UNIVERSITY OF IBADAN

The Anaemias: A Peep Through The Looking Glass Of Genes

Adeyinka Gladys Falusi,
B. Sc. M. Phil. Ph.D

Professor & Director,
Institute for Advanced Medical Research & Training,
College of Medicine,
University of Ibadan
Ibadan, Nigeria

2003/2004
INAUGURAL LECTURE

UNIVERSITY OF IBADAN

The Anaemias: A Peep Through The Looking Glass Of Genes

Adeyinka Gladys Falusi,
B. Sc. M. Phil. Ph.D

Professor & Director,
Institute for Advanced Medical Research & Training
College of Medicine,
University of Ibadan,
Ibadan, Nigeria.

THURSDAY 29TH JULY, 2004

2003/2004
INAUGURAL LECTURE

First Published 2004

ISBN: 978-36756-4-8

Printed by: Corporate Graphics Ltd, Ibadan.

Biographical Sketch

PROFESSOR ADEYINKA GLADYS FALUSI

Lecture Title: **"THE ANAEMIAS: A PEEP THROUGH THE LOOKING GLASS OF GENES"**

The Inaugural Lecturer, Professor Adeyinka Gladys FALUSI was born on 21st May 1945 in Efon Alaaye, Ekiti State to late Chief Isaiah and Mama Monica Ologbenla. She was educated at St. Paul's Anglican School, Idagba, Efon Alaaye and Queen's School, Ede, 1959-63, 64-65. In 1966, she was admitted to the University of Ibadan and graduated in1969 with Honours degree in Chemistry. She later obtained the M.Phil. (1981) and Ph.D (1986) in Haematology from the University of Ibadan.

Professor Adeyinka Falusi started in 1970 as a Research Associate in Langmuir Laboratory, Cornell University under the World renowned Scientist, Professor Gene Lickens. In Aug. 1973, she joined the Department of Haematology, University of Ibadan, as a Research Technician working in the laboratory of Professor L.L. Luzzatto. Professor Adeyinka Falusi began her academic career in October 1987, when she was appointed Research Fellow I in the then Postgraduate Institute of Medical Research and Training (PIMRAT). Her priority was to establish the Medical Genetic Research Unit. She was promoted Senior Research Fellow in 1990, Reader in 1995 and Professor in October 2000. In March 2002, she was appointed Director of the Institute for Advanced Medical Research and Training (IMRAT) and thereby became the CHAIRPERSON of the UI/UCH Institution Review Committee (IRC).

Professor Adeyinka Falusi has solidified her reputation as the foremost Scientist in the Molecular Genetics of Sickle Cell Disease and alpha thalassaemia in Nigeria. Her work has contributed tremendously to the genetics of sickle cell disease, alpha-thalassaemias, Glucose-6-

phosphate Dehydrogenase (G6PD) deficiency and malaria. Her current research effort is on the genetics of hypertension.

Professor Adeyinka Falusi has been privileged to carry out research in collaboration with renowned scientists such Prof. L.L. Luzzatto an authority on G6PD worldwide and Prof. D.J. Weatherall a first authority world wide on thalassaemias. She has collaborated with scientists in institutions such as Hammersmith Hospital, London; John Radcliffe Hospital, Oxford; University of Malta; University of Milan; Institute for Tropical Medicine Berlin; the Sloan Kettering Cancer Center in New York City and the University of Chicago Cancer Research Centre, Chicago.

Professor Adeyinka Falusi has received several Awards and Honours which include the British Council/National Universities Commission Staff Development Scholarship (1983); the Ministry of Science & Technology Travelling fellowship (1988); the International Cell Research Organisation (ICRO)/(UNESCO) Training Fellowship (1991); the Medical Research Council (MRC) Grant(1994); the Deutsche Volkswagen-Stiftung Research Grant (1997);the Deutsche Volkswagen-Stiftung Travelling Fellowship (1998); the Christopher Kolade Foundation Grant (1998); the College of Medicine Research Grant (2000); the Falk Clinical Research Trust of the USA (2002 to date) and the University of Ibadan Senate Research Grant (2003).

Professor Adeyinka Falusi is a productive scholar. She has to her credit 42 articles in peer-reviewed national and international journals with over 95% referenced on the internet. She also has over twenty five published abstracts, conference proceedings, monographs and technical reports, as well as contributions to chapters in Books.

Professor Adeyinka Falusi's research output revealed NOVEL findings resulting in global changes in existing literature on megaloblastic anaemia and alpha thalassaemia in particular. She received an international recognition of approval, when she was conferred with the prestigious L'OREAL/UNESCO AWARD for Outstanding Woman in Science (Africa) on 28th February 2001. In 2002, Professor Adeyinka Falusi was appointed a UNESCO Resource Person for the UNESCO Young Women Scientists Grants Awards for Africa. In 2003, she

received the Rare Gems Award in the category of Science & Technology from Women's Optimum Development Foundation (WODEF) in Lagos, Nigeria to mark the International Women's Day.

Professor Adeyinka Falusi is a member of several distinguished bodies including: The American Society of Haematology; The New York Academy of Science; The Nigerian Society of Haematology and Blood Transfusion; The Sickle Cell Association of Nigeria; The Nigerian Institute of Biology; The National Association of Medical Scientists; and The Nigerian Bioethics Initiative of which she is the Protem Chairperson.

Professor Adeyinka Falusi is married to Professor Abiodun Falusi and they are blessed with children and grandchildren.

THE ANAEMIAS: A PEEP THROUGH THE LOOKING GLASS OF GENES

The Vice Chancellor,
Deputy Vice Chancellors,
Registrar,
Provost of the College of Medicine,
Deans of Faculties, of the Postgraduate School and of Students,
Directors of Institutes,
All other Principal Officers of the University of Ibadan,
Heads of Departments,
Professors of the University of Ibadan and other sister institutions of higher learning,
Members of the Academic Community of the University of Ibadan,
Distinguished Ladies & Gentlemen,

As I prepared the Award-winning paper on Molecular Genetics in Nigerians for presentation before the French Academy of Science in Paris on 28th February 2001, I was stuck by the uncanny feeling that, perhaps, the topic of an Inaugural lecture was also in gestation. I am blessed and to God be the Glory for making that dream a reality today.

Mr. Vice Chancellor let me also thank the Dean of the Faculty of Basic Medical Sciences, Prof F.A.A. Adeniyi, and all other members of my Faculty for honour of selecting the Institute for Advanced Medical Research & Training (IAMRAT), the only Institute within its domain, as the source of this year's inaugural lecturer.

IAMRAT had its beginning in the Postgraduate Institute for Medical Research and Training (PIMRAT) which was established in 1980 as one of the academic divisions of the College of Medicine of our University, for capacity building in research, for the coordination of multidisciplinary research programmes and to serve as the channel for the dissemination of research outputs through the organization of courses, workshops and public lectures.

The Institute houses the following units: the Medical Genetic & Bioethics Research Unit, Malaria Unit, Environmental Sciences and

Biotechnology Research & Service, Health and Information Systems Unit, Cancer Research and Service Unit, Epidemiology and Biostatistics Unit, Pharmaceutical/Pharmacognosy Unit, Behavioural/Social Sciences Unit, UI/UCH Ethical Committee secretariat, African Journal of Medicine & Medical Sciences office as well as Centre for HIV/AIDS in Nigeria (CEHAIN). The Institute collaborates with other scientists both locally and internationally and attracts research funds from donor agencies like the WHO, International Atomic Energy Agency (IAEA), Volkswagen - Stiftung Foundation, United States Agency for International Development (USAID), Christopher Kolade Foundation, Ralph and Marion Falk Cancer Trust of the US,A among others. The Institute is a designated centre of excellence for malaria research in Africa.

The Medical Genetic & Bioethics Research Unit focuses on:

I. Population genetics of sickle cell disease, the thalassaemias and other factors, which modify the pathology of these diseases.
II. Identification of genetic factors modifying the incidence and prognosis of Hypertension.
III. Molecular Epidemiology of the genetic markers, which affect the prognosis, drug resistance and treatment of malaria.
IV. Antimalaria drug disposition and Kinetics.
V. Awareness programmes of Education, Genetic counseling and diagnosis of the haemoglobinopathies in Nigerians.
VI. Training programmes in the Ethics of Human subjects research at various levels.

I stand before you all today, a proud successor to twelve distinguished men like the late Professor Siji Osunkoya, Professor Oyewale Tomori (a Nigerian National Order of Merit recipient) and Professor O. Odejide, who among others were either Directors or Ag. Directors in the twenty four years of the Institute's existence and some of who have given inaugural lectures from their base departments. My pride, however, has the additional adornments in being the first female to hold the office of Director of the Institute, the first core staff and incumbent Director to give an inaugural Lecture.

Medical Genetics

A specialty of Medicine that deals with Diagnosis, Treatment and Management of Hereditary Disorders

My interest in Medical Genetics (SLIDE) led me to the journey of the Genetics of Anaemia through the looking glass of genes. The journey commenced in the Haematology Research Laboratory of Professor Lucio Luzzatto in August1973 on the recommendation of Professor G.B. Ogunmola of the Department of Chemistry. I had opted for a technical staff position instead of an academic Junior Research Fellowship in the mistaken belief that domestic responsibilities would not be unduly compromised. I found myself in the library daily and in the laboratory all day! It was difficult to fault Professor Luzzatto by his penchant for leadership qualities. My husband had accompanied me to our research laboratory to read the result of an experiment at 12 mid-night. Emerging from the UCH Blood Bank as we arrived was Professor Luzzatto who had just checked the experiment for me. What a leader!!! Up till today, I still wonder how I managed to cope with the pace of tutelage under Luzzatto. That initial experiment unraveled for the first time the basis of Megaloblastic Anaemia in Pregnant Nigerians. Inspired by Luzzatto, my curiosity into the common causes of anaemia in Nigerians took the first breath of life.

