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Relationship between Haemoglobin Genotype, Incidence of Diabetes Mellitus and Some Clinical Complications in Southern Nigeria Tertiary Health Institutions

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Abstract: *Established association exist between haemoglobin genotype and protection against or susceptibility to some disease conditions. We investigated the relationship between haemoglobin genotypes, incidence of diabetes mellitus and its clinical complications. A cross sectional survey design was adopted for this study; questionnaires were developed and filled accordingly. 445 subjects took part in the study, with 244 making up the test group (diabetics) and 221 as control group (non-diabetics). Subjects were interviewed and samples collected during their weekly clinic visit. Analyses were done to determine the blood sugar concentration in both groups. Data were analyzed using Instat Graph pad 2.5a and Chi-Square. Student T-test were employed to compare two set of data. The result showed that fasting blood sugar (FBS) for the diabetics group was 7.3±0.68mmol/l. Random blood sugar (RBS) for the diabetics group was 18.96±0.089mmol/l and the FBS for the control was 4.2±0.41mmol/l. RBS for the control was 10.1±0.72mmol/l. Diabetics had a significantly higher fasting and random blood sugar concentration than the non-diabetics. The prevalent of diabetes mellitus with AA genotype was significantly higher than patient with AS genotype. Neuromuscular complication was significantly higher than eye complication. Heamoglobin AS appears to offer protection against diabetes mellitus. Eye and neuromuscular complications were most common.*

Keywords: *Clinical Complications, Diabetes Mellitus, Genotype, Haemoglogin, Incidence*

I. Introduction

Haemoglobinopathies are among the most common genetic disorders worldwide. Typically inherited as autosomal recessive disorders from healthy-carrier parents, the most common are the sickle cell disorders and the thalassemia syndromes [1]. Sickle cell disease is an autosomal recessive disease caused by hemoglobin S (HbSS), an oxygen carrying protein in blood cells. It is characterize by a single Point mutation in the nuclear base sequence of chromosomes II. Point mutation on the β-globin gene result in glutamic acid substituting for valine at position 6 of amino acid sequence resulting in the formation of sickle cell haemoglobin [2] with structural deformity. Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by abnormal hemoglobin production, hemolytic anemia, and intermittent occlusion of small vessels leading to acute and chronic tissue ischemia, chronic organ damage and organ dysfunction [3]. Hemoglobin A is a tetrameric protein that is composed of two α-globin chains and two β-globin chains.

Several research studies have shown that individuals with HBAS and HBSS have a genetic advantage over those with HBAA for protection against plasmodium falciparium[4]. Growth failure has been reported as the most frequent endocrine abnormality in patients with sickle cell disease (SCD) [5, 6] Children with sickle cell disease have significantly decreased height, weight, and body mass index (BMI) when compared with healthy, central subject of comparable age, sex and ethnicity [7]. Factors for decreased growth are multifactorial with contributions from abnormal endocrine function [8], suboptimal nutrition [9], an increase in metabolism because of hyperactivity of the bone marrow and chronic inflammation [10, 11] and hypoganadism [7]. Recent evidence suggests abnormalities in the GH-IGF-IGFBP3 (growth hormone-insulin-like growth factor I-IGF-binding protein) axis as an important etiologic factor of impaired growth in sickle cell disease [12, 13].

Diabetes is a group of metabolic disease characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels.

Long term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, Charcot joint and autonomic neuropathy causing gastro-intestinal, genitourinary, cardiovascular symptoms and sexual dysfunction.

Diabetes is gradually becoming pandemic according to the international diabetes federation [14]. Diabetes affects at least 285 million people worldwide and that number is expected to reach 438 million by the end of the year 2030, with two-third of all diabetes cases occurring in low to middle income countries [15]. It is estimated that approximately 72,000 Americans are homozygous for the sickle cell gene. (i.e SS genotype) and have sickle cell disease and 2 million are heterozygous carriers (i.e AS genotype) and therefore have sickle cell traits [2].

