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Molecular Characterization of Interleukin-8 Polymorphism among Patients with Black Grain Mycetoma, Gezira Mycetoma Center, Sudan (2019-2020)

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Abstract: ***Interleukin 8 (IL-8) is a chemokine, mainly involved in the initiation of inflammatory reactions. It attracts and activates neutrophils that are unable to clear eumycetoma inflammatory regions. In endemic areas the majority of individuals have antibodies, and therefore, a small proportion develops the disease. The objective of the study was to identify Polymorphism in the Allele distribution for gene encoding to IL-8(CXCL8) and associated with the risk of Mycetoma infection due to black grain. A cross-sectional hospital-based study was conducted. DNA extraction was performed for 41 blood samples. Genotyping for the IL8-251 T-A polymorphism was accomplished using the PCR - ARMS method. A fragment of 349 bp of IL8 was amplified. Three specimens were detected by standard Sanger sequencing. A percent of 28 (68.3%) and 13 (31.7%) were males and females, with ages ranging (13 -36 years) consecutively. The PCR results revealed 36 (87.8%) were amplified whereas, 5(12.2%) were not. The sequencing of IL8 -251 T fi: A (66.6%) initiated an (A: allele) and, (33.3%) are excluded. In conclusion, the study abstracted that the IL8 -251 T fi A (87.8%), polymorphism prevalent in Mycetoma patients in the Gezira. Further studies are recommended for surveillance, characterization, and mapping the target pockets.***

Keywords: Interleukin 8 (IL-8), Mycetoma, eumycetoma, Gezira and Sudan

1. Introduction

Mycetoma is a chronic granulomatous disease of the skin, subcutaneous tissue, and bones that is present worldwide and is endemic in tropical and subtropical regions. The infection occurs commonly in the foot and was described by Gill in the Indian Madura district in1842, hence the name Madura Foot.[1]It is a slow- growing infection presenting with characteristic symptomatic triad of swelling, draining sinuses and extrusion of colonial grains in the exudates.[2, 3] As the disease has slow and relatively pain- free progression, it is usually diagnosed at an advanced stage.[4, 5] The most common site of occurrence is the foot (70%cases), explaining the synonym 'Madura foot'.Mycetoma is commonly seen in agricultural workers and barefoot walkers in dry and dusty areas. Repeated trauma or implantation by thorns and splinters provides a portal of entry for the organism. Infection can be caused by true fungi (eumycetoma) in 40% cases and by filamentous bacteria (actinomycetes) in 60% cases. Since the treatment of these two etiologies is entirely different, definite diagnosis after a histopathological and microbiological examination is mandatory.[6, 7]Histopathology remains one of the major tools of diagnosis in mycology. The advantages of histopathology are speed, low cost and ability to provide a presumptive identification of the infecting fungus as well as demonstrating the tissue reaction.[8, 9]Although rare, Mycetoma should be diagnosed as early as possible to avoid long-lasting complications. Primary care physicians in European countries are frequently in the first line of care of migrant patients and therefore should be aware of the common and uncommon clinical presentations of Mycetoma.[10]

Madurella mycetomatis is the most common fungal causative agent of eumycetoma Sudan.[11, 12] This agent is abundantly present in the soil and on the vegetation in the endemic region.[13, 14]Chances for coming into contact with this micro-organism are being high for inhabitants of endemic and co-endemic areas.[15, 16]Recently, some reconstructive techniques were performed to address essential aspects concerning Mycetoma surgical management. These practices are including coverage of large skin and soft tissue defects left after local excisions. These procedures were enhancing the rate of chronic Mycetoma wound healing and restoration of a functional status of the affected limbs. [17] The objective of the current study is to identify the Polymorphism in the Allele distribution for gene encoding to IL-8(CXCL8) Associated with risk of Mycetoma infection due to black grain

