International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Molecular Characterization of Complement Receptor1 (Sl) and (McC) Polymorphisms in Patients with Black Grain Mycetoma, Gezira Mycetoma Center, Sudan (2019-2020)

Mona Alfadil Elamin[1], Tahra Yahia Hassan[1], Adam Dawoud[1], Azhari Mohammed Tyara[2], Mohammed Abdelwahed[3], Aliya Elamin[4, 6], Mahjoub Makki[6], Aya. A.M. H Hassan[7], Alaa. A. M. Hamid[8]

[1]Department of Medical Parasitology, Faculty of Laboratory Medical Sciences, University of Gezira

[2]Department of Molecular Biology and Biotechnology University of Gezira

[3]Department of Molecular Biology, NCI, University of Gezira

[4]Department of Physiology, Faculty of Medicine, University of Gezira

[5]Faculty of Medicine, University of king Fisal (KFU), KSA

[6]Faculty of Medicine, Omdurman Islamic University

[7]Medical Student, faculty of Medicine, University of Khartoum (U OF K), Sudan

[8]Medical Student, faculty of Medicine (WMCT), Sudan

Abstract: ***The complement receptor1 (CR1) gene encodes for CR1protein as a component of the activity regulators. The Swain Langley (SL) and McCoy (McC) polymorphisms are common in CR1 and defined as the knob of blood group antigen. A Cross-sectional laboratory-based study was conducted to detect molecular characterization of complement receptor1 (SL) and (McC) Polymorphisms in black grain Mycetoma patients, Gezira Mycetoma Center, Sudan. Forty-one blood samples (3-5ml) were collected in EDTA tube. DNA was extracted using iNtRON's extraction kit. PCR reaction was done by using MyTaqTM Red Mix Kit. The polymorphisms for selected specimens (4) were determined by BGI Sanger sequencing. The participants were 28/41 (68.3%) males and 13/41 (31.7%) females consecutively. The age is ranging from (13-36) years. The PCR results revealed 34/41 (83%) were amplified whereas 7/41(17%) were not. The sequencing of McC (SNP A4795G) shows a 50% is G allele and 50% an A-type. Whereas SL (SNPA4828G) bears 75 % G and 25% represents an A allele. In conclusion, the study supports the hypothesis of the SLallele and the McC allele and polymorphisms evolve in the Mycetoma predisposing factor. Further studies are recommended for screening and mapping the target sinuses surrounding the endemic and co-endemic areas.***

Keywords: The Swain Langley (SL), McCoy (McC) Polymorphisms, Black Grain, Mycetoma, Gezira,Sudan

1. Introduction

Mycetoma is a chronic granulomatous disease of the skin, subcutaneous tissue, and bones that are 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 a characteristic symptomatic triad of swelling, draining sinuses, and extrusion of colonial grains in the exudates[2, 3]. As the disease has a 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 in 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 about 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 this study to identify the Polymorphism in the Allele distribution for gene encoding to complement receptor- 1 (CR 1), Associated with Risk of Mycetoma

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 have participated in the standing study. The 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 was labeled and centrifuged to obtain serum then transferred to an Eppendorf tube and stored frozen at -20C^0. Genotyping for the McC and Sl polymorphism was performed using the PCR. Briefly, the oligonucleotide forward primer 24KnNde: 5'-ACC AGT GCC ACA CTG GAC CAG ATG GAG AAC AGC TGT TTG AGC AT-3' and reverse primer 25Rb: 5'-GGA GGA GTG TGG CAG CTT G-3', were used to amplify a 305 bp fragment of CR1 exon 29 by PCR required to the genotype of the polymorphismusing purified genomic DNA as a template. 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 obtain 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 board, faculty of medical laboratory sciences, University of Gezira. A 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) is also used. 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. The 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

Clinical and Epidemiological Survey

The diagnosis of Mycetoma is based on clinical presentation and identification of the etiologic agents. Clinical diagnosis is characterized by the asymptomatic triad: a subcutaneous mass (tumefaction), draining sinuses, and grain discharge. The most specific diagnostic tool is the examination of the grains discharged from sinuses. Both spontaneous drainage and manually expressed material from sinus is carefully examined macro and microscopically to visualize the grains. Table:1 and (Fig**.**1.A and Fig**.**:1.B) are illustrating the frequency of gender, age class, `and duration of Mycetoma infection per year among the study populations. The frequency of the gender among the study populationreveals that about 28 **(**68.3%) for males and13 (31.7%) females correspondingly. The highest percentages were 23(56.1%) recorded for 21-28 years age classes whereas, 12(56.1%) and 12(29.3%) for 13-20 and 6(14.6%) for 29-36 age classes respectively. The infection duration per year is shown in (Table: 1) and (Fig**.**1.C). In the present study data collected regarding the typical clinical presentation by interviewing patients and co patients. The contemporary study originates a painless subcutaneous mass of slow progression forming multiple sinuses that drain pus and clusters of bacterial and fungal structures called "grains (Fig**.**2. Fig**.**3A:.B: C: D: E and G).The first part of the study demonstrated that the host's inflammatory response consists of a neutrophilic infiltrate and these outcomes may help the formation of granulomas that typically contain grains. The clusters of fungal hyphae will vary in color according to the causative agent. Untreated infections will eventually destroy the tissues, including bones (Fig: 3A:.B: C and D). The most frequent radiological finding among interviewed patients in the current study is that Mycetoma is soft tissue swelling followed by bone sclerosis, bone cavities and periosteal reaction. After the inoculation of the causative agent (fungi or bacteria), a subcutaneous infection develops. The analysis of collecting data concerning the questionnaire sections disclose that the infection is usually painless and processes slowly, over months and years. The standing study initiates 21/41(50%) of Mycetoma in infected participants are eumycetoma. Moreover, the most common species are *Madurella mycetomatis* 28.7/41(70%), *Madurella grisea*. The study institutes that malnourished patients and patients with associated illnesses such as (malaria, leishmaniasis, diabetes mellitus and other immunological diseases) are the more intense and faster progression of the disease occurs.

