PARATHYROID STATUS AND ITS ASSOCIATION WITH HAEMOGLOBINOPATHIES AMONG TYPE 2 DIABETES MELLITUS IN SOUTHERN NIGERIA

Funmilola Aduke Mapayi* 1
1Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.

Mabel Ayebatonyo Charles-Davies2
2Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.

Taiwo Rachel Kotila3
3Department of Haematology, College of Medicine, University of Ibadan, Nigeria.

Jokotade Oluremikeun Adeleye4
4Department of Medicine, College of Medicine, University of Ibadan, Nigeria.

Matthew Ogunlakin5
5Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.

Felix Rotimi Afolabi6
6Department of Epidemiology and Medical Statistics, College of Medicine, University of Ibadan, Nigeria.

Emmanuel Oluyemi Agbedana7
7Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.

*For correspondence: Funmilola Aduke Mapayi

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ABSTRACT

Background: Endocrine action is integrative and an endocrine dysfunction of one gland is known to affect other endocrine glands. Parathyroid glands are associated with the beta cell function. Thus, insulin resistance observed in type 2 diabetes mellitus (T2DM) may be associated with alterations of parathyroid hormones and their metabolic pathways. These have been reported to have a genetic root, postulated to be aberrant haemoglobin gene resulting in haemoglobin variants. This has not been fully explored in Sub-Saharan Africa, which has significant population of haemoglobin variants.

Aim: The aim of this study was to evaluate the status of parathyroid dysfunction and its association with haemoglobinopathies among Sub-Saharan Africans with type 2 diabetes mellitus.

Method: A total of 204 individuals aged 25 – 80 years which comprised 100 T2DM and 104 Controls without T2DM were enrolled from a tertiary hospital, in Ibadan, Nigeria and environs. 10mL intravenous blood was obtained from each participant. Parathyroid Hormone (PTH) was measured using enzyme linked immunosorbent assay (ELISA). Calcium, Phosphate, Albumin and Fasting Plasma Glucose (FPG) were analysed spectrophotometrically. Haemoglobin A2 (HbA2), Haemoglobin A (HbA), Haemoglobin C (HbC) and Haemoglobin S (HbS) and Glycated haemoglobin (HbA1c) were determined by High Performance Liquid Chromatography (HPLC) method using Variant Haemoglobin Testing System (Bio-Rad Variant II). Data analysed using appropriate statistical analysis were significant at p<0.05.
**1.0 INTRODUCTION**

Recent epidemiological studies have shown increasing prevalence of Type 2 diabetes mellitus (T2DM) in developed and developing countries (Dianna et al., 2019). T2DM is principally linked with insulin secretory defects, which may be related to inflammation, metabolic stress and genetic factors, manifesting clinically as hyperglycemia (Diabetes Care, 2019). Diabetes and osteoporosis are prevalent chronic diseases with serious clinical complications (Cipriani et al., 2020). Reduced metabolic control of diabetes mellitus may possibly prompt defects in calcium homeostasis and affect bone mineral metabolism (Cipriani et al., 2020).

Calcium (Ca) is an essential mineral that exerts a wide range of biological functions, including bone and tooth mineralisation, blood coagulation, muscle contraction, nerve impulse transmission and cellular signalling transduction (Serova et al., 1990; Peacock, 2010; Becerra-Tomás et al., 2014; Rooney et al., 2016 & Sing et al., 2016). Ca also plays a fundamental role in insulin secretion and glucose homeostasis (Mears, 2004; Becerra-Tomás et al., 2014 and Zaccardi et al., 2015). Glucose-dependent insulin secretion is a Ca regulated process, which is dependent on intracellular Ca concentration in pancreatic β-cells (Wollheim et al., 1981 & Zaccardi et al., 2015). Additionally, increased cytosolic Ca also affects glucose uptake in the myocyte (Begum et al., 1993; Ojuka et al., 2002 & Zaccardi et al., 2015).

Ca homeostasis abnormality could therefore, be potentially involved in insulin action defects and disorders in glucose homeostasis, causing T2DM advancement (Procopio et al., 2002; Mears, 2004; Becerra-Tomás et al., 2014; Zaccardi et al., 2015). An increase in parathyroid hormone (PTH) secretion may occur to correct the chance of reduction in calcium (Seino et al., 1995).

