and progression of gastric carcinogenesis [7]. Also, the *IL-17F 7488GA* genotype that reported to increase gastric cancer risk from the age of 40 years [7].

The significant association between *IL-17A* and participants ethnicity could be attributed to several factors, remarkably, based on 2009-2010 cancer prevalence in Sudan, 37.8 % of the total cancer patients were diagnosed with breast cancer [26]. Although, less is known about patient’s ethnicity, nevertheless based on Mahmoud et al., (unpublished data), the rate of breast and gastric cancer incidence in Sudan since 2010 to 2015 were approximately 20% and 5% respectively. Among both cancer groups, 60% and 70% were noted to be of Arab ethnicity (unpublished data). This is suggesting that *IL-17A* polymorphism is taking role in cancer susceptibility among different Sudanese ethnic groups. This was well discussed previously by Li et al. 2015, indicating the role of ethnicity in gastric cancer susceptibility, where found that *IL-17A* increases gastric cancer susceptibility among Japanese population but not with the Chinese population [21].

This study highlights the need for further investigations towards addressing *IL-17A* and *IL-17F* polymorphisms among Sudanese population diagnosed with different types of cancers, in order to further understand the role of this SNP polymorphism in cancer susceptibility and further cancer incidence. However, such prediction could also be ambiguous and misleading since cancer susceptibility may not be linked to a single nucleotide polymorphism at a single gene [27, 28].

**Conclusion**

This study provides the first data on the Sudanese population structure on *IL-17A* and *IL-17F* gene polymorphisms. This might be of help to identify the association of these polymorphisms with different patients’ groups and could benefit in the understanding of those genes’ involvement in cancer or other types of diseases susceptibility.
Limitations:

- This study focused on healthy Sudanese participants, limiting the natural variation within the population structure, which might be biasly selected against the \textit{IL-17A} and \textit{IL-17F} polymorphisms and resulted in the underestimation of their prevalence. Therefore, the need for more diverse study population including unhealthy population is extremely important to further understand the role of these SNPs in different diseases’ susceptibility.

Abbreviations

HWE: Hardy-Weinberg Equilibrium; ILs: Interleukins; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; SNP: Single Nucleotide Polymorphism; SPSS: Statistical Package for Social Sciences: SPSS; Th17: T helper 17.

Declarations

Ethics approval and consent to participate

The protocol was approved by the Ethical Committee of Nile University Research Ethics Committee. Informed consent was obtained from each participant prior to enrollment using writing and verbal informed consent in case of illiterate patients.

Consent to publish

Not Applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

NSM, EES, and RAO provided conceptual framework for the project. AEA, MMAB, HA, ETA, and AA guidance for interpretation of the data, performed data analysis. AAA, SAA, MM, MSM, HAO and MMO
participated in the molecular performance. NSM, MSA, and AMME performed the statistical analysis and guidance for data interpretation. All authors read and approved the final manuscript.

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Author’s Information

Nouh S. Mohamed

Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Nile University, Khartoum, Sudan.

Alfarrabi College for Science and Technology, Khartoum, Sudan.

Department of Parasitology and Medical Entomology, Faculty of Medicine, Sinnar University, Sinnar, Sudan. Email: nouh_saad@outlook.com.

Emmanuel E. Siddig

Alfarrabi College for Science and Technology, Khartoum, Sudan.

School of medicine, Nile University, Khartoum, Sudan

Research Assistant, Mycetoma Research Center, University of Khartoum, Khartoum, Sudan. Email: Emanwell-eds3@hotmail.com.

Abdallah E. Ahmed

Alfarrabi College for Science and Technology, Khartoum, Sudan.

Email: abdallah_elseer@yahoo.com.

Musab MA. Albsheer

Department of Parasitology and Medical Entomology, Faculty of Medicine, Sinnar University, Sinnar, Sudan. Email: musabali39@yahoo.com.

Hanadi Abdelbagi

Department of Biotechnology, School of Pharmacy, Ahfad University for Women, Omdurman, Sudan. Email: hanadi3814@gmail.com.

Eman T. Ali
Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan. Email: eman2taha@gmail.com.

Anadel A. Alsubki

Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Nile University, Khartoum, Sudan. Email: anadilalsubki@gmail.com.

Sabah A. Abdalaziz

Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Nile University, Khartoum, Sudan. Email: sabahalhaj1991@gmail.com.

Madinna Mustafa

Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Nile University, Khartoum, Sudan. Email: mado93344@gmail.com.

Mohamed S. Muneer

Department of Neurology, Mayo Clinic, Jacksonville, FL, USA.
Department of Radiology, Mayo Clinic, Jacksonville, FL, USA
Department of Internal Medicine, Faculty of Medicine, University of Khartoum, Khartoum, Sudan. Email: mohamedsideeg@yahoo.com.

Hussam A. Osman

Department of Biotechnology, School of Pharmacy, Ahfad University for Women, Omdurman, Sudan. Email: hussomco@gmail.com.

Maha M. Osman

Alfarrabi College for Science and Technology, Khartoum, Sudan.
Email: maha_mahgoub2008@yahoo.com.

Mohamed S. Ali

Faculty of Medicine, Neelain University, Khartoum, Sudan.
Email: alkhatalmi@gmail.com.

Ali MM. Edris
Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

Department of Histopathology and Cytology, Faculty of Applied Medical Sciences, University of Bisha, Bisha, Kingdom of Saudi Arabia.

Email: aedris@ub.edu.sa.

Ayman Ahmed

Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

Email: ayman.ame.ahmed@gmail.com.

Rihab A. Omer

Department of Molecular Biology, Institute of Parasitology, University of Leipzig, Germany. Email: rihab.omer@yahoo.com.

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**Figures**
Figure 1

PCR-RFLP results of IL-17A and IL-17F genotyping.

Figure 2

Sequencing results of the amplified products for IL-17A and IL-17F polymorphisms.

Supplementary Files

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