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

Parameters	Data of study subjects	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 resultsin theCR1 gene in the patients:

Parameters	Frequency	Percent
Valid Percent	34	83%
Absent	7	17%
Total	41	100%

Table 3: Show the Frequency of (SNP A4795G) and (SNP A4795G) in Mycetoma patient:

	Frequency of (SNP A4795G)	Frequency of (SNP A4795G)	Percent of (SNP A4828G)	Percent of (SNP A4828G)
Valid present	4	6	50%	75%
Absent	4	2	50%	25%
Total	8	8	100%	100%

BGI Sanger Sequencing results

Sample Code	Sample Name	Primer Name	Found of (SNP A4795G)	Found of (SNP A4828G)
1904001678G	4	[19040296]RCR1	Found	Found
1904001678G	4	[19040293]FCR1	Found	Found
1904001679G	16	[19040296]RCR1	Not found	Found
1904001679G	16	[19040293]FCR1	Not found	Found
1904001680G	31	[19040293]FCR1	Found	Found
1904001680G	31	[19040296]RCR1	Found	Found
1904001681G	27	[19040293]FCR1	Not found	Not found
1904001681G	27	[19040296]RCR1	Not found	Not found

Molecular genotyping

The core of this study is vis-à-vis the molecular genotyping of the Mycetoma for identifying the polymorphism in the allele's distribution for the gene encoding to CR1polymorphism could enhance the chance of developing a Mycetoma infection. Although CR1 is expressed on neutrophils, it is not unique for this cell type. In fact, CR1 is a receptor expressed by a whole range of other cells including follicular dendritic cells, macrophages, T and B lymphocytes, and erythrocytes. Knops blood group polymorphisms in the CR1 gene are responsible for the Sl blood group Ag and the McC blood group Ag, At this juncture was showed that the Sl and the McCa alleles were more often found in Mycetoma patients than in the matched endemic control population. This was unique, because with other blood group typing systems such as the ABO blood groups and Rhesus factors no association was found with a predisposition to developing Mycetoma.As is seen the Sl and McC polymorphisms are present in the long, homologous repetitive D region of the CR1 gene. Two of these SNPS have now been identified as antigens in the KN blood group system. The K1590E polymorphism is associated with the McCa and McCb antigens, whereas the R1601G is associated with the Sl. This is the region that codes for the binding structure of the protein in which MBL and C1q binding occurs and could, therefore, cause conformational changes that could influence the function of the molecule, not only as implemented on erythrocytes but also other cell types. Neutrophil surfaces, CR1 binds pathogens such as *Escherichia coli* and *Staphylococcus aureus* and presents them to phagocytic cells It is, therefore, conceivable that conformational variations in the receptor also influence the efficacy of *M. mycetomatis* phagocytosis. However, this remains to be determined because the effects of these polymorphisms on the function of the receptors have not yet been defined in full detail. The differential diagnosis using DNA sequencing will be performed for 4 samples (4 for McC and 4 for SL) by Marcogen Company (Seoul, Korea).

Analysis of McC and Sl polymorphism gene sequences was done to determine their relationship to other McC and Sl polymorphism gene sequences available in the Gen-Bank database using the BLAST nucleotide algorithm (http://www.ncbi.nlm.nih.gov/).The BLAST analysis of McC and Sl polymorphism gene showed 98% identity to a nucleotide sequence of McC and Sl polymorphism During our studies to identify DNA sequence polymorphisms associated with Sl:2 and McC(b+), we showed that both heterozygosity for the CR1 K1590E (SNP A4795G) and R1601G (SNP A4828G) amino-acid substitutions and CR1 expression polymorphism.

Our mutant for the CR1 K1590E (SNP A4795G) and R1601G (SNP A4828G) was the same as the sequences that retrieved from a gene bank. The (SNP A4795G) show 50%posative and 50% negative and (SNP A4828G) show 75 % positive and 25% negative. CR1 receptor associated with risk of Mycetoma infection due to black grain Molecular genotyping is an update science of today and tomorrow that has also changed the clinical practices on the advance and rapidly changing globally. It also provides insight into the clinical features in affected individuals and also allows for accurate diagnosis, improved genetic counseling, prenatal genetic diagnosis, and can also be used to diagnose cutaneous infections with pathogens difficult to identify using standard culture techniques, such as mycobacterial or human Papilloma infections and Mycetoma as well. New molecular data also provide a platform to develop new drugs as well as drug repositioning to treat genetic skin diseases. Thus, understanding the molecular pathology diseases can have important implications that benefit patients. In the current study, the neutrophils are being potent attracted to the Mycetoma grains. Two main types of inflammatory reaction were observed. Both reactions could be seen in the same lesion and are not unique to M. mycetomatis. Deceptively, neutrophils are important in the early defense against Mycetoma. The study also shows that Mycetoma always occurs in all age classes. (Table: 2)bares the consequences of the PCR in CRI gene receptor in the patients.Figure: 4 displays the results of study subjects using the PCR technique and authenticate the 349 bps of band length in infected study participants. The frequency of (SNP A4795G) and SNP A4828G) have been recorded in (Table: 3). BGI Sanger Sequencing upshots are shown in

(Table: 4).The study outcome that the positive samples 34(83%) and 7(17%) are negative. Three isolates of patients sample were exposed to DNA sequencing by Marcogen Company (Seoul, Korea) for verification and differential diagnosis. Analysis of (SNP A4795G) polymorphism gene sequences was applied to determine their relationship to other SNP A4828G) polymorphism gene sequences available in Gen-Bank database using BLAST nucleotide algorithm that retrieved from (http://www.ncbi.nlm.nih.gov/).

The BLAST analysis of (SNP A4795G) polymorphism gene showed 98% identity to nucleotide sequence of (SNP A4795G) polymorphism.