Several inter-related and complex pathophysiologic mechanisms have been proffered for these complications. However, the central factor in the pathogenesis is prolonged hyperglycermia which is the "hall mark" of diabetes mellitus. The hypothesized nature of the contribution of the haemoglobin genotype trait in the protection against diabetes mellitus onset, or predisposition to it, is not yet documented. As a starting point therefore, this present research was aimed to assess the likely association of haemoglobin genotype and the incidence of diabetes mellitus with its clinical complications.

II. Materials And Methods

2.1 Sources of samples

Two major centers were chosen for this study. University of Uyo teaching hospital and St. Luke's hospital Anua-offot in Uyo local government area. Both the test-samples and control were obtained from these centers. A total of 445 subjects took part in this study, with 244 making up the test group (Diabetics) and 221 as the control group (Non Diabetics).

Inclusion and exclusion criteria were not applied as basically all those attending their routine diabetic clinic were very cooperative and eager to be involved in the study. However, some subjects failed to offer information which they considered to be confidential, like average monthly income.

Both the test and the control subject were of Nigeria nationality with various tribal/ethnical groups. However, indigenes of Akwa Ibom State made up the majority of the study population.

Application for permission to carry out the study was forwarded to the ethics and rules committee of the respective centers and the extent and purpose of the study was clearly explained and informed consent was obtained from the subjects. The rules on respect of person were duly observed. Subjects were interviewed and samples collected during their weekly diabetic clinic visit and research questionnaire filled accordingly. The control groups were randomly selected from those on routine medical/surgical check-ups confirmed to be non-diabetes.

2.2 Collection and treatment of samples

2.2.1 Urine:

Universal urine bottles were issued to subjects for urine collection using the clinic convenience. The urine samples were kept at normal room temperature ($25^{o}C$). Urinalysis was carried out on the samples within one hour of collection**.**

2.2.2 Blood:

Venepuncture was used in collecting the blood samples. A tourniquet was tied above the point chosen for venepuncture. The area was clean with spirit swap (cotton wool). Blood was withdrawn with 5mls syringe. About 2mls were emptied into each EDTA bottle and about 2-3mls also fixed in the fluoride bottle. Blood sample in the EDTA bottles were used for genotype determination, while that in the fluoride bottle was used for random blood sugar and fasting blood sugar as applicable.

2.3 Urinalysis

A strip of combi-10 was dipped into the urine in the universal urine container. Excess urine was wiped off with cotton wool, the colour changes on the strip was compared with the standard colour on the bottle within 30 seconds.

2.4 Genotype

Blood sample was washed with saline, a drop of saporine was added to lysed and red cells. Tris buffer was poured into the electrophoretic tank with enough water to cover the electrodes. The tank was plugged into the power pack then connected into the electricity source. The cellulose acetate paper was placed on the tank and wetted with buffer. The lysed blood was then placed on the paper with a known control blood and covered with its lid. The electricity source was switched on and the power pack was simultaneously switched on and allowed to run for 10-15 minute. After the specified period, the pack was switched off; the lid was then removed to inspect the movement. This was compared with the AS control and the genotype of the test sample determined.

2.5 Blood sugar estimation

The blood sample for estimation of blood sugar was collected into the fluoride bottle. One stop glucometer was switched on the sugar strip inserted into the glucometer. A drop of blood was placed on the sample spot. The meter automatically started timing, and after 45 seconds the result was issued out in mg/dl and converted to mmol/l.

2.6 Statistical analysis

The data was entered in micro soft excel for analysis. Further analysis was carried out using instat Graph-pad 2.5a. The chi-square and student T-test were employed to compare two sets of data.
Three or more variable were compared with the analysis of variance. $P<0.05$ was considered statistically significant.

III. Result

3.1 Blood sugar

The means of the blood sugar concentration as shown by fasting blood sugar (FBS) and Random blood sugar (RBS) for diabetics and control groups are shown in table 1. It shows that the diabetics had significantly higher fasting and Random blood sugar concentrations. ($P<0.01$) than the non-diabetics.