Mr. Vice Chancellor, Sir, this is the genesis of today's lecture: **"THE ANAEMIAS: A PEEP THROUGH THE LOOKING GLASS OF GENES"**

Kindly permit me to acquaint this audience with some terminologies and their relevance in today's lecture.

What is Anaemia?

Anaemia can be simply defined as a decrease in the haemoglobin parameters a s m easured by Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). It is functionally characterized by a haemoglobin concentration below Normal (Oski Table) (SLIDE).

OSKI TABLE

- **Anaemia**

Age(yrs)	Hb(g/gl)
<5	<11
5-8	<11.5
9-12 & >18	<14.

- **Microcytosis**

Age(yrs)	MCV (fl)
<2	<70
2-4	<73
5-7	<75
8-12	<76
>18	<80

- **Hypochromia**

Age(yrs)	MCH (pg)
<2	<23
2-5	<24
6-12	<25
≥ 18	<26.

Mr. Vice Chancellor, anaemias can be primarily classified as shown below

Classification of Anaemia

- Cause - Hemorrhagic, Haemolytic , Nutritional
- Marrow Reaction -Homoplastic, Megaloblastic, Aplastic
- Haemoglobin Content -Hypochromic, Orthochromic, Hyperchromic
- Size of red blood cells - Microcytic, Normocytic, Macrocytic
- Shape - Spherocytic, Sickle cell

on the basis of their causes, as hemorrhagic, hemolytic and nutritional. They can also be classified according to the marrow reaction and described as homoplastic, megaloblastic or aplastic. Within these main groups, the anaemias can be further subdivided according to the size of the red cells as microcytic, normocytic or macrocytic; or according to the shape as spherocytic or sickle cell. Anaemia can again be classified according to the hemoglobin content as hypochromic, orthochromic or hyperchromic. Anaemia is a disorder in which the patient suffers from tissue hypoxia, the consequence of a low oxygen-carrying capacity of blood. Absolute anaemia results in decreased red cell mass. The pathophysiological classification of anaemia also includes effects of decreased red cell production, red cell destruction and acute blood loss. Causes of anaemia can be due to iron or other nutritional deficiencies, hereditary haemoglobinopathies such as sickle cell disease, alpha thalassaemias; enzymopathies such as Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency and infections such as malaria. These issues are the highlights of the present discourse. In as much as diseases affect people and not merely their blood forming organs, classification is not pushed to its logical limits. The more common causes of anaemia in this environment will be our subject of discussion today. (SLIDE)

SOME COMMON DISORDERS RESULTING IN ANAEMIA IN NIGERIANS

MEGALOBLASTOSIS -	Abnormal ineffective DNA erythropoiesis (Common in Anaemia in pregnancy).
SICKLE CELL DISEASE -	Mutation of β6Glu→Val i.e (GAG→GTG) (2-3% incidence in Nigeria).
ALPHA THALASSAEMIA-	Decrease of some or all of the 4 α- genes (Microcytosis & hypochromic anaemia).
(G6PD) Deficiency -	Red cell enzymopathy qualitative abnormality & quantitative deficiency neonatal jaundice chronic non-spherocytic haemolytic anaemia (CNSHA) haemolytic attacks on ingestion of certain drugs (oxidants)
MALARIA INFECTION -	Severe anaemia caused by Plasmodium species

Distribution of these anaemias predominantly occurs along the same equatorial malaria belt as shown in the next Slide below.

Malaria Distribution in the World

WORLD DISTIBUTION OF MALARIA, SICKLE CELL, ALPHA- THAL, G6PD,

Causes of Anaemia and their effects are quite varied as are outlined below:

Megaloblastic anaemia is characterized by many large immature and dysfunctional red blood cells (megaloblasts) in the bone marrow, and it is associated with pernicious **anaemia**. Megaloblastic Anaemia is caused by ineffective DNA erythropoiesis. It is quite common in pregnancy.

Causes of Megaloblastic Anaemia can be due to nutritional deficiency, malabsorption, increased Folate requirement and defective utilization of Folate.

The haemoglobinopathies represents abnormalities in the blood such as in sickle cell anaemia resulting from a change in the amino acid chain, and the thalassaemias which result from reduction or absence of synthesis of a particular portion of the haemoglobin.

Sickle cell anaemia occurs in three million people while sickle cell trait occurs in about 25-28% of our population of 120 million which means that a large number of children are born and perish with the disease (Fleming et al 1979). Sickle Cell Disease (SCD) results from the inheritance of two sickle cell genes (homozygous inheritance). It manifests as a chronic haemolytic anaemia with severe pain and wide spread organ damage with varying morbidity & mortality patterns. In this condition infection is increased, growth and development retarded and puberty delayed in some cases. The various types of crises among children and adults have been evaluated in Nigerian patients by Flemming in 1982.

The frequency of sickling appears to be directly related to that of malaria due to a higher resistance of the heterozygous AS individual to malaria than the normal haemoglobin AA individuals. The disease ranges from the hardly symptomatic patients discovered during health or other screening programmes to those who are severely ill and regularly admitted to hospitals for one or other complications (Steinberg & Hebbel 1983). (Falusi 1986). Although environmental and social factors do play some part in the severity pattern, the inheritance of genes is critical in the sickling of the red cells which result in these abnormalities.

Mr. Vice Chancellor, **the Thalassaemias** are blood abnormalities that result from the reduced or absent synthesis of the alpha or beta chains of haemoglobin ($\alpha 2\ \beta 2$). There are two α-globin chains each with 2α globin genes i.e. a total of 4α globin genes on each chromosome 16 ($\alpha\alpha/\alpha\alpha$) in humans. There are 2 major types of α thalassaemias, the α-thal-1 in the Mediterraneans and α-thal-2 in black populations. In Mediterraneans the α-thal-1 type has extremely severe effects in its homozygous form which can result in the loss of 3 or 4 of the alpha genes. The α-thal-2 in its heterozygous form results in a loss of 1 or 2 of the alpha genes. Misconception that there is no α thal in Nigerians stems from the fact that only the milder form i.e. the α-thal-2 has been recorded in blacks. The clinical effects of the α-thal-2 found in Nigerians are modest as it causes only mild anaemia (1-2g/dl lower than normals)(Weatherall & Clegg 1981).

The Enzymopathies are abnormalities in enzymes such as **Glucose – 6 Phosphate Dehydrogenase (G6PD) deficiency.** G6PD is an inherited condition. G6PD deficiency is the most common enzyme deficiency in the world, with about 400 million people living with it. It is most prevalent in peoples of African, Mediterranean, and Asian ancestry. The incidence in different populations varies from 0% in South American Indians to less than 0.1% among Northern Europeans and up to 14% in African American males. This enzyme deficiency has qualitative and quantitative deficiencies leading to neonatal jaundice, Chronic Non-Spherocytic Hemolytic Anaemia (CNSHA) and haemolytic attacks on ingestion of certain drugs which act as oxidants in the blood.

G6PD enzyme is present in all human cells but is particularly important to red blood cells. It is required to synthesise NADPH in red blood cells and glutathione. Glutathione and NADPH both help to protect red blood cells against oxidative damage. Thus, when G6PD is defective, oxidative damage to red blood cells readily occurs, and cells break open as a result. This event is called hemolysis, and multiple hemolyses in a short time span constitute an episode of hemolytic anaemia which varies in severity from life-long anaemia, to rare bouts of anaemia or to lack of symptoms. The episodes of hemolytic anemia are usually triggered by oxidants such as, primaquine and many of the quinoline antimalarials and infection such as malaria. The hemolytic anaemia of G6PD is self-limiting because; new synthesized red blood cells possess more functional G6PD enzymes than the older cells (Luzzatto & Mehta 1995).

G6PD deficiency is a recessive trait found on the X linked (sex linked) chromosome. Thus, males have only one copy of the G6PD gene, while females have two. Accordingly, females with one copy of the gene for G6PD deficiency usually appear normal, while males with one copy exhibit a greater effect of the deficiency. It has **remained relatively more difficult to assess the frequency of female heterozygotes for a long time in our population since previous research on G6PD had earlier employed the use of microscopy, electrophoresis and enzyme activity by**

spectrophotometry only. Viewing through the looking glass of genes in 1986 and 1999, Falusi and her associates were able to provide a clearer picture on **the precise genetic classification** of G6PD types both in males and females, **but in females in particular in Nigeria for the very first time** (Fey et. al. 1990, May et. al. 1999).

Mr. Vice Chancellor, **Malaria** parasites are transmitted from one person to another by the female anopheles mosquito. The malaria infection is caused by the plasmodium species and **results in severe anaemia**. Approximately 300 million people worldwide are affected by malaria and between 1-1.5 million people die from it every year (WHO 2003). Previously extremely widespread, malaria is now mainly confined to Africa, Asia and Latin America. The problems of controlling malaria in these countries are exacerbated by inadequate health structures and poor socioeconomic conditions. The situation has become even more complex over the last few years with the increase in resistance to the drugs normally used to combat the parasite that causes the disease. Four species of *Plasmodium* can produce the disease in its various forms worldwide. These are:

- *Plasmodium falciparum*
- *Plasmodium malaria*
- *Plasmodium ovale*
- *Plasmodium vivax*

P. falciparum is the most widespread and most dangerous of the four. Malaria infection if untreated can lead to fatal cerebral malaria.

Plasmodium feeding on a sleeping child

After 9-16 days, Plasmodium parasites return from the liver to the blood and penetrate the red cells, where they multiply again, progressively breaking down the red cells. This induces bouts of fever and anaemia in the infected individual.