The mutant type for the (SNP A4795G) was found to bear the same sequences that retrieved from a gene bank(Fig: 5) exemplified the mutant, shown by Finch TV sets, and software packages (SNP A4795G).

The polymorphisms for selected specimens (4) were determined by BGI Sanger sequencing. The participants were 28/41 (68.3%) males and 13/41 (31.7%) females consecutively. The age is ranging from (13-36) years. The PCR results revealed 34/41 (83%) were amplified whereas, the rest of 7/41(17%) were not amplified. The result of sequencing of McC (SNP A4795G) harbor a 50% G allele and 50% take an A type. Whereas Sl (SNPA4828G) bears 75 % G and 25% an A alleles. In conclusion, the study supports the hypothesis of the SL allele and the McC allele, polymorphisms and evolved in the context of Mycetoma predisposing factor. The overall results of the current study regarding molecular basis and bioinformatics packages have been abstracted in the following figures (8, 9, 10, 11, and 12). The protein alignment and s E-K Polymorphism have been publicized in (Fig: 7).the wild type of the predicted amino acid was found to be Lysine (Lys) with the intention of position 14. The secondary protein structure of the wild gene type is drawn by Phyre2 software has been illustrated in Fig: A1.The Tertiary protein structure of the wild gene is drained by Chimera software version 1.8 andhas been demonstrated in Fig: A2.Whereas, Fig: 8 and 9) exemplified the mutant type of thepredicted amino acid Lysine Glutamic acid (Glu) at position 14. The multiple sequence alignment of the mutant isolate with similar nucleotide sequences with the purpose obtained from BLASTn was carried out to find the homology and evolutionary relationship between these sequences as it shown by BioEdit software. The results displayed that there aresubstitutionsSl blood group in and R1601G (SNP A 4828 G).Likewise **(**Fig.10) have shown evidence of themutantexposed by Finch TV software CR1 R1601G (SNP A4828G**).**The mutant type of the predicted amino acid Lysine Glycine (Gly) at position 14(Fig: 11).The secondary protein structure of the mutant gene pinched by Phyre2 software Fig: A1 .Tertiary protein structure of themutant genes strained by Chimera software version 1.8 Fig: A2 and Fig:. 12.

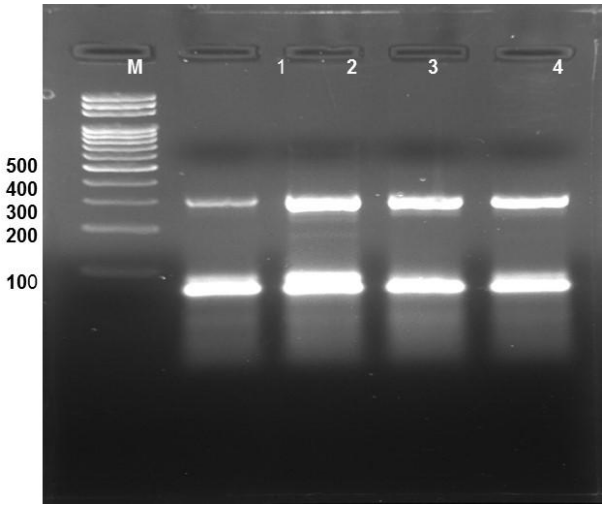

Figure 4: Explains the electrophoresis gel of the CR1 for McC and Sl polymorphisms in 41Mycetoma patient by using one primer (24KnNde,25Rb) for two polymorphisms (McC and Sl) 1; 2; 3; and 4 bands of PCR product length 305 bp:A**:** 305 bp DNA Ladder (size marker 100 bp).

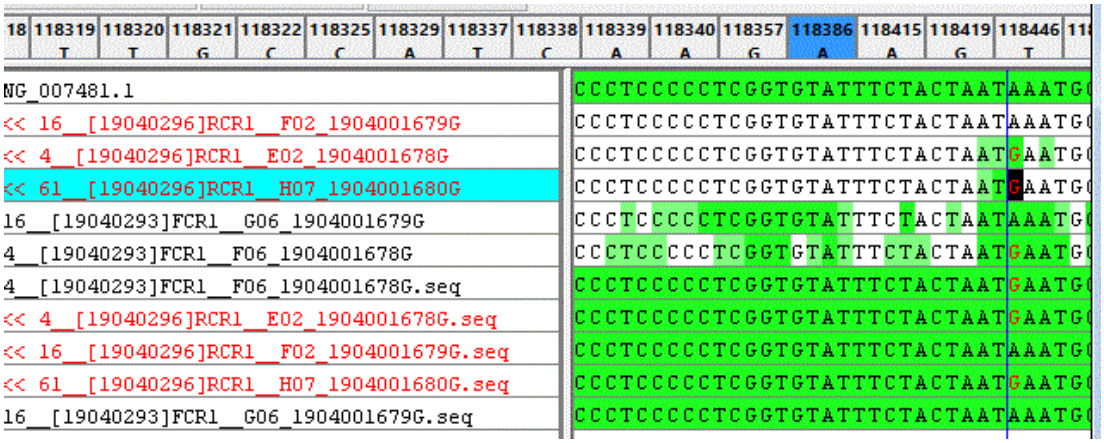

Figure 2: Displays the multiple sequence alignment of the mutant sample with similar nucleotide sequences obtained from BLASTn was carried out to find the homology and evolutionary relation between these sequences. As shown by BioEdit software there aresubstitutionsin McC blood group K1590E (SNP A4795G*)*.