Table 1: mean of blood sugar concentration

Blood sugar concentration (mmol/l)	Diabetics Mean ± SD	Nondiabetics Mean ± SD	P value
FBS	7.3 ± 0.68	4.2 ± 0.41	**
RBS	18.96 ± 0.89	10.1 ± 0.72	**

** = significant at $P<0.01$

3.2 Genotype

Table 2 shows that 93.75% of the test population (diabetics) had AA genotype compared to the control population (nondiabetics) with 72.39% that had AA genotype ($P<0.01$). However, AS genotype was less among the diabetics than in control (6.25% versus 27.6%; $P<0.01$).

Table 2: Distribution of genotype among diabetic and non-diabetic subjects

Genotype	Diabetics (N = 224)	Non-diabetics (N 221)	p-value
AA	210 (93.75%)	160 (72.39%)	**
AS	14 (6.25%)	61 (27.60%)	**

** = significant at $p<0.01$

3.3 Clinical complication

Associated clinical complications were compared with duration of illness; the duration of illness was grouped into those below 5 years, 6 – 10 years and those above 11 years. (Fig. 1) There was no direct relationship between the duration of illness and the onset of complications. Only the eye and neuromuscular complications were considered, as these were the commonest.

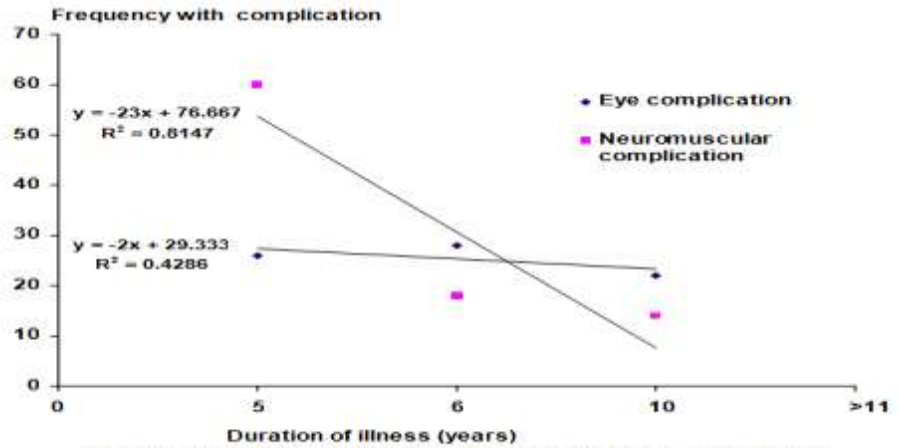

Figure 1: Relationship between clinical complications with duration of illness of diabetics subjects

IV. Discussion

The result showed that the prevalence of diabetes mellitus in patient with AA genotype was significantly higher than patient with AS genotype. This difference is of enormous clinical implication especially, when compared to the prevalence in the general population of this region. This finding is related with the report of [16] that studied the beneficial effect of glycosylated sickle haemoglobin (HbSS) that failed to detect a single case of diabetes mellitus in sickle cell patient. Similarly, from Orissa, India, where the frequency of sickle cell gene is very high (15.1%) diabetes has not been reported among patients with homozygous and heterozygous sickle cell gene [17]. From our result, it is observed that haemoglobin-AS (Hb-AS) offers protection against diabetes mellitus. One explanation for these findings could be that majority of the patients with sickle cell anaemia died early, therefore relatively small number of patients survive for the clinical manifestation of diabetes [17]. The beta-globin gene and insulin gene are located in the short arm of chromosome 11 [16], but it is not known whether the genetic loci of insulin and beta-globin gene have any inhibitory effect on the inheritance pattern of these patients. However, decrease rate of obesity due to increase frequency of illness among the sickle cell disease population may also be responsible for the observed low prevalence of diabetes mellitus.

It is widely accepted that sickle cell traits (AS) protects the carrier from malaria (both symptomatic and asymptomatic), though the mechanism are still not fully understood. However, a number of mechanisms have been proposed. One of which is related to that of plasmodium falciparium on sickle cell haemoglobin. Since haemoglobin AS offers resistance to malaria by phagocytosing the infected sickle cells, it could be suggested that changes in haemoglobin AS erythrocyte, such as low intracellular potassium, and osmotic shrinkage of the red blood cells may provide an unfavourable environment for antibodies to grow.