Parathyroid Hormone (PTH) is a polypeptide that comprises 84 amino acids (Chang et al., 2009), which preserves the extracellular calcium levels within a narrow normal range and controls plasma calcium homeostasis (Stanley et al., 2013). The parathyroid glands secrete PTH in response to low calcium levels causing an increase in bone resorption and maintaining extracellular calcium through direct effect on the kidneys and bones as well as an indirect impact on the small intestine.

The association of parathyroid hormone (PTH) with insulin resistance has been demonstrated (Chang et al., 2009). The development of diabetes mellitus has been linked with elevated PTH concentrations with insulin resistance, beta cell dysfunction, and abnormal glucose levels (Chiu et al., 2000; Reis et al., 2007; Reis et al., 2008; Kramer et al., 2014).

High levels of PTH is predominant in diabetes mellitus by two to four times the levels in individuals without T2DM (Rahimi, 2014). Studies of patients with primary hyperparathyroidism have shown a higher prevalence of diabetes compared to control populations (Werner et al., 1974; Ljunghall et al., 1983; Cheung et al., 1986 & Taylor, 1991).

Hyperparathyroidism is a disease characterized by autonomous excess production of PTH resulting in hypercalcemia. Overproduction of PTH results in mobilization of calcium from bone and inhibition of the renal reabsorption of phosphate, resulting to hypercalcemia and hypophosphatemia (Dariusz et al., 2012). PTH downregulates the insulin receptors peripherally, increases insulin resistance and has a direct effect on beta cells (Murray, 2005). Both primary and secondary hyperparathyroidism are involved in abnormal glucose metabolism. Ivarsson et al. (2014) reported a higher prevalence of diabetes mellitus in patients with primary hyperparathyroidism while the removal of parathyroid glands improve glucose tolerance in these individuals (Hamed et al., 2011).

**Results:** Normal parathyroid function, hyperparathyroidism and hypoparathyroidism were present in 93% vs 96%, 3% vs 0.96% and 4% vs 6.73% in T2DM and controls respectively. T2DM and controls with AA and Non AA had 62% vs 31% normoparathyroidism, 3% vs 0% hyperparathyroidism and 2% vs 2% hypoparathyroidism respectively. The association between parathyroid gland disorder in T2DM and controls with the various haemoglobin variants was not significant (p>0.05) but the difference between parathyroid function in the control group with and without Beta Thalassaemia Trait was significant (p<0.05).

**Conclusion:** Hypoparathyroidism and hyperparathyroidism were revealed in Type 2 Diabetes Mellitus and control individuals with haemoglobin genotype AA (HbAA). Hypoparathyroidism was also found among controls with Beta Thalassaemia Trait. Timely identification of these disorders may be helpful in appropriate therapeutic regimen to facilitate bone growth, prevent fractures and complications of parathyroid gland in these individuals.

**KEYWORDS:** Haemoglobin Variants, Parathyroid Dysfunction, Type 2 Diabetes Mellitus (T2DM).
decreased calcium and calcitonin. Deficient parathyroid hormone leads to hypocalcaemia symptoms including pain and muscle cramp,, numbness, tingling, seizures, carpopedal spasms, Trouseau and Chvostek signs (Yavari, 2014; Ferrara et al., 2002; Al-Akhras et al., 2016).

Genetic factors appear to be important in the development of reduced bone mass and osteoporotic fractures (Voskaridou et al., 2013), which are often prominent in T2DM (Hothersall et al., 2014; Sellmeyer et al., 2016). T2DM and parathyroid dysfunction are associated endocrinopathies with genetic basis, largely attributed to haemoglobinopathies.  

Hemoglobinopathies are the most common genetically inherited single-gene disorders in the world (Goonasekera et al., 2018). Haemoglobin disorders are divided into qualitative and quantitative defects in globin synthesis. Quantitative defects result to thalassemia syndromes, often with normal globin structure. Hemoglobin variants result from qualitative defects with point mutations in globins (Kohne, 2011; Payandeh et al., 2014; Brancaloni et al., 2016). The two major types are structural haemoglobin variants (HbC, Haemoglobin E (HbE) and Hbs) and thalassemia syndromes (α- and β-thalassaemia) are known (Kohne, 2011). Several Investigators have identified about 700 structural haemoglobin variants but only three (HB S, Hb C, and Hb E) are predominant (Lal et al., 2010).

