Dedication

To

My

Family

Acknowledgements

I would like to express my deepest and sincere appreciation to my previous supervisor **Dr. Adil Mahgoub** may, Allah rest his soul in peace-who guided me through the early stage of this work, the knowledge you passed to me and my colleagues will stay as will your memory which will live in our hearts forever.

Thank you **Dr. Galal Mustafa** my main and principal supervisor, for his constant encouragement, guidance and support during my PhD studies. Our discussions and argument, helped greatly in refining the ideas in this thesis from their original hazy form into ones that can be elucidated clearly and concisely.

Special thanks also due to **Dr. Bakhita Attalla**, for helping in samples collection, I am continually grateful for her support and motivation.

My dear **Neveen Abdel Wahab,** who was my inspiration in this work , her determination improve this work , helped me see that I could do this that I could have time out finish it and that it was worth it . Her friendship support and vision are important components of this dissertation

My thanks **Meryam Tagavi** From Iran, Pasteir Institute – she helped and gave me valuable advices during the practical period in Iran.

My colleagues in National Health Laboratory, Sudan International University, laboratory C15 who provided the vision, enthusiasm and determination to make the study actually happen.

My family I have an amazing family, unique in many ways, and the stereotype of a perfect family in many others. Their support has been unconditional all these years; they have given up many things for me, and supported me whenever I needed it…..

My Dear colleague Elfatih Seifeldein for his great efforts.

Perhaps, I forgot someone …….so; just in case thank you to whom it concerns!

Abstract

Sickle cell disease is a major health problem worldwide particularly in developing countries including Sudan. The disease has a great impact on both individual and society. Sickle cell disease disorder produces prominent clinical manifestations. Subjects with heterozygous form (AS) are designated sickle cell trait; they are essentially healthy unless exposed to extreme conditions.

Further more AS subjects are known for their immune resistance to justify malaria Patients of sickle cell anemia are known not to survive beyond their 20^{th} birthday. Sickle-cell hemoglobin (HbS) is found as an inherited abnormality with relatively high frequency in many races and tribes, particularly that resident in, or originating from, the malarial regions of the world. Molecular diagnosis of the disease, genetic and family studies of patients becomes an important tool for management of sickle cell disease patients. In this study we compare the efficiency of conventional cellulose acetate electrophoresis techniques with recently developed molecular biology methods i.e. (RFLP, ARMS/PCR, RAPD).We also estimated the level of SCD amongst Sudanese populations living in central Sudan. The objectives of the study also is to provide a database for the establishment of the most appropriate genetic counseling services for SCD patients and their families and to identify areas of research and collaboration.

The result of this study shows higher frequencies of sickle cell disease in certain Sudanese tribes. The level of sickle cell disease was found with higher frequencies in the Afro/Arabs groups living in western part of the country. The hemoglobin levels of the Sudanese sickle cell patient was found significantly lower than normal percentages in individuals with normal hemoglobin, the significant elevated hemoglobin F level was also observed among sickle cell patients which help making the disease less severe and contribute to the mildness of the SCD among Sudanese patients.

The sickle cell gene was found with higher frequencies in certain Sudanese tribes. In the results of ARMS PCR, we have two lines M for mutation bands and N for normal bands. If we have two bands in M&N sample is heterozygous but if we have one band in M that means it is a homozygous for Hb S and if we have one in N line that means it is normal. Discrimination of HbAA, HbAS and HbSS using ARMS-PCR shows amplification of HbA and HbS genotypes using ARMSPCR, typing of the AA, AS, SS genotypes yielded the 207bp.

The results of RFLP In this method after the amplification of DNA with primers1 (mutant), primer 2 (normal primer), the 281bp fragment was achieved, after that we digested this fragment with DdeI restriction enzyme in two fragment (200bp&81 bp) so +/+ shows that our sample is normal and we had 200 & 81 bp both of them but we couldn't see 80 bp so we just had 200 bp! For heterozygous

carrier we had 3 bands 200&281&81 that we saw just 2 bands (200 and 281), that was -/+! for homozygous disease we had just one band 281 bp that shows our restriction sits disturb and our enzyme couldn't digest it

ملخص البحث

يعتبر مرض فـ قر الدم المنجلي من أكبر المشكلات الصحية على نطاق العالم لاسيما في البلدان النامية بما فيها السودان . ويلـ قي المرض باثاره السالبة على الفرد والمجتمع .

يؤدي الى homozygous HB SS يبرز المرض في مظهرين , أحدهما ينتج عن حلل وراثي مزدوج أعراض مرضية حادة تنتهي بالوفاة ببلوغ العشرين . مـ قارنة بالخلل الوراثي الجزئي heterozygous AS وتختفي فيه الاعراض ما لم يتعرض المريض لظروف خاصة " قلة توفر الاكسجين" وهؤلاء يكتسبون مناعة ضد الملاريا .

وبما أن المرض وراثي فنجده يكثر في شعوب و قبائل بعينها . كما أن طبيعة المرض الوراثية جعلت من التشخيص الجيني المعتمد على التـ قدم الكبير للعلوم في الأحياء الجزيئية أداة هامة للتشخيص.

مرض فـ قر الدم المنجلي يورث المريض هيمو قلوبين غير طبيعي ذو قابلية عالية للتبلور في حال قلة الاكسجين محدثاً إنسداداً في الاوعية الدموية الصغيرة وتحللاً في خلايا الدم الحمراء.

في هذا البحث نـ قارن كفاءة تـ قنية الرحلان الكهربي خلال السليلوز وهي تـ قنية تـ قليدية, وتـ قنيات RFLP- ARMS PCR RAPD - حديثة هي كما أننا بصدد تـ قدير حالات الاصابة معتمدين. علي سكان أواسط السودان كـ قاعدة إحصائية وتوفير بيانات تساعد علي تحديد أفضل الخدمات الإستشارية للمصابين وعائلاتهم.

و قد أظهرت دراساتنا إرتفاع نسب حالات الاصابة بالمرض في الـ قبائل العربية الافريـ قية في غرب البلاد.

F كما ان نسب الهيمو قلوبين في المرضى أ قل بكثير من الاصحاء الا ان زيادة نسبة هيمو قلوبين بين المصابين أدت الي التـ قليل من حدة أعراض المرض.

وتشير الي عدم N وتشير الي الطفرة أوالمرض و M لدينا حزمتين ARMS PCR في نتائج ال فانها تدل علي ال N و ال M وجود المرض فاذا ما أظهرت العينة حزمتين في ال homozygous اما heterozygous فذلک يدل علي ال M ولكن اذا ظهرت حزمة واحدة في ال .فذلک يعني ان العينة سليمة N اذا ظهرت حزمة واحدة في ال

) Primer 2 الطفرة) و) Primer 1 وبعد مضاعفة الحمض النووي باستخدام RFLP في نتائج ال حصلنا Ddel-RE ومن ثم وبأستخدام الأنزيم ال قاطع bpالطبيعي) حصلنا علي جزء مكون من 281 .وهذا يعني +/+ أن العينة طبيعية bpو 81 bpعلي جزئين 200

و bpحصلنا علي ثلاثة أجزاء بظهور حزمتين ف قط من 281 heterozygous وفي حالة ال .+/- ويعبر عن ذلک ب 200bp

لأن الانزيم ال قاطع لم يجد bpفنحصل علي حزمة واحدة 281 homozygous وفي حالة ال .موضعه الطبيعي لا قطع نتيجة للطفرة المرضية

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