Cold stage- 1hr, intense, shivering, temp 39.5 – 40°C

Hot stage- 3hrs; shivering stops; burning temp 40-41°C (106°F)

Sweating stage - 3 hrs fever disappears, profuse sweating; sensation of relief & tiredness.

In cerebral malaria, the infected red cells obstruct the blood vessels in the brain. Other vital organs can also be damaged often leading to unrousable coma, collapse and sometimes, the death of the patient.(see Fig below)

Collapse of a child

Malaria is generally diagnosed by the clinical symptoms and microscopic examination of the blood.

Epidemics of malaria now occur around traditionally endemic zones in areas where transmission had been eliminated. **These outbreaks are generally associated with deteriorating social and economic conditions, and the main victims are underprivileged rural populations.** Unfortunately, population

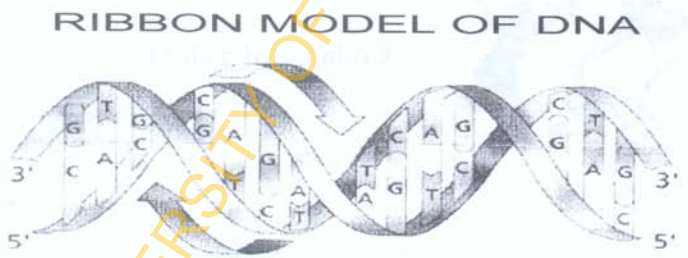
movements and intensive urbanization are not always accompanied by adequate development of sanitation and health care(WHO).

These disorders of anaemia namely: sickle cell anaemia, alpha thal, G6PD and malaria **have been examined through the looking glass of genes** as will be shown later in this lecture.

Mr. Vice Chancellor, all diseases have a genetic link, although, some more than others for the simple reason that all living things are alive and function normally because of genes. Even in the common disease as a viral infection, the different genetic constitution of humans determine whether they will be affected or immune and if affected whether they will die or not.

What then are Genes?

Genes can simply be defined as stretches of deoxyribonucleic acid (DNA) assigned specific biochemical places on the cell's chromosomes and performing specific functions of heredity. (See fig. below)



DNA is made up of two complimentary strands of nucleotides twisted about each other to form a double helix. There are 4 nucleotides namely adenine (A), thymine (T), guanine (G) and cytosine (C). A pairs with T and C pairs with G, the pair held together by hydrogen bonds while the sugar is in the deoxyribose form. In Ribonucleic Acid (RNA) another genetic material, the

nucleoitides are A, U, CG. The relevance of this distinction is seen later.

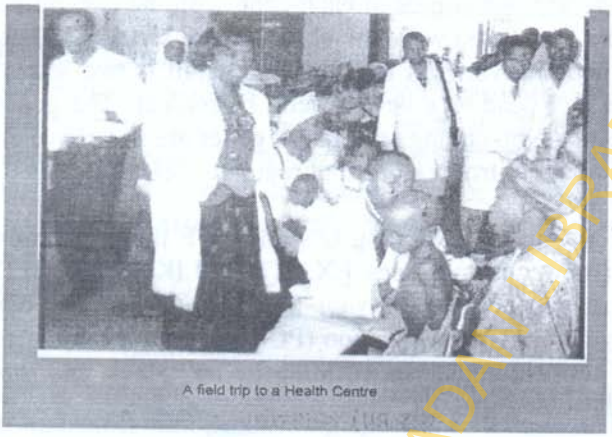
Genes are arranged on long DNA molecules called chromosomes. Animals and larger plants have two sets of chromosomes in each cell; so that there are two copies of each gene with the exceptions of the germ cells (sex cells) with one chromosome set each. Thus, when a sperm and an ovum get together, the result is two sets of chromosomes which lead to a new genetic individual. The genes have a coding and a Non coding region. All genes are not active all the time; genes have control regions that regulate their activities.

Genes are responsible for switching ON and OFF the production and copying of new chromosomes EXACTLY LIKE THE OLD ONES otherwise you could have your nose coming out of your ear!!!. The Polymerase Chain Reaction (PCR) technique simulates the process of new chromosomes building by the genes, and this model will be discussed shortly.

Mr. Vice Chancellor, the topic of today's discussion involves **megaloblastic anaemias, sickle cell anaemia, α thalassaemia, Glucose 6 Phosphate Dehydrogenase Deficiency and malaria**. In each of these conditions, the life span of the red cells is reduced. The red cells contain haemoglobin to transport oxygen to the tissues. The most economical shape of a container is a sphere; the most efficient shape for diffusion would be a flat disk, a couple of molecules thick. The normal blood cell is a compromise. Departures from this ideal shape are liable to be regarded by the body as abnormal with consequent premature destruction of the red cells. Such is the sickle cell situation where the cells are unable to manoeuvre through the small blood vessles which thereby become blocked and the cells are destroyed. Haemoglobin SS, G6PD deficient cells, α thal cells and malaria infested cells could have a life span as low as 50 days resulting in anaemia, while the normal red cells have an average life span of 120 days. Some of the quantitative & qualitative abnormalities of these conditions will be discussed further in this lecture.

METHODS

Mr. Vice Chancellor, to visualize anaemia through the looking glass of genes, I had to carry out several field studies in Ibadan and its rural environs (see PIC below).



A field trip to a Health Centre

To process the blood samples, two basic molecular biology techniques were applied in the study. They are: The Southern Blot technique and the Polymerase Chain Reaction (PCR) technique.

WHAT IS THE SOUTHERN BLOT ?

Southern Blotting Techniques

The Southern Blot is a technique first perfected by E.M. Southern in 1975. (Southern 1975). It was developed to detect and examine specific DNA among complex mixtures of contaminating molecules. It

has allowed the identification and characterization of genes involving numerous diseases. The technique can be summarized in the following steps:

- DNA preparation from nucleated cells of hair, blood, tissue, saliva etc.
- Lysis of nuclear pellet
- Extraction of DNA in phenol- chloroform-isoamyl alchol
- Estimation of DNA purity (DNA/Protein OD ratio)
- Restriction enzyme digestion
- Agarose gel electrophoresis
- Blotting, Baking, H ybridization with radioactive probes
- Autoradiography on X-ray films.(bands indicate desired genes)

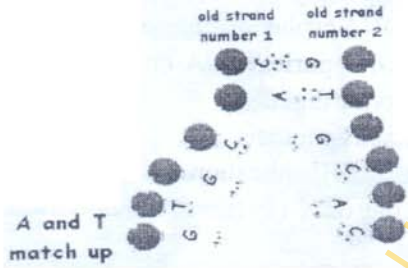
WHAT IS POLYMERASE CHAIN REACTION (PCR)?

The Scientist setting up a PCR for Malaria in Berlin Germany

Setting up the Polymerase Chain Reaction (PCR) is a simple process as shown above. PCR is an *in vitro* concept reminiscent of the way that cells duplicate their DNA to expand their numbers *in vivo*. The PCR which was invented by Kary Mullis in 1983 is today the greatest tool of Molecular Biology.

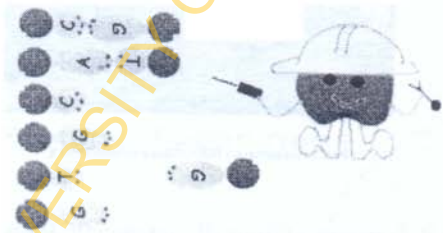
The Procedure is summarized in three basic steps:

First Step: ***Denaturation*** : The DNA containing the target genetic material must be denatured by heating to just between 90 96°C, when the strands unwind and separate.(SLIDE)

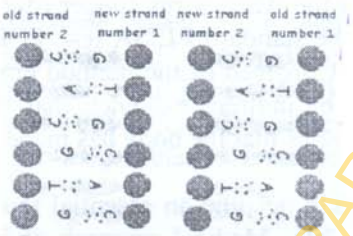


DNA replication team unwinds (un-zips) a small section of the old chromosome pulling the two strands apart. DNA has four nucleotides namely A C T G,n ormally held together by hydrogen bonds.

Second Step ***Hybridization or Annealing***



In hybridization (or annealing), the primers now bind to their complementary bases on the new single-stranded DNA. The nucleotide A lines opposite T while C lines opposite G. The free nucleotides float in and line up in their proper order all by themselves once the conditions are exactly right.



Starting from the primer, the polymerase reads the template strand and matches it specifically with complementary nucleotides. This results in new strands in place of the first, each composed of one of the original strands plus its newly assembled complementary strand. There is now twice as much double stranded DNA sample present in the tube as there was to start with. The cycle is repeated and the amount of sample DNA doubles further with every cycle. The doubling is in 2, 4, 8, 16, 32-fold etc. By repeating the process for just 45 minutes, millions of copies of a specific DNA strand are generated.

Usefulness of PCR

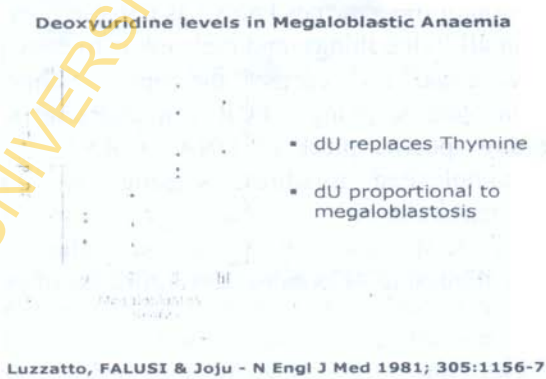
PCR is a delightfully simple concept which exploits the remarkable natural function of the enzymes known as polymerases. These enzymes are present in all living things, and their job is to copy genetic material (and also "proofread" and "correct" the copies). Sometimes referred to as "molecular photocopying", PCR can characterize, analyze, and synthesize any specific piece of DNA or RNA. It works even on extremely complicated mixtures, seeking out, identifying, and duplicating a particular bit of genetic material from blood, hair, buccal washings or tissue specimens, from microbes, animals, or plants, some of them many thousands or possibly even millions of years old.