Bone disease has been characterized as an evident cause of morbidity in individuals with thalassaemia and other haemoglobin disorders. The risk of T2DM, gestational diabetes, osteoporosis, renal diseases, decreasing pulmonary functions and dental problems is shown to be increased in a individuals with beta thalassemia minor (Leung & Lao, 2012; Giusti et al., 2016; Helmi et al., 2017 and Nickavar et al., 2017. (Voskaridou et al., 2013). Hypoparathyroidism with reduced serum calcium levels in thalassemia patients was improved with vitamin D and calcium supplementation (Goyal et al., 2016). Several studies have reported parathyroid gland status in diabetes mellitus in different populace but reports on the effect of haemoglobin disorders on parathyroid function in T2DM individuals in African blacks, are unavailable. Hence, the aim of this study is to determine the association of haemoglobin variants with parathyroid dysfunction among T2DM in Southern Nigeria.

2.2u Participants
204 (two hundred and four) participants consisting of 100 (one hundred) T2DM and 104 (one hundred and four) without T2DM (controls) volunteers who gave their consent were enrolled from a tertiary hospital, in Ibadan, Nigeria and environs. The diabetic group were confirmed diabetics by a Consultant Endocrinologist using the World Health Organisation Criteria (Venous fasting plasma glucose (FPG) values of ≥ 7.0 mmol/L (126 mg/dL), 2-h post-load plasma glucose ≥ 11.1 mmol/L (200 mg/dL) (WHO, 2006), HbA1c ≥ 6.5% (48 mmol/mol); or a random blood glucose ≥ 11.1 mmol/L (200 mg/ dL) in the presence of signs and symptoms are considered to have diabetes (WHO, 2016). The individuals without T2DM and history of diabetes were volunteer staff of the same tertiary hospital where the cases were recruited and persons without history of diabetes from the environs were selected. All the participants fasted overnight (10 – 12hrs) and details on biodata, lifestyle, diet and medical history were obtained through a pre- test semi-structured questionnaire.

2.3 Sample Collection
Intravenous blood (8mL) sample was aseptically obtained by venepuncture from each of the participants after an overnight fast (10 –12 hours). Four millilitres (4mL) of blood was dispensed into labelled vacuum collection tube containing potassium ethylene diamine tetra acid (K3EDTA), stored at 2–8oC and processed within 7 days of sample collection for the determination of haemoglobin variants (HbA2, HbA, HbC and HbS) without sample centrifugation. 4 mL of blood was dispensed into gold-topped serum separator gel tubes for the determination of PTH, Calcium, Albumin, and Phosphorus. Serum/plasma was obtained by centrifuging blood and spun at 300g for five minutes. These were stored in small aliquots at -200C until analysis was done.

2.4 Biochemical Investigations
Biochemical parameters estimated were Fasting Plasma Glucose Serum Parathyroid Hormone (PTH), Calcium, Albumin and Phosphorus and Haemoglobin Variants (HbA2, HbA, HbC and HbS).

2.4.1 Fasting Plasma Glucose Estimation
Fasting Plasma Glucose was determined by glucose oxidase, an enzymatic method (Produktion, Austria), as described by Barham and Trinder, (1972). Participants were classified according to WHO criteria: Normal range for Fasting Plasma Glucose (FPG) was FPG ≤110mg/dL (≤6.1mmol /L) (Normal); FPG (110 – 125mg/dL (6.1 to 6.9mmol/L) (Prediabetes) for the control group and FPG ≥126mg /dL (≥7mmol /L) (Diabetes Mellitus) for the T2DM group (WHO, 2016).

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2.4.2 Calcium Estimation
Serum calcium ions was determined by colorimetric method with O-Cresolphthalein complexone without deproteinization medium (Randox Laboratories, Crumlin, United Kingdom) as described by Benedict et al., 1924). The reference range for normal calcium level was 8.10 – 10.4mg/dL.

2.4.3 Phosphate Estimation
Serum phosphate ions was determined by the formation of a yellow phosphorus molybdate complex when it reacts with ammonium molybdate and the concentration obtained photometrically (Dialab Produktion, Austria), as described by Thomas (1998). The reference range for normal phosphate was 2.6 –4.5 mg/dL.