2. Material and Method

A Prospective cross-sectional laboratory based study was applied in the study. The target populations were Mycetoma patients attending to Mycetoma Hospital in Wad-Madani. A total of 41 subjects living in Wad-Madani town and its surrounding outskirts with different ages and consist of both gender participated in the standing study. Based on statistical calculations, data collection. A structured tested questionnaire was used to collect data from study populations. The questionnaire was filled by the researcher and trained interviewers. Blood samples were collected by sterile syringe (puncture technique) and about 3-5ml of venous blood was withdrawn and dispensed in EDTA containers. The containers werelabeled and centrifuged to obtain serum then transferred to eppendorf tube and stored frozen at -20C^0. Genotyping for the IL8 -251 T _ A polymorphism was performed using the PCR tetra-primer ARMS method. Four primers are required to genotype the polymorphism by using purified genomic DNA as template. This PCR reaction was done by using MyTaqTM Red Mix Kit. The PCR reaction comprises three steps: denaturation, annealing and extension, with different temperature for each. The first step of PCR is the denaturation in which the DNA sample is heated up to 94$^o{}_C$ to Split the double- strands. The high temperature breaks down the hydrogen bond between the nucleotides that form the DNA code. The second step is annealing in which the two primer (forward and reverse) bind to build up an appropriate complementary strand. The temperature of this step various depending on the size of the primer and its homology to target DNA (the appropriate temperature for CXCL8 is 58^0 c). Finally, DNA polymerase extend the primers by its polymerase activity, this is done in an optimal temperature for the Taq polymerase which is 72C^0. These steps are repeated for 35 times. In sterile 0.2 ml micro- centrifuge tubes This PCR reaction was done by using MyTaqTM Red Mix Kit. Normal DNA sequencing was performed for 3 samples by Marcogen Company (Seoul, Korea). Bioinformatics programs that work with DNA sequences to identify the origin of a DNA sample**.** In a chromatogram file, the signal intensities are presented in a graph with the four bases, each identified by different colors. Like many sequence analysis programs, Finch TV uses green for adenine, red for thymine, black for guanine, and blue for cytosine *(Northwest Association 2012)***.** BioEdit. The multiple sequence alignment of the isolates with similar nucleotide sequences obtained from BLAST will carry out to find the homology and evolutionary relation among the sequences

Ethical consideration

The acquiescence to conduct this study was obtained from the Ministry of health Gezira state. The research approval was also gotten from the research University of Gezira. The Consensus of every participant was voluntarily obtained. Information was collected from the subjects under top privacy and the investigator undertakes using the data only the study purposes**.**

Statistical analysis: Statistical analysis was performed using Statistical software beside individual descriptive measures. The Chi-square tests(x2). Comparative data were also subjected to descriptive statistical analysis. Tests for equal proportion have been applied to explore the dependencies between the count variables where these were significant they were reported along with the corresponding p-value. A Comparative survey was entered into Ms. Excel and analyzed using SPSS version 21. Differences among studied parameters were explored under probability levels of 5% and 1%.

3. Results

Table: 1. The frequency of gender, age class and duration of Mycetoma infection among the study populations

Parameters		Frequency	Percent
Gender	Male	28	68.3
	Female	13	31.7
	Total	41	100
Age (Class/ Years)	13-20	12	29.3
	21-28	23	56.1
	29-36	6	14.6
	Total	41	100
Period of infection (duration/ years)	1-4 duration	23	56.1
	5-8 duration	15	36.6
	9-12 duration	3	7.3

Table 2: Shows PCR results I the IL8 gene in the patients

Parameters	Frequency	Percent
Valid Percent	36	87.8
Absent	5	12.2
Total	41	100

Molecular genotyping:

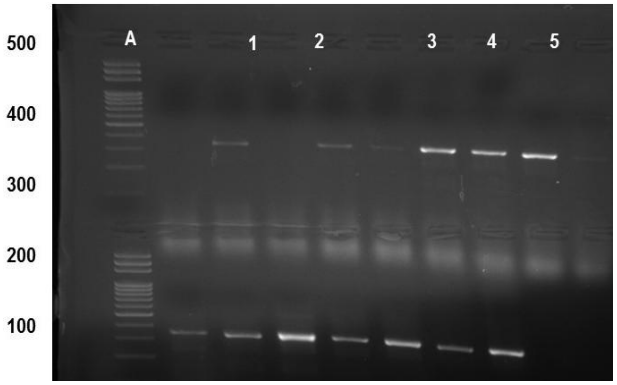

Figure 1: Explains the electrophoresis gel of the IL8 _251 T fi A PCR-tetra primer method .1; 2; 3; 4 and 5 bands of PCR product length 349 bp:A**:** 349 bps DNA Ladder (size marker 100 bp)