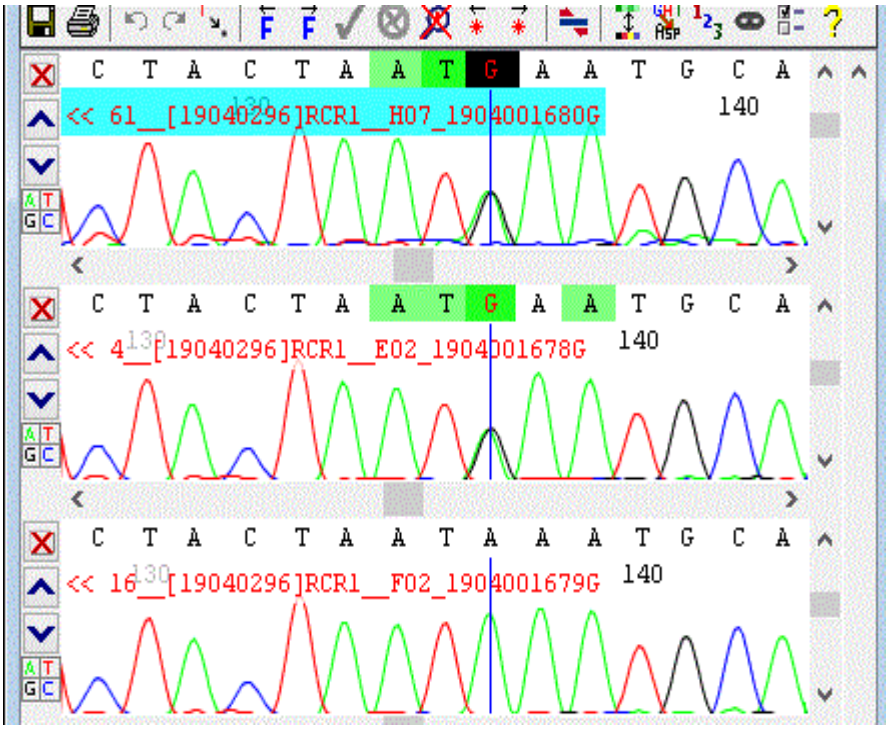

Figure 3: Demonstrates the mutant, shown by Finch TV software CR1 K1590E (SNP A4795G).

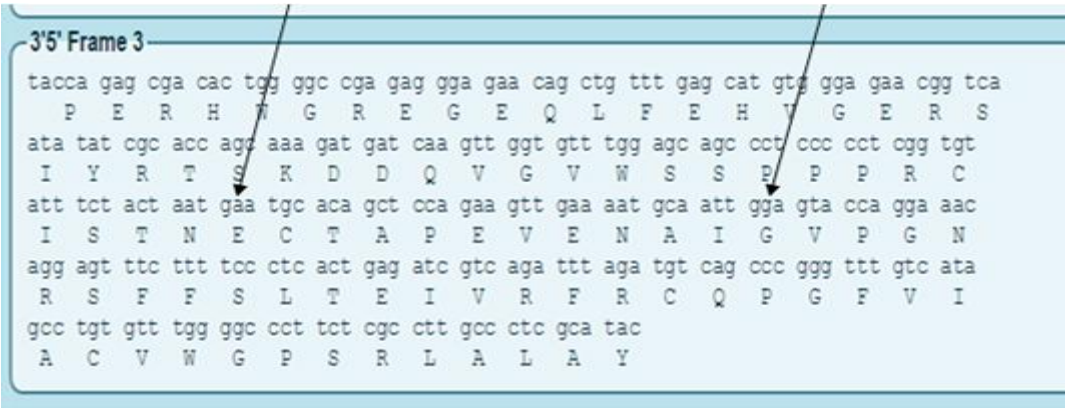

Figure 4: Mutant, shown by ExPASy - Translate tool CR1 K1590E (SNP A4795G) and R1601G (SNP A 4828 G).