The method is especially useful for searching out disease organisms that are difficult or impossible to culture, such as many kinds of bacteria, fungi, and viruses, because it can generate analyzable quantities of the organism's genetic material for identification. It can, for example, detect the AIDS virus sooner (during the first few weeks of infection) than the standard ELISA test. PCR looks directly for the virus's unique DNA, instead of the method employed by the standard test, which looks for indirect evidence that the virus is present by searching for antibodies that the body has made against it.

PCR has very quickly become an essential tool for improving human health and human life. Medical research and clinical medicine are **profiting from PCR in two main areas: in the detection of infectious disease organisms, and of variations and mutations in genes, especially human genes**. Because PCR can amplify unimaginably tiny amounts of DNA, even that from just one cell, physicians and researchers can examine a single sperm, or track down the elusive source of a puzzling infection such as malaria. These PCR-based analyses are proving to be just as reliable as previous methods sometimes more so and often much faster and cheaper.

Mr. Vice Chancellor, "What exactly did we find on applying the techniques just described"? (SLIDE MEGALOBLASIS)

MEGALOBLASTIC ANAEMIA – RESULTS



The morphological changes resulting from ineffective cell production is compatible with qualitative abnormality in the DNA of megaloblastic red cells. To identify the cause of the problem we embarked on a study in some anaemic pregnant women in UCH, Ibadan, in 1973. We found that Uridine normally present in RNA was present in the DNA (Luzzatto, Joju, Falusi 1973). It was also found that increasing amounts of Uridine in DNA correlated well with the morphological grading of megaloblastic anaemia.(Luzzatto, Falusi, Joju. 1981).

Mr. Vice Chancellor, at the time (1975 – 1981), the finding was unprecedented and was quickly grabbed for publication in the New England Journal of Medicine (Luzzatto et. al. 1981). This novel finding formed part of the bedrock data for internationally accepted biochemical basis of megaloblastic anaemias worldwide. As I said earlier, the fact of such data emanating from our own UCH here in Ibadan, provided the impetus for me to now search for other possible causes of anaemia through the looking glass of genes.

SICKLE CELL – RESULTS

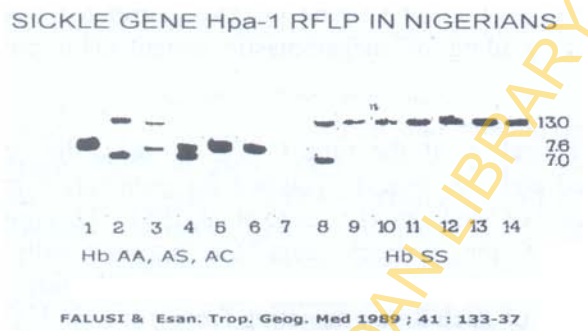
A search for Prenatal Diagnosis of Sickle Cell Disease.

Due to the problems encountered by sickle cell sufferers, the annual cost of treatment needed almost daily throughout life is enormous. Furthermore, the financial burden of sickle cell disease is only a pale reflection on the burden on family and society. These considerations explain part of the great interest among health care providers and families at risk in preventing or avoiding the disease.

The options available to us in Nigeria are:

- Education and Genetic counseling on awareness of sickle cell disease (a topic for another day).
- Phenotyping of haemoglobin to ascertain the individuals and families at risk.
- Prenatal diagnosis of the genes of sickle cell disease (as early as three weeks of gestation with modern technology)

Education, genetic counseling and Phenotyping are routinely done but prenatal diagnosis requires the more sophisticated molecular biology technique. This brings us to the search for a prenatal diagnosis of sickle cell diseases through the looking glass of genes with **Haplotype** analyses.



Mr. Vice Chancellor, Sickle cell disease is so far not yet curable. To reduce the toll of this disease on patients, an avenue for prenatal diagnosis of this disease. Kan & Dozy used the DNA βs-HpaI enzyme to evaluate prenatal diagnosis of Sickle Cell Disease (Kan & Dozy 1978). HpaI linkage is located 5kb to the 3' site of the βglobin gene. β^A-HpaI is expected to be linked to the 7.6kb or 7.0kb fragment, the β^s –HpaI to the 13.0kb. and (HbAS) to the 13.0/7.6 or 13.0/7.0kb fragment. The application of Hpa-I linkage analysis *in vitro* in Nigerian samples shows representative autoradiogram of the DNA from some of the subjects as shown above (Falusi & Esan 1989). (SLIDE MAP)

Distribution of HpaI -13kb polymorphism in Africa Falusi & Esan 1989

A further look at this gene in the four neighbouring West African countries showed **that HpaI enzyme did not have a complete (i.e. 100%) linkage to the 13.0kb fragment as expected thus detracting from the diagnostic value.**

Table Frequency of gene linkage 13.0kb OR 7.6/7.0kb Hpa-I fragment in different populations

Source	$\beta^{A7.6}$	$\beta^{A7.0}$	$\beta^{A13.0}$	$\beta^{S7.6}$	$\beta^{S7.0}$	$\beta^{S13.0}$	Reference
San Francisco	-	-	-	0.134	-	0.866	Kan and Dozy, 1978
San Francisco	0.879	0.087	0.034	0.306	0.015	0.679	Kan and Dozy, 1980
New York	-	-	-	0.420	-	0.580	Feldenzer *et al.*, 1979
Baltimore	0.912	-	0.088	0.425	-	0.575	Panny *et al.*, 1981
Gabon	1.00	-	-	**1.00**	-	0.00	Kan and Dozy, 1980
Kenya	0.786	0.071	0.143	**1.00**	-	0.00	Kan and Dozy, 1980
Togo	0.820	0.010	0.170	-	-	**1.00**	Mears *et al.*, 1981b
Algeria	0.95	0.05	-	-	-	**1.00**	Mears *et al.*, 1981a
Morocco	1.00	-	-	-	-	**1.00**	Mears *et al.*, 1981a
Ghana	0.957	0.00	0.043	0.029	-	0.971	**Esan and Falusi, 1986**
Cote D' Ivoire	0.647	0.294	0.959	0.457	-	0.543	**Esan and Falusi, 1986**
Sierra Leone	0.846	0.077	0.077	0.385	-	0.615	**Esan and Falusi, 1986**
Nigeria	0.774	0.168	0.053	0.016	-	**0.984**	**Falusi and Esan,1989**

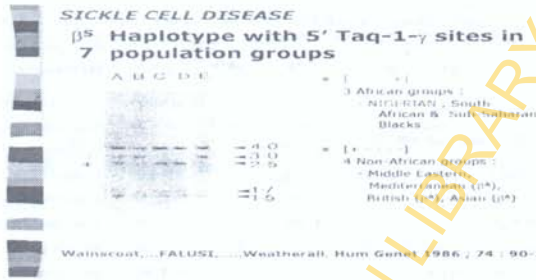
β^A, β^S linkages that could vitiate the use of Hpa-I restriction site polymorphism for prenatal diagnosis. Falusi and Esan, 1989

The Table shows 100% linkage of HpaI-13kb enzyme site for β^S in Algeria, Morocco, and Togo while Ghana, Cote D'ivore, Siera Leone, Nigeria show a 97.1, 54.3, 61.5 and 98.4% linkage respectively. Kenya and Gabon have a 100% HpaI- β^s-7.6kb linkage. All these variations within the African Continent!!!

The study shows a tight linkage (98.4%) of the β^s gene to the HpaI-13kb fragment in Nigerians. With only 1.6% of all the β^s genes not linked in this way, the method would at first glance appear to be very suitable for prenatal diagnosis. However, when the proportion (5.8%) of β^A linkage to the 13kb is considered, it is obvious that a considerable number of false positives will occur. To reduce this margin of error, both parents as well as their near relatives would need to have their Hpa-I linkage pattern determined. The increase in cost necessitated by family studies and the elongated time factor for accurate diagnosis of linkage polymorphism make the use of Hpa-I enzyme less suitable than previously imagined. Hence the use of Mst II, Dde I and Mnl I enzymes with 100% accuracy is recommended for prenatal diagnosis

in Nigeria. Our own genetic data on these enzyme polymorphisms were generated directly for the first time in Nigerians and not simply inferred from peoples of "African descent". (See fig below)

Genetic Basis of Poor Prognosis of Sickle Cell Disease.



Mr. Vice Chancellor, attempts made to examine the Genetic Basis of Poor Prognosis of Sickle Cell Disease in Nigerians cast the searchlight on the local environment surrounding the β^S gene itself. This requires the typing of β^S Haplotypes with different specific enzymes. The result of such a typing is as shown in the Fig above in which seven different populations were examined with Taq-1-γ enzyme. The African group clearly shows a difference from those of non Africans.

A similar study in 10 other different populations, on β^A haplotypes (not shown here) indicated that the four African populations have a different haplotypes when compared with those of other six non African populations (Wainscoat et al 1986).

In yet a separate study, the β^s haplotype profile from four different populations using a different set of enzymes (Hind II, Hind III, Ava II, HpaI, Bam HI) is as shown below. The Indian and Eastern Saudi Arabs show a distinctly different profile from those of Riyadh (Western Saudi Arabia) and Nigeria. This further explained the disparity in the prognosis of sickle cell disease in these populations (Kulozik et al. 1986).