Table 3: Illuminates the frequency of (SNP IL8 -251 T fi A)

Parameters	Frequency of (SNP IL8 -251 T fi A)	Percent of (SNP IL8 -251 T fi A)
Valid present	2	66.7
Excluded	1	33.3
Total	3	100%

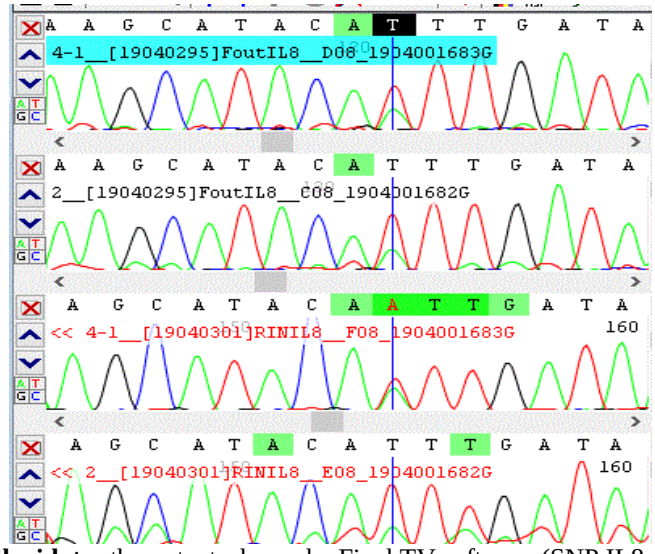

Figure 2: Elucidates themutant, shown by FinchTV software (SNP IL8 -251 T fi A)

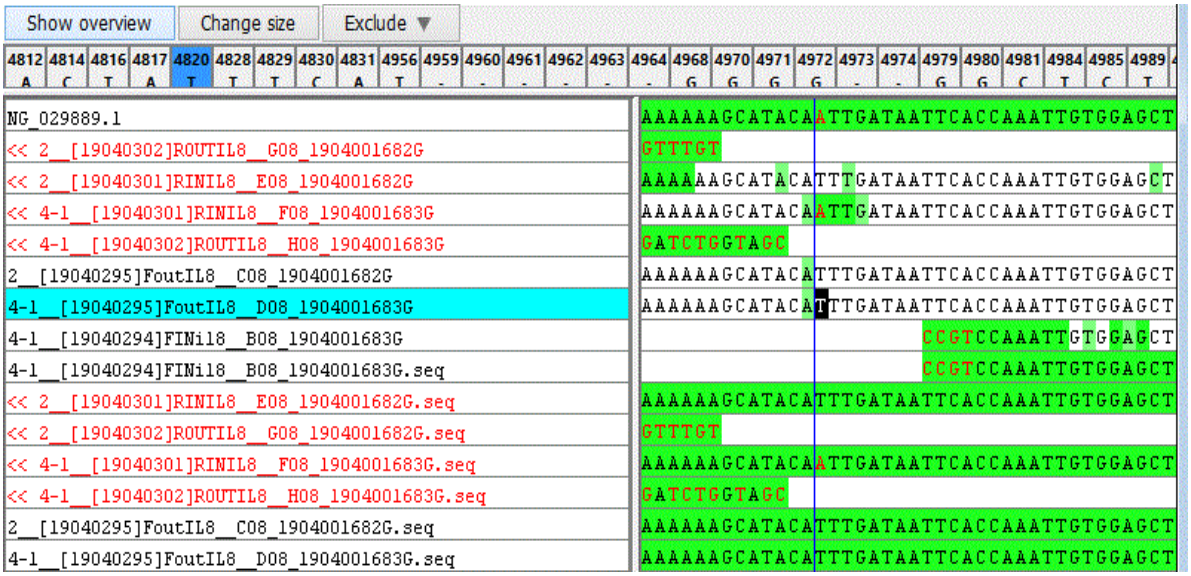

Figure 3: Reveals the multiple sequence alignment of the mutant sample with similar nucleotide sequences obtained from BLASTn was carried out to find the homology and evolutionary relation between these sequences. As shown by BioEdit software there are (SNP IL8 -251 T fi A) substitutions.