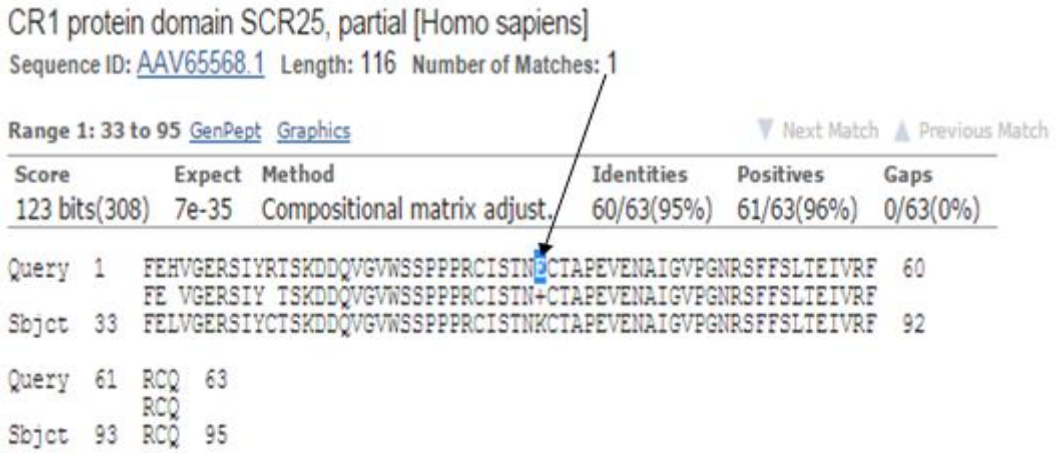

Figure 5: Shows the protein alignment and CR1 K1590E (SNP A4795G) Polymorphism

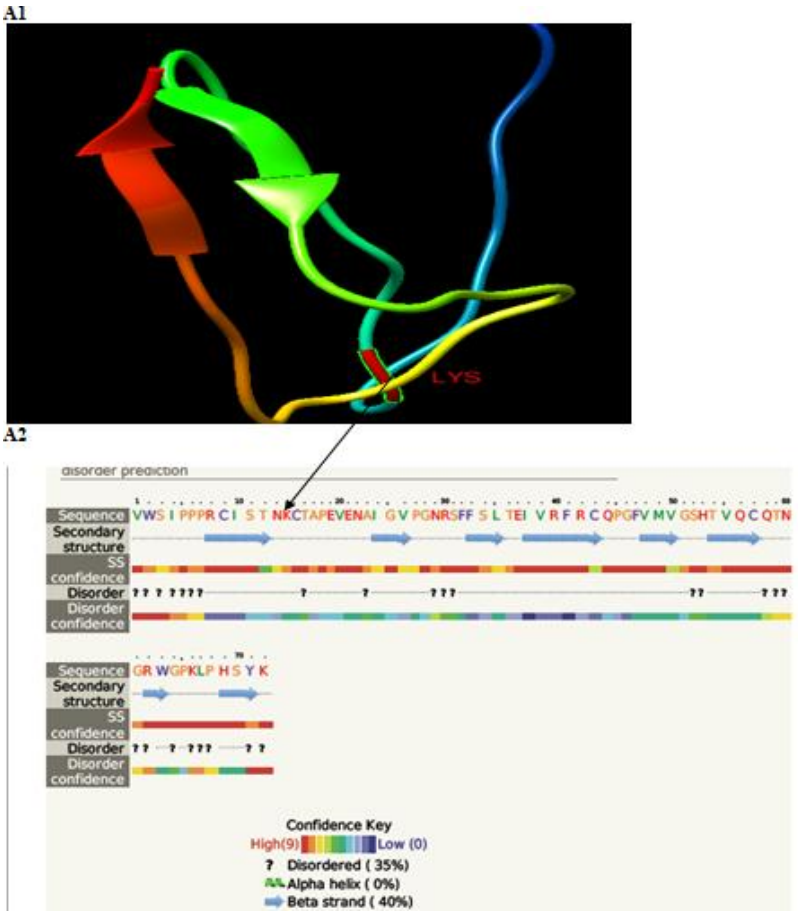

Figure 6: Clarifies the wild type of K1590E (SNP A4795G) of the predicted amino acid Lysine (Lys) at position 14. The secondary protein structure of the wild gene that is drawn by Phyre2 software .Fig: A2. Tertiary protein structure of the wild genes that is drawn by Chimera software version 1.8 Fig: A1

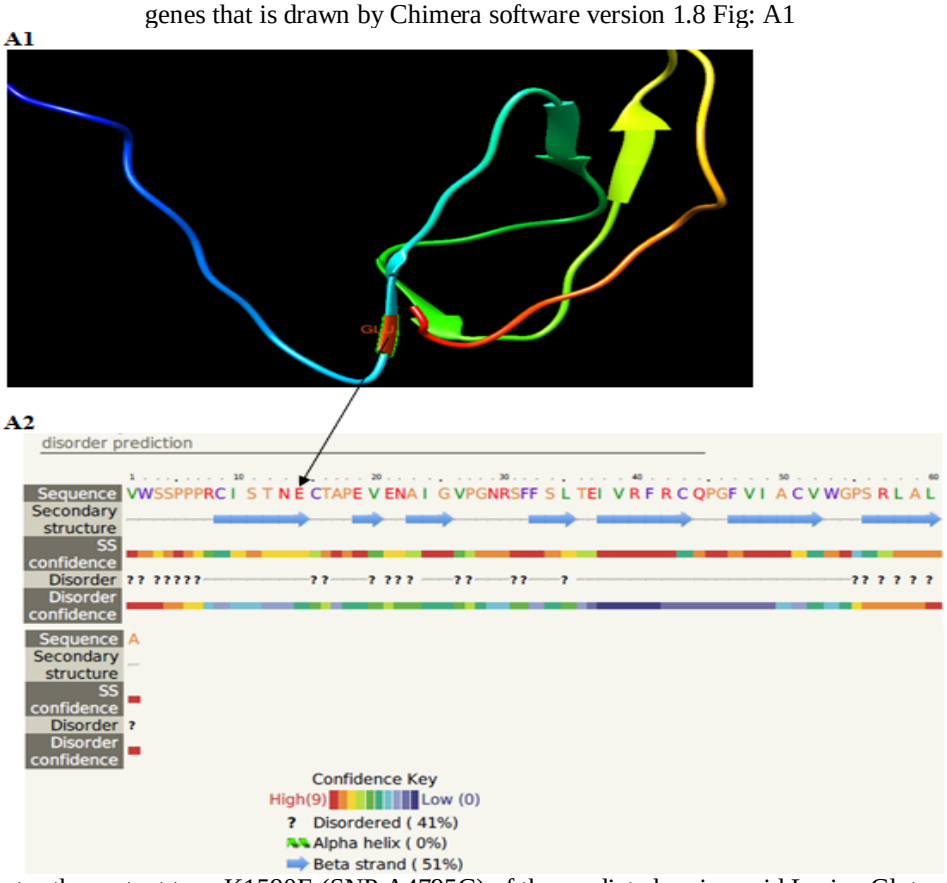

Figure 7: Illustrates the mutant type K1590E (SNP A4795G) of the predicted amino acid Lysine Glutamic acid (Glu) at position 14 .The secondary protein structure of the mutant gene that is drawn by Phyre2 software Fig:A2. Tertiary protein structure of the genes mutant that is drawn by Chimera software version 1.8 Fig: A1.