Table - Sickle cell disease βS-globin gene haplotypes in different population groups)

Haplotypes	India Orissa (tribal)	Saudi Arabia Eastern Oases	Saudi Arabia Riyadh	Nigeria Ibadan
+ + – + + + –	22	45	-	-
+ + – + + + +	-	2	-	-
+ – – + + + –	1	-	-	-
+ – – + + + +	1	-	-	-
+ – – – – + +	1	2	-	-
+ – – – – + –	-	-	-	-
– + – + + + –	-	1	-	-
– + – + + – +	-	-	-	-
– + + – + + –	-	-	-	1
– – – – + + –	-	-	17	33
Total	25	50	22	34

Haplotype classified by the (+) absence(-) of an array of polymorphic restriction sites using H=Hind II, Hd= Hind III, A=Ava II, Hp=Hpal, B=Bam HI Enzymes.
(Kulozik..... Falusi.....Weatherall Am J. Hum. Genet. 1986)

Genetic distance analysis based on these nuclear DNA polymorphisms indicates a major division of human populations into an African and a Euro-Asian group hence, the differences observed in the poor prognosis of Sickle Cell Anaemia in Nigerians as compared with people in Eastern Saudi Arabia and India.

Foetal Haemoglobin levels

To further examine the basis for the severity of sickle cell diseases in Nigerians, foetal haemoglobin levels examined showed HbF levels of 0.9 – 16.7 % with a mean of 5.6% while in a comparative group in Eastern Saudi Arabia & India levels were found as high to 46% with a mean of 28%. In short, the proportion of HbF in Nigerians is far too low to reach the threshold of 20% required to adequately reduce the severity of sickle cell disease (**Falusi** & Esan 1989).

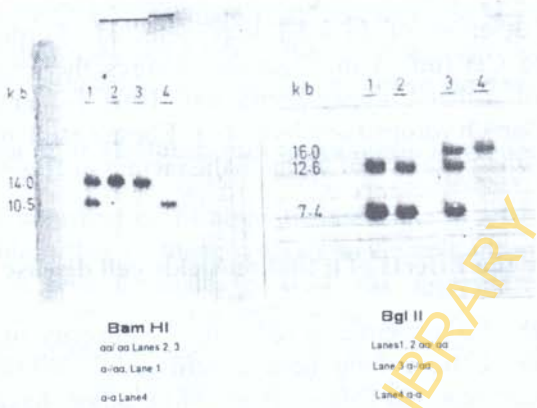
In summary, the series of studies on **DNA haplotypes polymorphisms indicate a major division of human** populations **in archeological evolutionary terms**. They also, for the very first time highlight the genetic basis of the severity of the poor prognosis of anaemia of sickle cell disease in Nigerians when compared with the much better prognosis observed in sickle cell individuals of Eastern Saudi Arabia, and India.

IS α-THAL PRESENT IN NIGERIANS?

Mr. Vice Chancellor, beneficial effects of Alpha-thalassaemia has been previously reported in some populations (Mears *et al.*, 1981), while others have reported a lack of effect (Felice *et al.* 1979). A study on individuals from different African countries living in the USA showed a prolonged survival of patients with Alpha-thalassaemia albeit with a mixed African population and very few numbers of subjects studied. (Mears *et al.*, 1983). Prior to this time, however, the literature showed that a complete absence of Alpha-thalassaemia (α-thal) was postulated in Nigerians and in Tanzania (Nhonoli *et al.*, 1979) This was due to the use of a less sensitive method of Hb Barts only (Esan, 1972). I thank my predecessor Prof. G.J.F Esan who made all attempts to redress the situation regarding the existing literature on α-thal in Nigerians by encouraging me to go out in search of a "looking glass of genes" to correct this false reference in the world literature (i.e. that no Alpha thal exists in Nigerians).

Mr. Vice Chancellor, It is therefore obvious to ask the question "Is there alpha thalassaemias in Nigerians"? An attempt to clarify the issue with respect to Nigerians led to the series of experiments which I was able to conduct in the foremost α-thal-laboratory in the world (MRC laboratory at the University of Oxford under the able leadership of Professor D.J. Weatherall). It is noteworthy that Professor D.J. Weatherall has received the Queen of England's Award for being the first authority world wide on α thal in which the more modern techniques of DNA analysis are used.

The analysis of alpha- thalassaemia is as shown with two specific enzymes Bam HI and Bgl II. (SLIDE).



Autoradiographs of alpha globin gene using Bam HI & Bgl II

Falusi 1986

Our Results on the profile of α THAL IN NIGERIANS is as shown in the next table

PROFILE OF α THAL IN NIGERIANS.

- Novel finding of α thal in Nigerians
- A thal -2 deletion type only
- Rightward deletion (α-3.7/)
- Leftward deletion (α-4.2)absent
- Prevalence : 32.5% heterozygous (α-/ α α)
  6.7% homozygous (α-/ α-)
- Frequency of 0.24 in SS and Normals (AA, AS, AC)

Falusi..... Higgs..... Weatherall 1987

Mr. Vice Chancellor, our looking glass actually found α thal in abundance albeit and thanks to natures compensation of a milder α-thal-2 type found in blacks as distinct from the more severe α-thal-1 found in the Mediterraneans. Our results showed that α-thal is not only present in Nigerians but that its frequency is as high as 0.24 with a prevalence of 32.5% in its heterozygous (α-/ α α) and 6.7 % in its homozygous (α-/ α-) form. Only the thal-2 type, with a "rightward"

deletion due to a loss of 3.7kb of DNA fragment (α-3.7/), was found. No leftward deletion of (α-4.2/) was detected. Furthermore, the absence of the Cis (αα/--) in Nigerians ensures the absence of the severe form of α-thal-1 thalassaemia trait HbH (--/-α) and that of Hemoglobin Barts hydrops foetalis (--/--). These result represent the true picture of the presence of alpha thalassaemia in Nigerians (Falusi et al. 1987).

What then are the Effects of α thal on sickle cell disease?

Effects of Alpha thalassaemia in reducing the severity of anaemia in sickle cell disease have long been controversial. While beneficial effects have been reported (Mears et al 1981), others have reported a lack of effect (Felice et al 1979). The interaction of α thal was demonstrated by examining the effect on the prognosis of sickle cell disease. The effect of α thal on the over 3 milion sickle cell patients in Nigeria is worth examining (SLIDE- Effect of α thal)

Table: :EFFECT OF α–THAL on SS Red Cells

- MCV, MCH MCHC ↓
- Polymerization, ISC ↓
- Intracellular Sickling ↓
- Retics Counts ↓
- Total Bilirubin ↓
- Hb ↑
- Deformability ↑

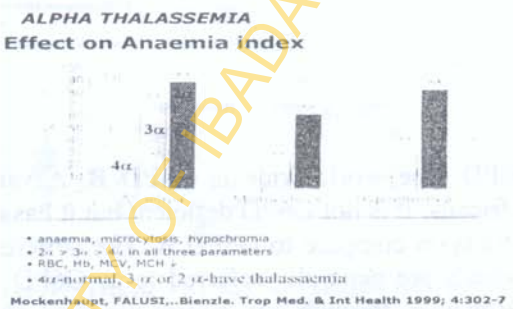
Higgs et al 1982, Falusi et al 1987

Mr. Vice Chancellor after our looking through the glass of genes we found alpha thalassaemia in high proportions. We also assessed the effects of alpha gene deletions on the red cell parameters, sickle cell prognosis and anaemia in children in general. One notable effect on the haematological parameters is as shown above (Higgs et al 1982, Falusi et al 1987). The decreased MCHC inhibits the red cell polymerization, total haemoglobin is increased but total bilirubin level is decreased due to a milder cell breakdown, with all of these leading to a greater red

cell survival which is an advantage to sickle cell patients. In a complementary study by Falusi and Olatunji in 1994, a significant reduction in the blood transfusion rate was found in Sickle cell patients with coinheritance of alpha thal. This is yet another benefit of α thal coinheritance with sickle cell anaemia (Falusi & Olatunji 1994).

Contribution of α Thalassaemia to Anaemia in General

The level of contribution of alpha thalassaemia to anaemia in normal African children that is, those without sickle cell anaemia is not well recognized. A gene frequency of 0.24 was observed respectively in 1987, 1999 & 2000, using the PCR in the later years by the different studies of Falusi and her team. In 1999, it was again showed that 36.5 % were heterozygous (α-/αα) while 9% were homozygous (α-/α-) in normal HbAA children corroborating her previous findings (Mockenhaupt 1999). (SLIDE)

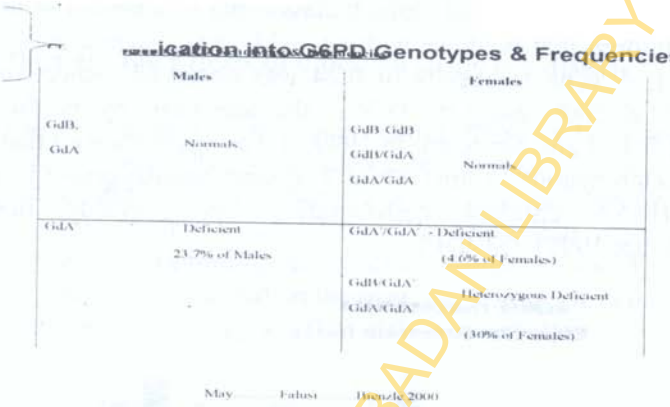


Alpha-thalassaemia was shown to contribute to anaemia, microcytosis and hypochromia in Nigerian children more than in those without alpha thalassaemia as shown above. With alpha thalassaemia, mean haemoglobin levels were 10% lower in homozygotes (α-/ α-) and 3.7% lower in heterozygotes (-α/ αα) than in those with normal (αα/αα) genotype (Mockenhaupt et al 1999)

Anaemia, classified as a lowering of haemoglobin levels, was seen in **54.7% of the** children with **normal (αα/αα)** globin genes but in **69.9% of heterozygotes with three α globin genes (α-/αα)** and in **88.4% of homozygotes with 2α globin genes (α-/α-) only.**

G6PD – RESULTS

G6PD deficiency of red blood cells is a health problem in Nigeria and other developing countries, causing neonatal jaundice, chronic haemolytic anaemia and haemolytic attacks after the ingestion of certain oxidants (Luzzatto & Mehta 1995).