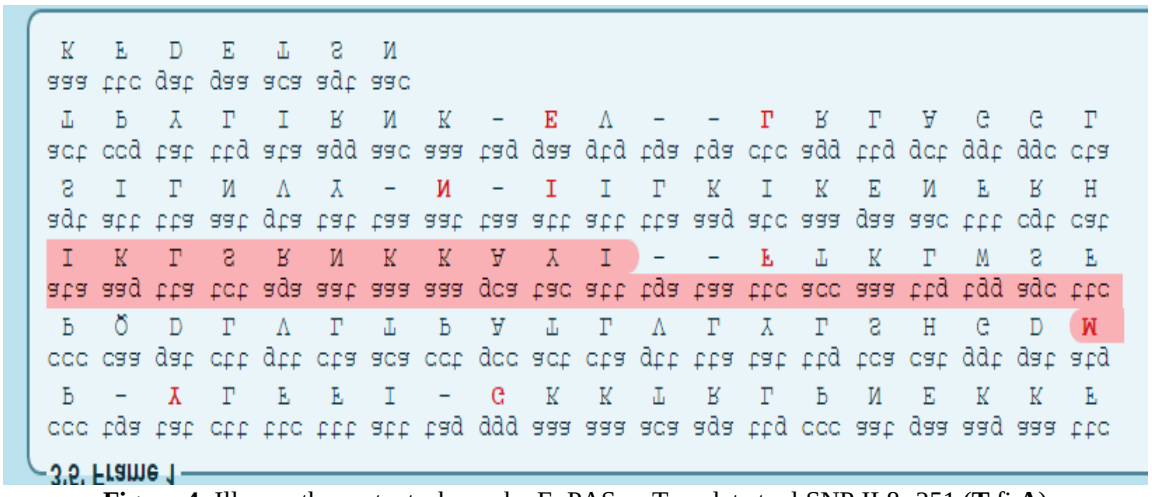

Figure 4: Illumes the mutant, shown by ExPASy - Translate tool SNP IL8 -251 (**T** fi **A**)