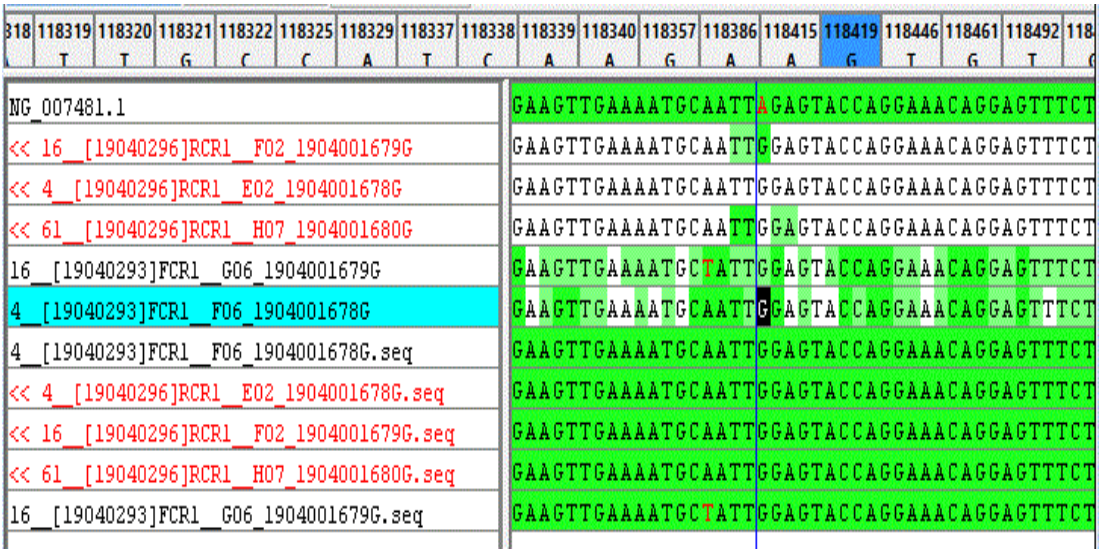

Figure 8: Shown the multiple sequence alignment of the mutant isolate with similar nucleotide sequences obtained from BLASTn was carried out to find the homology and evolutionary relation between these sequences. As shown by BioEdit software there aresubstitutionsSl blood group in and R1601G (SNP A 4828 G).

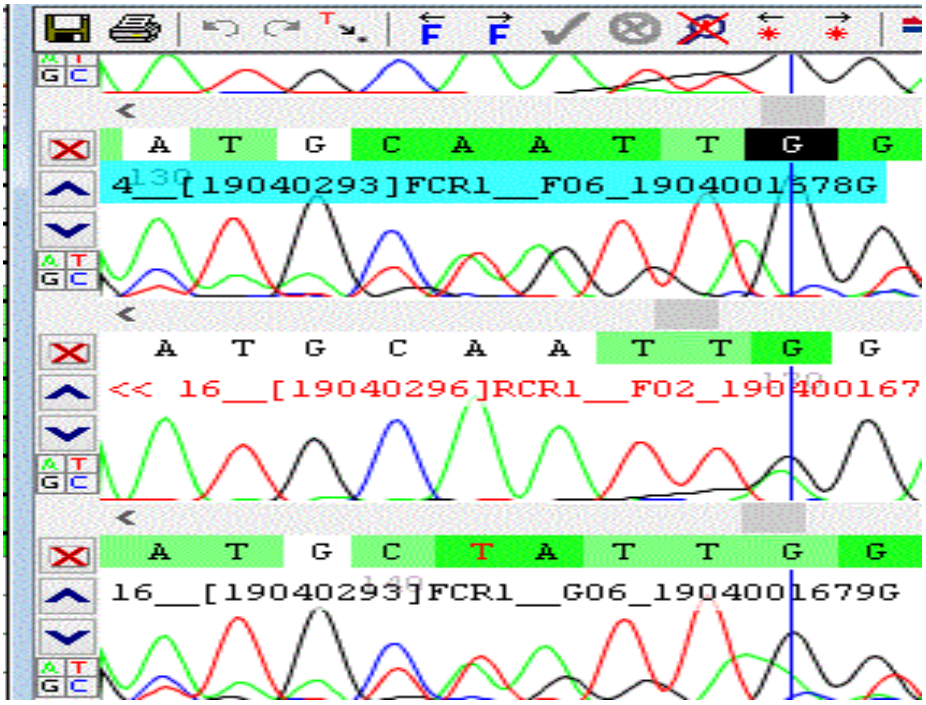

Figure 9: Illuminates the mutant, shown by Finch TV software CR1 R1601G (SNP A4828G)