Hence, haemoglobin AS offers protection against diabetes mellitus by phagocytosing the antibodies that causes autoimmune destruction of the beta cells of the pancreas and anti-insulin receptor antibodies that binds to insulin receptor and prevents its responsiveness. Similarly it could be suggested that, MicroRNAs present in sickle cell erythrocyte also offer protection against diabetes by blocking the mRNA translation of the antibodies genome that causes autoimmune destruction of the pancreas as seen in the case of malaria.

Neuromuscular complication and eye complication were considered as these were the commonest, but it was observed that neuromuscular complication was the earliest occurring complication. The mechanism for the early onset of neuromuscular complication may possibly be attributed to persistent hyperglycaemia, micro vascular damage and changes in the interaction between neuronal and immunological systems in parallel with glial activation [18, 19]. Persistent hyperglycaemia is responsible for the enhance activation of polyol pathway.

In hyperglycaemic state, the affinity of aldose reductase for glucose is increased, leading to increase production of sorbitol. Sorbitol does not cross cell membranes and accumulate intracellularly in the nervous tissue, thus generating osmotic stress. Osmotic stress increases intracellular fluid molarity as well as water influx, causing schwan cell damage and nerve fiber degeneration [20]. Furthermore, upregulation of the NADPH oxidase complex result in oxidative stress through reduce glutathione production, decrease nitric oxide concentrations and increased reactive oxygen species concentration [21]. Free radicals, oxidants and some unidentified metabolic factors activate nuclear enzyme poly (ADP-ribose) polymerase (PARP), which is a fundamental mechanism in the development of diabetes neuromuscular complication [18]. Nitric oxide deficit and increased oxygen free radical activity are responsible for micro vascular damage [19]. Growing body of evidence indicates that the activation of non-neuronal cells (microglia, astrocytes and immune cells) plays an important role in the development of diabetic neuromuscular complication [22]. *Myo*-inositol depletion also causes diabetic neuropathy [23]. In diabetes mellitus, excess sorbitol accumulates in nervous tissue, which leads to an increase in osmotic stress and tissue damage. Simultaneously, decreases in the concentration of *myo*-inositol reduce Na+/K+ ATPase activity, which is important in impulse conduction. Under normal conditions, the *myo*-inositol content is approximately 30-fold higher in peripheral nerves than in plasma [24].

Phosphoinositides are metabolically active cell phospholipids associated with the cell membrane. The phosphatidylinositol cycle involves the transformation of phospholipids accompanied by cell activation, and this cycle is important for the conduction of nerve impulses [25]. Under normal conditions, the Na+/K+ ATPase activity in the nerve maintains a lower concentration of sodium in the peripheral nerves compared to the plasma [26]. In diabetes, *myo*-inositol deficiency is observed in the nerves, resulting from the inhibition of the sodium-dependent uptake of *myo*-inositol and severe changes in the polyol pathway. The reduced *myo*-inositol concentration causes the insufficiency of Na+/K+ ATPase, the enzyme necessary to generate nerve depolarization. As a result, the conduction of stimuli is reduced [27]. Sundkvist et al; showed that high *myo*-inositol levels were associated with nerve regeneration. Therefore, the elevation of *myo*-inositol levels might be considered a compensatory mechanism to prevent nerve damage [28].

Persistent hyperglycemia results in damage to retinal capillaries. This weakens the capillary walls and results in small outpouchings of the vessels lumen, this is known as microaneurysms. The weakened vessels also become leaky, causing fluid to leak into the retina. Fluid deposition under the macula, or macular edema,

interferes with the macula's normal function and is a common cause of vision loss in those with diabetes retinopathy [29].

Several of the diabetics in this study had fairly good control of their blood glucose level, though the majority of them tilted to the above normal range. It has been noted that the risk of chronic complications increases as a function of the duration of hyperglycemia [30]. The fairly good control of the blood sugar of subject undergoing regular clinic appointment, possibly explains the low incidence of chronic complications reported in this study.

V. Conclusion

The number of AS genotype subject with diabetes mellitus was less than in the control population. Thus, suggesting that the sickle cell trait may very likely protect against diabetes mellitus. Eye and neuromuscular complications were the most common in this study location.

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