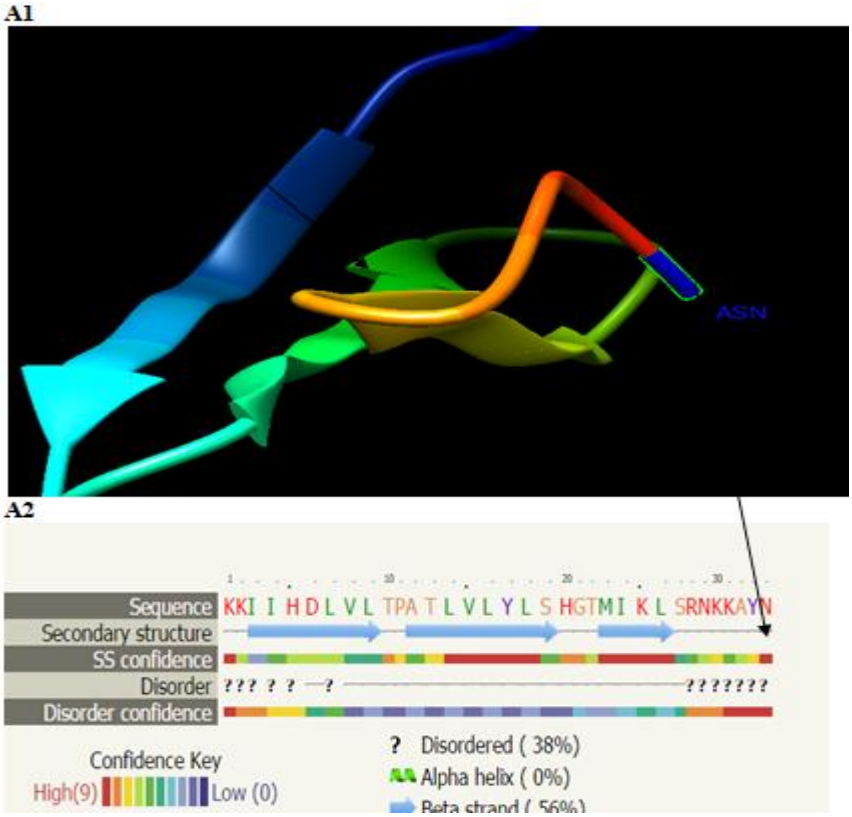

Figure 5: Clarifies thewild type of predicted amino acid Asparagine (ASN) at position 14 the secondary protein structure of the wild gene is drawn by Phyre2 software fig (A 2). The tertiary protein structure of the wild type genes that is drawn by Chimera software version 1.8 Fig (A 1)

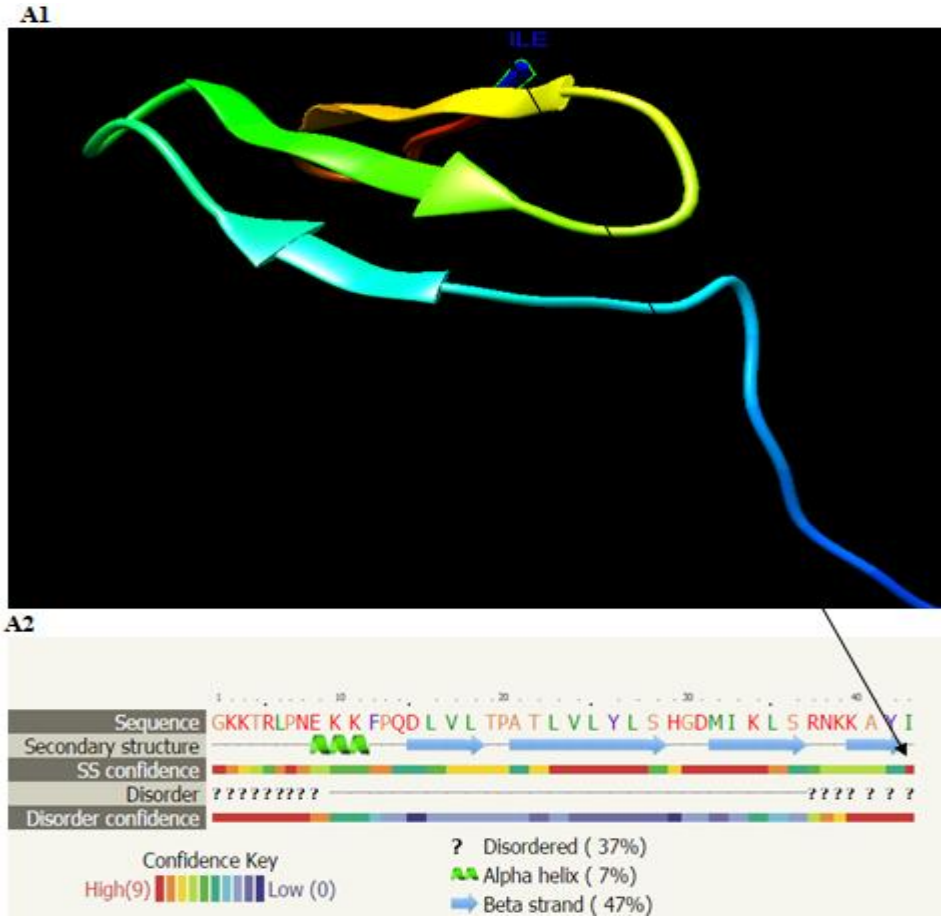

Figure 6: Shown the mutant type of predicted amino acid Isoleucine (ILE) at position 43.The secondary protein structure of the mutant gene that is drawn by Phyre2 software fig (A1). Tertiary protein structure of genes mutant that drawn by Chimera software version 1.8 fig (A2).