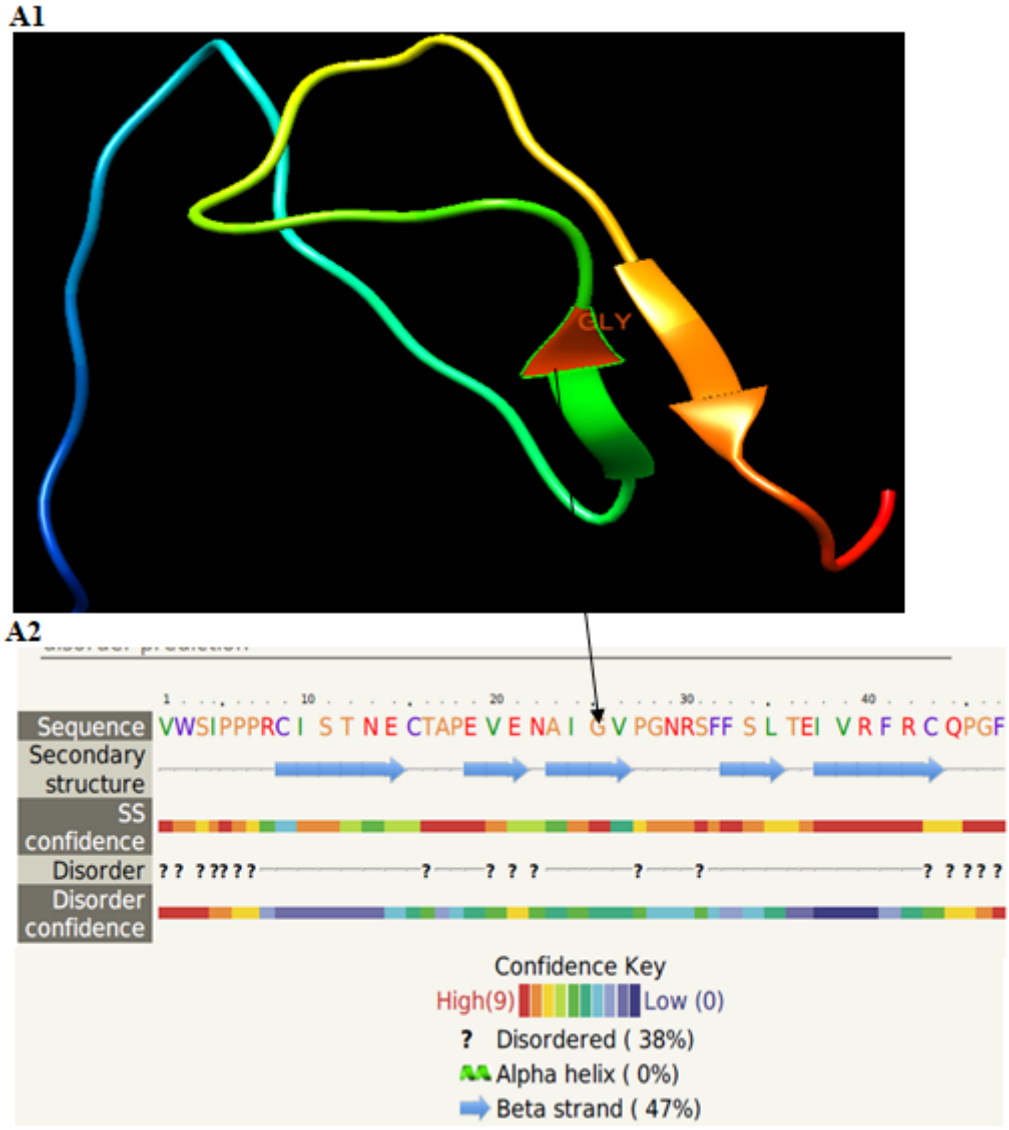

Figure 10: Elucidates themutant type CR1 R1601G (SNP A4828G)of the predicted amino acid Lysine Glycine (Gly) at position 25. The secondary protein structure of the mutant gene that is drawn by Phyre2 software Fig: A2, Tertiary protein structure of themutant genes is drawn by Chimera software version 1.8 fig: A1.

4. Discussion

Complement reseptor1 is expressed on neutrophils; it is not unique for this cell type. Knops blood group polymorphisms in the CR1 gene are responsible for the SL blood group Ag and the McC blood group Ag, SL, and McC polymorphisms are present in the long, homologous repetitive D region of the CR1 gene. The K1590E polymorphism is associated with the McCa and McCb antigens, whereas the R1601G is associated with the SL. Neutrophil are important in the early defense against Mycetoma. In Mycetoma endemic areas, most individuals have antibodies against the causative agents, however only sequences available in the Gen-Bank database using BLAST nucleotide algorithm (http://www.ncbi.nlm.nih.gov/). BLAST analysis of CR1 K1590E and R1601G polymorphisms gene showed 99% identity to a nucleotide sequence of CR1 K1590E and R1601G polymorphisms. Interestingly, the existing study validates that the CR1 K1590E and R1601G were found to bear the same sequences retrieved from the gene bank. Moreover, two polymorphisms were embodied with the multiple sequence alignment of the sample with similar nucleotide sequences are obtained from BLASTn (Fig: 2 and Fig: 8) respectively. This process was carried out to find the homology and evolutionary relation between these sequences shown by BioEdit software and those shown by Finch TV software (Fig: 3 and Fig9). The protein sequence of CR1 K1590E and R1601G retrieved from the ExPASy Database (http://www.expasy.org/) (Fig; 4). The protein alignment and E-K Polymorphism have been publicized in (Fig: 5). The (SNP A4795G) show 50%posative and 50% negative and (SNPA4828G) show 75 % positive and 25% negative. The wild type K1590E (SNP A4795G) of the predicted amino acid Lysine (Lys) at position 14 (Fig: 4.6).The secondary protein structure of the wild gene is drawn by Phyre2 software (Fig: A2.6); the tertiary protein structure of the wild genes is careworn by Chimera software version 1.8 (Fig: A1.6). Whereas, Fig:.7 exemplified the mutant type K1590E (SNP A4795G) of the predicted amino acid Lysine and Glutamic acid (Glu) at position 14; the secondary protein structure of the mutant gene is drawn by Phyre2 software Fig: A2.7, the tertiary protein structure of the mutant genes is drawn by Chimera software version 1.8 (Fig: A1.7 and Fig: 10) show the mutant type CR1 R1601G

of the predicted amino acid Lysine Glycine (Gly) at position 25. The secondary protein structure of the mutant gene is drawn by Phyre2 software (Fig: A2 10); the tertiary protein structure of the mutant genes is drawn by Chimera software version 1.8 (Fig: A1.10).In conclusion, further studies are recommended for surveillance, screening, characterization, and mapping the target sinuses surrounding the endemic and co-endemic areas.

References

[1] **Reis, C.M.S. and E.G.d.M. Reis-Filho**, *Mycetomas: an epidemiological, etiological, clinical, laboratory and therapeutic review.* Anais brasileiros de dermatologia, 2018. **93**(1): p. 8-18. Available from:http://dx.doi.org/10.1590/abd1806-4841.20187075

[2] **Pérez-Alfonzo, R., et al.**, *Oral Signs of Tropical, Fungal, and Parasitic Diseases*, in *Oral Signs of Systemic Disease*. 2019, Springer. p. 193-225. Available from:https://link.springer.com/chapter/10.1007/978-3-030-10863-2_10.