Normal G6PD type world wide is **G6PD B**. A variant **G6PD A** is found in Africans. It is not G6PD deficient but it has a slightly reduced enzyme activity in compare to G6PD B. The deficient form is **G6PD A-** . Individuals are generally referred to as G6PD deficient or non-deficient. However, through our studies we were able to classify the non-deficient and deficient individuals according to their genotypes using PCR techniques. Nine genotypes of G6PD were identified as **Gd^B, Gd^A, Gd^B/Gd^B, Gd^B/Gd^A and Gd^A/Gd^A** normal genotypes in males & females. **Gd^{A-}, Gd^{A-}/Gd^{A-}** were homozygous deficient males and females while **Gd^B/Gd^{A-} and Gd^A/Gd^{A-}** were heterozygous females only. These genotypes were then correlated with enzyme activities.

Characterization of G6PD deficiency gene analysis shows that 30% of the Females were heterozygous deficient i.e. (GdB/GdA-) and

(GdA/GdA-) while 4.6% were homozygous deficient with (GdA-/GdA-). A total of 23.7% of males were deficient i.e. (GdA-). This is the first frequency determination of female G6PD heterozygotes in Nigerians (May et al 2000) The characterization of heterozygous G6PD deficiency in females is of great importance, as deficiency leads to a lower RBC, Hb, increased reticulocyte counts and chronic sub-clinical haemolysis, leading to anaemia. This has great implications for new born male infants care in this population as males must inherit one of their mothers X- chromosomes due to the X- linked inheritance pattern of the G6PD enzyme. Once the male inherits the deficient G6PD gene A- from his mother, then there is a need to carefully watch him for haemolytic anaemia.(SLIDE of G6PD activity)

The relationship of G6PD genotype with enzyme activity levels were compared and enzyme activity directly correlated with anaemia. The genotyping clearly explained some genetic basis of neonatal jaundice, haemolytic attack even when the subject is phenotypically normal.

G6PD DEFICIENCY

Table : Frequency of Pvu II G6PD RFLP in 8 different populations

Population (unrelated subjects)	No. of X chromosomes tested	f(+) (type 2)	f(-) (type 1)
Zambians	40	0.38	0.62
Kenyans	20	0.40	0.60
Nigerians	57	0.32	0.68
West Indians	22	0.36	0.64
British	74	0.00	1.00
Saudis	20	0.00	1.00
Icelanders	10	0.00	1.00
Filipinos	20	0.00	1.00

•Nigerians – PVU II site type 2 (f+) PRESENT
- Zambians, Kenyans, West Indians similar

•Saudis – PVU II site type 2 (f+) ABSENT
- British, Icelanders, Filipinos similar

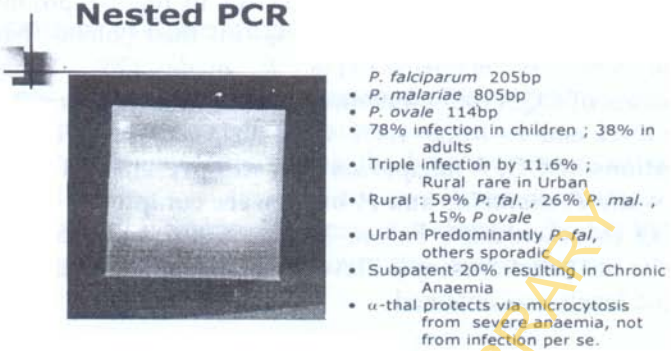
Fey,...FALUSI,...Luzzatto. Human Genetics 1990 ; 84 : 471-2

Our attention was again turned to examining some other specific enzyme (Pvu II) in eight different populations as shown in the fig above. It is interesting to note that all people of Africa and descendants of Africa from Zambia, Kenya, West Indies and Nigeria had a different polymorphism from the other four populations of Western Saudi Arabia, Britain, Iceland and the Philippines. This polymorphic site now serves as an African G6PD specific marker.

RESULTS ON MALARIA STUDY

Incidence of Plasmodium Species in Rural / Urban Populations

Generally malaria infection assessment is made by microscopy,a ntibody titres as well as clinical examinations. Our research has used the molecular biology techniques to unravel some genetic basis of malaria prognosis in this environment with species-specific PCR techniques. This allows detection of Subpatent parasitaemia much below the threshold of conventional microscopy. (Snounou et al. 1993). Our results showed that: (See Fig below)



May..... Falusi..... Meyer 1999

- A higher rate of 82% Plasmodium infection was observed in rural compared to the 77% in urban populations.
- A high rate of 19.6% Subpatent *Plasmodium falciparum* parasitaemia was found in the cohort.
- Mixed infection is found to be more common in rural areas than in urban areas
- Triple Plasmodium infection of 11.6% was found in the rural populations.
- 59% *P. falciparum* , 26% *P. malariae* , 15% *P. ovale* species were found in Rural but rare in Urban population.
- Double infection of *P. malariae* 26.1% and 14.8% *P. ovale* were observed in rural areas.
- Spleen enlargements correlated with parasite density.

Subpatent (PCR positive but microscopically negative) infection as high as 20% has important health consequences as it results in underlying chronic anaemia with drug resistant complications.

What is the effect of Treatment on *Plasmodium* Species?

Chloroquine (CQ) is one of the most widely consumed antimalarial drugs worldwide, and is certainly so in Nigeria. (Foster et al. 1994).

When the parasite densities of *Plasmodium* species, were determined at consumption of Choroquine (CQ) equivalent to regular prophylaxis levels, children without detectable CQ in their blood had, *P. falciparum* 87%, *P. malariae* 31%, *P. ovale* 17%. With low concentrations of CQ, (100 – 499 nmoles/litre) *P. falciparum* 74%, *P. malariae* 1.9% and *P. ovale* 1.4% were observed. **At prophylactic concentrations of CQ, *P falciparum* was still present in 13% of the children while *P. malariae* and *P. ovale* were completely destroyed by the CQ** (Mockenhaupt, Falusi, Meyer 1999). A spread of 20% parasitaemia at "submicroscopic" level and 48% at low and moderate microscopic levels was obtained.

The clearance was effective with high doses of CQ for *P. malariae* and *P. ovale* while *P. falciparum* remained resistant even at the prophylactic levels.

This very important finding further explains the reasons why *P. falciparum* is extremely deadly especially here in Nigeria where almost most people are their own doctors, prescribing regularly without any blood test, and using Suboptimal levels of CQ, which further aggravate the situation. Self medication, inadequate dosing and subtherapeutic levels of CQ in blood partly explain the CQ resistance in Plasmodium falciparum infection. (Mockenhaupt, Falusi & Bienzle et al 2000).The mode of use of chloroquine among Nigerians leads to high parasite resistance (Falade et al. 1997).

CONCLUDING REMARKS

Contributions of Research to Health and Disease

Sickle cell disease, the thalassaemias, G6PD deficiency and malaria occurring in the same geographical zone of Africa have been shown to have an interrelationship in which the high prevalence of sickle cell gene and thalassaemia are said to have been maintained by high malaria prevalence. Sickle cell anaemia is clearly a disease begging for gene therapy since Nigeria harbours the largest number of affected people worldwide.

Through our studies, Falusi and her collaborators have provided some remarkable insights first into the biochemical basis of **accumulation of abnormal deoxyuridine in megaloblastic anaemia** (Luzzatto, Falusi, Joju 1973; 1981).

Our studies of the β^s haplotype profile clearly explain the differential manifestation of the poor prognosis of sickle cell disease in Nigeria. The findings have also shown the effect of showing the effect of modifying factors of alpha thalassaemia, Glucose-6-Phosphate Dehydrogenase deficiency and malaria. The findings were similar to those in respect of the Western Saudi Arabia, Jamaica and Black Americans but clearly shown to be dissimilar to a much milder form of sickle cell disease in Eastern Saudi Arabia, East and West Coasts of India.

To further identify the underlying cause of genetic diversity in the Euro-Asian-African populations, other specific enzymes were used. **The four African countries were shown to have a common enzyme profile which was lacking in the Euro-Asian populations, making the Taq-1-γ a specific African genetic marker.**

An attempt at a search for prenatal diagnosis of sickle cell disease showed the Hpa-I-linkage for the first time in Nigerians (Falusi and Esan; 1989). However, its use for prenatal diagnosis was unviable owing to its incomplete linkage to the relevant fragment. Other enzymes were then recommended for **prenatal diagnosis of Sickle cell**

disease in Nigeria. This sets of experiments paved the way for prenatal diagnosis here in Nigeria.

In addition, Falusi and her collaborators presented the first unequivocal evidence on the frequency of alpha thalassaemia in Nigeria (Falusi et al. 1987). The findings showed similar proportion in both sickle cell anaemia and normal non homozygous individuals, **for the first time in Nigeria.** The exact frequencies of alpha thalassaemia were shown and confirmed again in her other separate studies in 1989 and 2000. The determinant for alpha thalassaemia in Nigerians was identified.

Our study on the level of foetal haemoglobins provided again for the first time, data on the levels of foetal haemoglobin in Nigerians. It also explained the relationships between low levels of HbF and a #19-Benin-haplotype profile which predict the more severe form of sickle cell disease among Nigerians.

Molecular genetics of heterozygous deficiency in female individuals was classified into its appropriate G6PD genotypes. This demonstrated the importance of the classification as it relates to health care of linked inheritance of neonatal jaundice or chronic anaemia.