4. Discussion

Mycetoma is a chronic, progressive, infectious and neglected disease caused by the variety of microorganisms including fungi and higher bacteria. It has uneven cosmopolitan distribution with health hazard concerns and socioeconomic significance (WHO, 2019). Mycetoma represents a major health problem in many tropical and subtropical areas, there are no prevention or control measures for this neglected disease.[17-19] In Mycetoma endemic areas, most individuals have antibodies against the causative agents, however only a few develop the disease.[19-21]

Fewer researchers believed that patients who develop Mycetoma seem to be deficient in their cell-mediated immunity.[19, 21] Mycetoma patients express high levels of CXCL8 during infection. The sequences are in agreement with the preceding studies conducted by.[22]The contemporary study publicized that CXCL8 is also abundantly present in the Mycetoma lesions. The extent of CXCL8 expression appeared to be dependent on the inflammation type, with more CXCL8-positive cells present during the type II reaction, a reaction characterized by a higher amount of histiocytes and giant cells. Macrophages with hemosiderin deposits and high CXCL8 expression were found in the vascular zone CXCL8 was also found on hyphae within the grain CXCL8 was not only expressed in the skin, but also recorded high amounts of CXCL8 were detected in the serum of the Mycetoma patients. CXCL8 concentrations have been successfully employed for different groups of fungi. There upon, by our standing research.[22-24]

Interestingly, it has also become apparent that it is very difficult to determine the true burden of Mycetoma, to identify its primary reservoir and monitor therapeutic responses because there are currently no safe, reliable, fast, and cheap diagnostic tools to determine Mycetoma causative agents. It was hoped that isothermal DNA amplification techniques, and serological-based procedures would be developed for Mycetoma causative agent's identification. In the past 10 years efforts have been made to develop or test such assays. This study addressing the relation between CXCL8 and Mycetoma infection in Gezira state. The current study was thus to assess the accuracy of molecular techniques to identify the mutations link with the immune response packages. In this cross-sectional laboratory-based study 41 blood samples were collected from Gezira Mycetoma Center, there were 28 (68.3%) males and 13 (31.7%) females.The age of the study group ranging between (13-36) years. The majority of Mycetoma in this study were (13-20) years (29.3%), (21-28) (56.1%) and (14.6%) are (29-36) years. The collected sample isolates share the same identity, for sequencing regions (349 bp). DNA sequencing was performed by 3 samples by Marcogen Company (Seoul, Korea). The outcomes regarding the (SNP IL8 -251 T fi A) were showing 100% positive of two samples after excluded the third sample because it is simple for verification and differential to diagnose. Analysis of the (SNP IL8 -251 T fi A) polymorphism gene sequences was applied to determine their relationship to the other (SNP IL8 -251 T fi A) polymorphism of gene sequences available in Gen-Bank database using BLAST nucleotide algorithm (http://www.ncbi.nlm.nih.gov/).BLAST analysis of (SNP IL8 -251 T fi A) polymorphism gene showed 96% identity to a nucleotide sequence of (SNP IL8 -251 T fi A) polymorphisms. The (SNP IL8 -251 T fi A) was found to bear the same sequences that retrieved from gene bank (Fig: 4.4) embodied the multiple sequence alignment of the sample with similar nucleotide sequences that obtained from BLASTn. This process was carried out to find the homology and evolutionary relation between these sequences shown by BioEdit software, then shown by Finch TV software (Fig: 4.3).The mutant type of predicted amino acid Asparagine Isoleucine (ILE)at position 43(Fig: 4.7). The secondary protein structure of wild gene type that drawn by Phyre2 software has been illustrated in fig A2 (4.7). The Tertiary protein structure of wild genes that drained by Chimera software version 1.8 has been demonstrated in fig A1 (4.7).

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