[3] **Giansante, E. and A. Guzmán-Fawcett, *Ricardo Pérez-Alfonzo, Silvio Alencar-Marques***. Oral Signs of Systemic Disease, 2019. **1**: p. 193 . Available from:https://link.springer.com/chapter/10.1007/978-3-030-10863-2_10

[4] **Margaritopoulos, G.A., M.A. Kokosi, and A.U. Wells**, *Diagnosing complications and co-morbidities of fibrotic interstitial lung disease.* Expert Review of Respiratory Medicine, 2019. **13**(7): p. 645-658. Available from:https://doi.org/10.1080/17476348.2019.1632196

[5] **Singh, V., *Fungal Rhinosinusitis**: Unravelling the Disease Spectrum.* Journal of maxillofacial and oral surgery, 2019. **18**(2): p. 164-179. Available from:https://link.springer.com/article/10.1007/s12663-018-01182-w

[6] **Bysani, V., A.L. Pabbathi, and D. Gopineni,***A Rare Case Report on Madura Foot.* Indian Journal of Pharmacy Practice, 2019. **12**(1). Available from:http://www.ijopp.org/sites/default/files/InJPharPract-12-1-50_0.pdf

[7] **Kumar, A. and J.S. Mehta**, *Diagnosis and Management of CMV Endotheliitis.* Current Ophthalmology Reports, 2019. **7**(2): p. 98-109.Available from:https://link.springer.com/article/10.1007/s40135-019-00205-0

[8] **Almeida-Silva, F., et al.,** *Current Aspects of Diagnosis and Therapeutics of Histoplasmosis and Future Trends: Moving onto a New Immune (Diagnosis and Therapeutic) Era?* Current Clinical Microbiology Reports, 2019: p. 1-10. Available from:https://link.springer.com/article/10.1007/s40588-019-00118-3#citeas.

[9] **Pendl, H. and K. Kreyenbühl,** *Clinical pathology*, in *BSAVA Manual of Backyard Poultry Medicine and Surgery*. 2019, BSAVA Library. p. 85-104. Available from:https://www.bsavalibrary.com/content/chapter/10.22233/9781910443194.chap10?crawler=true

[10] **Mekoguem, C., C. Triboulet, and A. Gouveia**, *Madurella mycetomatis infection of the buttock in an Eritrean refugee in Switzerland: a case report.* Journal of medical case reports, 2019. **13**(1): p. 32.

[11] **Bakhiet, S. M., N. A. Mhmoud, E. E. Siddig, D. M. Ibrahim, E. S. Ahmed and A. H. Fahal***Madurella mycetomatis causing multiple mycetoma lesions: A rare clinical presentation.* Khartoum Medical Journal, 2019. **12**(1). Available from:http://onlinejournals.uofk.edu/index.php/kmj/article/download/2386/2347

[12] **Siddig, E. E., N. A. Mhmoud, S. M. Bakhiet, O. B. Abdallah, S. O. Mekki, N. I. El Dawi, W. Van de Sande and A. H. Fahal***The Accuracy of Histopathological and Cytopathological Techniques in the Identification of the Mycetoma Causative Agents.* PLoS neglected tropical diseases, 2019. **13**(8): p. e0007056. Available from:https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007056

[13] **Mhmoud, N. A., A. Santona, M. Fiamma, E. E. Siddig, M. Deligios, S. M. Bakhiet, S. Rubino and A. H. Fahal***Chaetomium atrobrunneum causing human eumycetoma: The first report.* PLoS neglected tropical diseases, 2019. **13**(5): p. e0007276. Available from:https://scholar.googleusercontent.com/scholar.bib?q=info:ake983ag7vEJ

[14] Arkun, R., *Fungal and Higher Bacterial Infections.* Musculoskeletal Imaging Volume 2: Metabolic, Infectious, and Congenital Diseases; Internal Derangement of the Joints; and Arthrography and Ultrasound, 2019: p. 121. Available from:https://books.google.com.sa/books?hl=en&lr=&id=GJ-cDwAAQBAJ&oi=fnd&pg=PA121&dq

[15] **Shoham, S., E.A. Dominguez, and A.I.D.C.o.** Practice, Emerging fungal infections in solid organ transplant recipients: Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clinical transplantation, 2019: p. e13525. Available from:https://doi.org/10.1111/ctr.13525.

[16] **Casadevall, A.,** *Global Catastrophic Threats from the Fungal Kingdom.* 2019. Available from:https://link.springer.com/chapter/10.1007/82_2019_161

[17] **Abdelrahman, M., E. A. Saad, G. M. Abdulla and A. Mohamed***Reconstructive Surgery for Mycetoma: Is There a Need to Establish an Algorithm?* Plastic and Reconstructive Surgery–Global Open, 2019. **7**(4): p. e2197. Availablefrom:https://journals.lww.com/prsgo/Fulltext/2019/04000/Reconstructive_Surgery_for_Mycetoma__Is_There_a.17.aspx

[18] **Verma, R. and B. Vasudevan**, *Dermatological Emergencies*. 2019: CRC Press. Available from:https://books.google.com.sa/books?hl=en&lr=&id=zbyZDwAAQBAJ&oi=

[19] **Abushouk, A., A. Nasr, E. Masuadi, G. Allam, E. E. Siddig and A. H. Fahal***The Role of Interleukin-1 cytokine family (IL-1β, IL-37) and interleukin-12 cytokine family (IL-12, IL-35) in eumycetoma infection pathogenesis.* PLoS neglected tropical diseases, 2019. **13**(4): p. e0007098. Available

from:https://journals.plos.org/plosntds/article?rev=1&id=10.1371/journal.pntd.0007098

[20] **Siddig, E. E., A. M. Mohammed Edris, S. M. Bakhiet, W. W. van de Sande and A. H. Fahal,***Interleukin-17 and matrix metalloprotease-9 expression in the mycetoma granuloma.* PLoS neglected tropical diseases, 2019. **13**(7): p. e0007351. Available from:https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007351

[21] **Fahal AH, van De Sande WW.** The Epidemiology of mycetoma. Current Fungal Infection Reports. 2012 Dec 1;6(4):320-6. Availlable from https://link.springer.com/article/10.1007/s12281-012-0102-1