The molecular genetics of malaria also documented the frequency of the three plasmodium species *P. falciparium*, *P. malariae* and *P. ovale*. **While about 80% of healthy Nigerian children carry the parasite, only 58% of them are routinely diagnosed microscopically, with 20% remaining as submicroscopic infection**. The prevalence of mixed infection in rural areas compared to the urban was documented. The sub-optimal dosage and sub-therapeutic levels of chloroquine were found to be partly responsible for drug resistance, with important implications for the "Role Back Malaria" Programme in Nigeria.

In summary, our laboratory has generated qualitative and quantitative data on molecular genetics of sickle cell disease, alpha thalassaemia, glucose-6-phosphate Dehydrogenase deficiency and malaria in Nigeria, thus solidifying its reputation as a foremost laboratory in the Molecular Genetics of these sources of anaemia in Nigeria.

It was, Mr. Vice chancellor Sir, for the above significant findings among others that the "L'OREAL/UNESCO Award for Outstanding Woman in Science" was given to today's lecturer.

Adeyeye Gladys Funsho, Laureate 2001, receiving her Award from Koichiro Matsuura UNESCO Director General, and Lindsay Owen-Jones, L'OREAL President and CEO

Ethical Issues in Genetic Research

In all of this however, the question must remain "does the individual have the right to know, or the right to refuse to be told, about genetic abnormalities which may cause disease now or in decades later"? May the geneticist tell a young woman she should not marry a certain young man whose genomic map shows a defective gene which may shorten his life or would affect their children's lives seriously as may be the case in sickle cell disease?

Will the individual have the right to refuse to have the result of his or her study sample revealed? Will it be unethical to obtain an individual's genome map without his or her informed consent? Will parents have the right to refuse to have their children's genes mapped?

Many views on these aspects of human rights and dignity and human values must be harmonized by the researcher, the geneticist, the medical profession, ethicists, theologians, policymakers and society at large. (SLIDE)

Fundamental Principles of Human Research Ethics

- Respect for Persons

- Beneficence
- Justice

In doing this, certain basic principles must be observed as shown above: **Autonomy i.e. respect for the individual and his self determination Beneficence i.e. do good and not harm and ensure Justice in the equitable distribution of the benefits of research.** There is a need for rapid generation and dissemination of knowledge, while always protecting the rights and interests of the human patients or participants.

In 2002, when I assumed the position of Director of IMRAT, the greatest challenge for me as the Chairperson of the UI/UCH Ethical Review Board was to reorganize and overhaul the existing structure of Human Subjects Research in the University of Ibadan. I was to carefully guide the conduct of human subject research in this great University along proper ethical lines. The Ethical Review Board is now restructured and reconstituted. The new Board is made up of Faculty and non-Faculty members. To carry out these assignments, several international codes and laws (Nuremberg , Belmont, Helsinki, and CIOMS) guide the committees activities.

In the past two years, the UI/UCH IRB of which I have been fortunate to CHAIR has taken up the task of reviewing and approving all protocols on Human Subjects to ensure a balance of the benefits to the risks ratio to subjects, equitable selection of subjects and ensure documentation of informed consent. Our Federal Wide Assurance (FWA00003094) obtained in 2003 serves

as a pledge that all research activities involving human subjects are guided by the ethical principles set forth in the Belmont report regardless of funding.

To this end, the UI/UCH IRB with its new Secretariat at the IMRAT is functioning effectively, reviewing and approving several national and internationally funded projects. The committee has ensured the conduct of its monthly review & approval meetings. It has organized 3 major workshops in the last 7 months. These include " The Conflict of Interest in Biomedical Research in January 2004, the International Workshop on " New Trends in the Management of Breast Cancer & Cervical Cancers in collaboration with the University of Chicago in May 2004. In June 2004 the National Workshop on "Bioethics in Medical Research" was conducted and Nigerian Bioethics Initiative (NIBIN) was inaugurated.

Mr. Vice Chancellor, my conclusion is that our research has endeavoured to unravel the genetic basis of some anaemias in Nigerians. We are teaching ethics of good clinical practice in Human Subject research to younger scientists and researchers to pave the way to a better understanding and management of these diseases in Nigeria.

RECOMMENDATIONS

To "Colleagues"

President John F. Kennedy, the 35th President of the United States of America once said "**Do not ask what your country can do for you – ask what you can do for your country**" I have often wondered how a Professor can work in a laboratory for 20 – 30 years with the same old centrifuge with which he was taught?.

Do we have to hold our arms out and wait for the biblical manner to fall from heaven? No! we have gone past the days of Moses!!!. Let us search out far and wide to equip our laboratories nationwide. Today, I must say that College or Medicine, University of Ibadan is taking giant strides in this area. There, you see something close to the state of the art set up; it is due to Grant sourcing. The facilities put in place there cannot be taken to your homes as researchers. The best you can do is to encourage and mentor your younger colleagues to take over from you. There is a song that goes thus: "If I can help somebody then my living is not in vain". Likewise, "if I can hand over a good laboratory and train somebody then my living is not in vain".

I particularly thank God in this regard, that when in a few years from now, I have to sit back, my baton would have been safely handed over to the likes of Dr. O. G. Ademowo of IMRAT who is currently "looking through the glass of genes" in the genetics of malaria.

Dear Colleagues, we all must learn to reach out to the private sector with our laudable results. "Town and Gown" must go together not the "ivory tower" aloof from the private sector. Paradoxically, here in Nigeria, wealthy people have been seen to build retreat centres, donate one million Naira to the commissioning of a Hair Dressing Salon!!! Why can we not find the language of approach to make people set up Foundations like Christopher Kolade Foundation that has taken the lead to benefit humanity by funding research on sickle cell disease?

To the Government of Nigeria

The Government of Nigeria, if they ever listen to academics and implement findings which they often tag "esoteric" research, Nigeria would be a better place. These so called "esoteric research" are the likes of research that have taken us to fly with our heavy luggage in aeroplanes; to sophisticated computer networking, biochemical and genetic research for improving the health of nations.

To those in the corridor of power, my specific appeal is that for the millions of sufferers and carriers of Sickle Cell disease, People Living With HIV/AIDS (PLWA), Cancer patients, it is high time this Government funded a well equipped National Prenatal diagnostic laboratory.

Although our colleagues are geared to sourcing funds outside, our nation deserves to have stable power source, water supply which do not fail thereby burning up the equipment, researchers have worked so hard to source. Please note that funding agencies find it difficult to sponsor studies when there is no equipment maintenance culture.

If the basic essential facilities of water and power are available, Scientists and Researchers will not have to go cap in hand begging for minor to major essentials such as distilled or deionised water to carry out experiments. Nigeria can actually come very close to the forefront of technology and research if the conditions are favourable. Nigeria has a wealth of manpower but the lack of basic infrastructure has been the main reason behind the "brain drain". Good clinical practice in Research, if taken seriously by Government, should take Nigeria to greater heights.

ACKNOWLEDGEMENTS

Today marks an unforgettable landmark in my life. I must not forget to mention with gratitude those who assisted me through the journey with the Lords enabling grace.

First, my Best Teacher - Late Mr. R.L. Opedare my primary school headmaster at St. Paul's Anglican Primary School, Efon Alaye. He taught me to speak English and no vernacular in school always, insisted on no ink stains in my uniform, no crossing of the school lawn- even all these in the 50's.

My Mentor - Professor L. Luzzatto the Scientific Director, Science institute Tumori: Italy. My boss, who shared my dreams, organized my thoughts, piloted my steps in analytical haematology in the College of Medicine from 1975 – 81 and thereafter in Hammersmith Hospital London 1983 1984, at Sloan Kettering Memorial Cancer Center in New York City, USA 1994-1995. You even played a unique role when I was given the L'OREAL UNESCO Award in 2001 in Paris.

I also recognize and appreciate the contributions of Professor G.F. Esan, my thesis supervisor and Professor G.B. Ogunmola, co supervisor of my thesis and one of my earliest research collaborators, to my academic development and maturity as a scientist.

I say a big "thank you" to Professor Oladuni Olaniyan-Taylor for her encouragement and unwavering support over the years.

The professional support of Professor Olufunmilayo Olopade, the Director, Centre for Clinical Cancer Genetics, University of Chicago (USA) and that of Professor Folasade Akinkugbe is acknowledged.

I sincerely appreciate the editorial assistance of Professor O. Ajayi a renowned surgeon, Professor A. Adesanoye, Dr Edith Ajaiyeoba and Dr. C.P. Babalola in the preparation of this lecture.

Awarding Organizations - To **L'OREAL/UNESCO Organisation**, for spotting the value in my little contributions to science and humanity in the field of Human Genetics and giving me a lifetime honour of

Outstanding Woman of Science (2001). The CEDPA/WODEF Organizations for the **Rare Gems Award** in the category of Science and Technology for the International Women's day on 8th March 2003 and the Rotary Club of Iyaganku for giving me the **Excellence Award for the year 2003.**

To His Royal Majesty Dr. Aladejare the Alaaye of Efonland and the Oba-in-council. I say a big thank you for including me in the Efon Alaaye Roll of Honour.

My Scientific collaborators Professors L. Luzzatto, GJF Esan, D.J. Weatherall, D.R. Higgs, U. Bienzle, G.B. Ogunmola, J.S. Wainscoat, CG Meyer and Drs F.P. Mockenhaupt, J. May, O.G. Ademowo, O.G. Arinola, P.E. Olumese, CP Babalola, Edith Ajaiyeoba, A.Oni, A. Ajuwon for sharing my scientific dreams and making me realize them.

IMRAT S taff : Academic, Administrative, Technical and support staff of IMRAT, I appreciate your cooperation especially in the past two and half years as Director of the Institute. I am particularly grateful to the staff of IRC represented by Mr. Dayo Adepoju for the preparation and computer output of this lecture.

My Parents - Late Chief I.S. Ologbenla and Madam M.O. Ologbenla, I thank you for my moral upbringing, teaching me to be bold and courageous with confidence in myself, to be the best that I always can be. Thank you for your sacrifice from your meagre income in those tough days to ensure that I attended the best high school Queens School Ede and the best University in Nigeria – the University of Ibadan. To Papa Rev. Cannon J.B. Falusi of blessed memory and Mama Mrs D.I. Falusi for your constant encouragement for me to be 'your child' and not just your daughter in law.

Amb. & Mrs Adeyemi, Chief & Chief (Mrs.) Cole, I thank you for making your homes comfortable for my children.

My Children Toyin and Gbenga Adeyemi, Titi and Wale Cole, Anuoluwapo, Ebunoluwa and Ibukunolu Falusi .**My Grand Children** – Tosin, Funmi, Ayodele & Femi – you are welcome to a world of hardwork and dedication to duty. I cannot gloss over mentioning your

names for your forbearance when mum took a large percentage of her time for research.

My Husband Professor Abiodun Falusi a great man of virtue and value for being my confidant, constant source of support and encouragement. You are always there for me and eternally grateful

"What shall we say unto the Lord, all we have to say is thank you Lord. Thank you Lord, thank you Lord, all we have to say is Thank you Lord." Finally to God Almighty, the Creator who is all knowing, who has made all things well and has made this day a reality, I bow in humility and I say a VERY BIG THANK YOU LORD for unto you belong all Glory and Honour and Majesty.

My. Vice Chancellor, Deputy Vice Chancellors, Registrar, Deans, Directors, Professors from UI and other Sister Universities, Colleagues, Ladies & Gentlemen. I thank you all for your attention.

References

1. Esan, **(1972)** Haemoglobin Barts in newborn Nigerians. Brit. J. Haematol. 19: 47-55

2. Falade C.O., L.A. Salako, A Sowunmi, A.M. Oduola, P. Larcier **(1997)**. Comparative efficacy of halofantrine, chloroquine and sulphadoxine-pyrimethamine for treatement of acute uncomplicated falciparum malaria in Nigerian children. Trans. R. Soc. Trop. Med Hyg. 91:58-62.

3. **Falusi A.G. (1986)** Alpha thalassaemia in Nigerians (Ph.D.) Thesis)

4. **Falusi A.G.,** Esan G.J.F., Ayyub H. and Higgs D.R. **(1987)** Alpha Thalassaemia in Nigerians: Its interaction with sickle cell disease. Euro. J. Haematol. 38: 370-375.

5. **Falusi A.G.,** Esan G.J.F. **(1989)**. Hpa 1 Polymorphism and the sickle gene in Nigerians. Trop. Geogr. Med. 41: 133-137.

6. **Falusi A.G.**, Esan G.J.F. **(1989)**. Foetal haemoglobin levels in sickle cell anaemia in. Nigerians. Afr. J. Med. Med. Sci. 18: 145-149.

7. **Falusi A.G.** and Kulozik E.A. **(1990)**: Relationship of foetal haemoglobin levels and β-haplotypes in homozygous sickle cell disease. Eur. J. Haematol. 45: 1- 4.

8. **Falusi** A.G & Esan GJF **(1989)** Foetal haemoglobin levels in sickle cell anaemia in Nigerians. Afr J. Med. Sci. 145-149.

9. Fey, M.F. Wainscoat, J.S. Mukwala E.C., **Falusi A. G.**, Vulliamy T.J. and Luzzatto L. **(1990)** A PVU II Restriction fragment length polymorphism of the glucose -6- phosphate dehydrogenase gene is an African-specific marker. Human Genetics 84: 471-472.

10. **Foster (1994)** Economic prospects for a new antimalarial drug. Trans. R. Soc. Trop. Med. Hyg. 88(Suppl) 55-56.

11. Higgs D.R., Aldridge, B.E., Lamb, J., Clegg, J.B., Weatherall, D.J., Hayes, R.J., Grandison, Y., Serjeant. G.R.: **(1982)**. The interaction of alpha-thalassaemia and homozygous sickle cell disease. New England Journal of Medicine 306: 1441 - 1446

12. Kan Y.W. and Dozy A.M. **(1978)**. Antenatal diagnosis of sickle cell anaemia by DNA analysis of amniotic fluid cells. Lancet ii: 910-912

13. Kan Y.W. and Dozy A.M. **(1980)**. Evolution of the haemoglobin S and C genes in world populations. Science (Washington D.C) 209: 388-391

14. Kulozik A.E., Wainscoat J.S., Serjeant G.R., Al-Awamy B., Esan G.F.J., **Falusi A.G.**, Haque S.K., Hilali A.M. Kate S., Ranasinghe W.A.E.P., and Weatherall D.J. **(1986)**. Geographical survey of βs - globin gene Haplotypes: Evidence for an independent Asian Origin of the Sickle cell mutation. Am. J. Hum. Genet. 39: 239-244.

15. Luzzatto L, E.A. Joju and **A. Falusi (1974)**. Synthesis of Abnormal Nucleic acids in megaloblastic cells: Quantitation of Deoxyuridine in DNA. Nig. Med. J. 4: 133-4

16. Luzzatto L., **A.G. Falusi**, E.A. Joju . **(1981)**. Uracil in DNA in megaloblastic anaemia. New Eng. J. Med. 305: 1156 - 57 .

17. Luzzatto, Lucio and Atul Mehta **(1995).** "Glucose 6-Phosphate Dehydrogenase Deficiency." In *The Metabolic and Molecular Bases of Inherited Disease,* 7th ed. Edited by Scriver, Charles R., Arthur L. Beaudet, William S. Sly, and David Valle. New York: McGraw-Hill,

18. May J., Mockenhaupt F. P., Ademowo O. G., **Falusi A. G.,** Olumese P. E., Bienzle U., Meyer C. G. (**1999**) High Rate of Mixed and Subpatent Malarial Infections in South West. Nigeria. Am. J. Trop. Med. & Hyg. 61 (2) 339 - 343 .

19. May J., Meyer C. G., Ademowo O. G., Mockenhaupt F. P., Olumese P. E., **Falusi A. G.**, Bienzle U., **(2000).** Red Cell glucose-6-phosphate dehydrogenase status and Pyruvate Kinase activity in a Nigerian population. Trop. Med. Int. Health. 5: (2) 119-123.

20. Mears J.G., Beldjord C., Benadadji M., Belghiti Y.A., Baddou M.A., Labie D. and Nagel R.L.: (**1981**). Sickle Gene: its origin and diffusion from W. Afr. J. Clin. Invest. 68: 606-610.

21. Mears J.G, Lachman H.M., Labie D. and Nagel R.L.: (**1983**). Alpha Thalassaemia is Related to Prolonged survival in sickle cell anaemia. Blood 62: 286-290.

22. Mockenhaupt F. P., **Falusi A. G.,** May J., Ademowo O. G., Olumese P. E., Meyer C. G., Bienzle U. (**1999**) The contribution of alpha $^{+}$-thalassaemia to anaemia in a Nigerian population exposed to Intense malaria transmission. Trop. Med. Int. Health. 4, (4):302.-307.

23. Mockenhaupt F.P, Bienzle U, May J., **Falusi A. G,** Ademowo O. G, Olumese P.E. Meyer C. G. (**1999**). *Plasmodium falciparum* infection: influence on hemoglobin levels in alpha-thalassemia and microcytosis. J Infect Dis. 180 (3) 925 - 928 .

24. Mockenhaupt F.P, May J, Bergqvist Y, Ademowo O.G, Olumese P.E **Falusi A.G.**, Grosbterlinden L, Meyer C.G, Bienzle U. (**2000**) Concentrations of Chloroquine and Malaria Parasites in Blood in Nigerian children. Antimicrob. Agents Chemother.44: (4) 835-839.

25. Nhonoli, A.M., Kujwalile, J.M., Nmari, P.W. and Shemaghoda, Y.: **(1979).** Heamoglobin Bart's in Newborn Tanzanians. Acta Haematologica, 61: 114 – 119.

26. Olatunji P.O., **Falusi A.G.**, **(1994)** Effect of Alpha Thalassaemia and haemoglobin F (HbF) levels on the clinical severity of sickle cell Anaemia in Nigerians. Eur. J. Haematology 52: 13-15.

27. **Oski** FA. Differential diagnosis of anaemia. In: Nathan DG, Oski FA. Eds. Hematology of infancy and childhood. Philadelphia: WB Saunders, 1992; 346-53.

28. Snounou G., Viriyakasols, Zhi XP, Jarra W, Pinhero L. DoRosario VE, Thaitong S, Brown K.N **(1993)**. High sensitivity of detection of human malaria parasites by the use of nested Polymerase Chain Reaction. Mol. Biochem. Parasitol 61:315-320).

29. Steinberg, M.H., Rosenstock, W., Coleman, M.B., Adams, J.G., Platica, O. Cedeno, M., Reider, R.F., Wilson, J.T., Milner, P., West, S. and the cooperative study of sickle cell disease **(1984)**. Effects of thalassaemia and microcytosis on the haematologic and vasoocclusive severity of sickle cell anaemia. Blood 63: 1353 – 1360.

30. Vulliamy T.J., Othman A., Town M., Nathwani A., **Falusi A.G.**, Mason P.J., Luzzatto. L **(1991)**. Polymorphic sites in the African population detected by sequence analysis of the glucose-6-phosphate dehydrogenase gene outline the evolution of the variants A and A-. Proc. Natl Acad. Sci. U.S.A. 88: 8568-8571.

31. Weatherall D.J. & Clegg J.B. **(1981)**. The Thalassaemia Syndromes.Blackwell Scientific Publications, Oxford -3rd Edition.