2.4.4 Albumin Estimation
Serum albumin was determined by a colour change of indicator in the presence of bromocresol green and the intensity of the colour obtained photometrically (Dialab Produktion, Austria) as described by Johnson et al., (1999). The normal reference range for albumin concentration was (3.5 - 5.2g/dL).

2.4.5 Determination of Haemoglobin Variants and Glycated Haemoglobin
Haemoglobin parameters (HbA2, HbA, HbC and HbS) were determined by high performance liquid chromatography method using Biorad Variant II (Bio-Rad Laboratories Inc., Hercules, CA, USA). The reference value for HbA2 variant was normal (2.0 -3.3%), Beta thalassemia Trait (BTT) (≥3.5%) (Buch et al., 2016). Normal adult percentage of Haemoglobin was HbA (≥98%), HbF (<1%), HbS (0%), HbC (0%); HbAS, Sickle Cell Trait percentage of normal form of Hb A was about 60% with a moderate amount of HbS (about 40%); HbAC, haemoglobin C trait percentage of normal form of HbA was about 60% and moderate amount of HbC (about 40%); HbCC, hemoglobin C disease percentage of HbC was (80% and above); HbSC, sickle-haemoglobin C disease percentage was 50% of HbC and 50% of HbS ; HbSS Sickle Cell Disease percentage of HbS was 80% and above (AACC, 2019). Participants were classified according to WHO criteria: Reference value for Hba1C was normal (4 – 5.6%) and Prediabetes (5.7 – 6.4%) for the control group and Diabetes ≥ 6.5% for T2DM group (WHO, 2016).

2.4.6 Estimation of Serum Parathyroid Hormone (PTH) Level
Serum PTH was estimated by quantitative enzyme immunoassay technique (Double-antibody sandwich) technique (Melsin Medical, Changchun, PTH, ELISA KIT CAT.NO: EKHU-1533). The reference range for PTH was between 11.0 and 70.6 pg/mL(Soubrielle et al 2016). Normal parathyroid status is defined when PTH levels fall within the normal range with normal calcium and phosphate. Hypoparathyroidism was defined by decreased serum calcium and increased phosphorous levels with reduced PTH (Ali Bazi, et al., 2018) while hyperparathyroidism by inappropriately high PTH level with increased calcium and low phosphate (Dariusz et al., 2012).

2.5 Statistical Analyses
Analysis of data was done using Statistical Package for Social Sciences (SPSS) version 21.0. Quantitative variables were presented as mean ± standard error of mean (SEM). Categorical variables were presented as absolute (n) and relative frequencies (in percentage). Comparison of means between two groups and among subdivided groups was done by statistical independent t-test while test of association was determined using Pearson’s Chi square test and Analysis of Variance (ANOVA). Post hoc was used to compare difference between the groups. Statistical significance was defined by p value < 0.05.

3.0 RESULTS
Table 1 shows the parathyroid function of the study participants. (p< 0.001). Among the T2DM and controls, 93 (93%) vs 96 (92.3%) were found to be normoparathyroid, 3 (3%) vs 1 (0.96%) were hyperparathyroid and 4 (4%) vs 7 (6.73%) were hypoparathyroid respectively. A high percentage of T2DM and healthy controls had normal parathyroid function and minority of the type 2 diabetics and controls had hyperparathyroidism and hypoparathyroidism. There was no significant difference in the parathyroid function among T2DM and controls (p>0.005).
Table 1 Parathyroid function among Type 2 Diabetes Mellitus and Control Group

| PARATHYROID FUNCTION | T2DM n =100 | CONTROL n = 104 | TOTAL N =204 | X2 | p  |
|----------------------|-------------|-----------------|--------------|----|----|
| Normoparathyroidism   | 93 (93.0)   | 96 (92.3)       | 189(92.6)    | 1.788 | 0.429 |
| Hyperparathyroidism   | 3 (3.0)     | 1 (0.96)        | 4 (1.96)     |     |    |
| Hypoparathyroidism    | 4 (4.0)     | 7 (6.73)        | 11 (5.4)     |     |    |
| Total                | 100 (100.0) | 104 (100.0)     | 204 (100.0)  |     |    |

Values are presented in number (n) and (%) = percentage in parentheses, N = total number, X2 = Chi-squared test, p = probability, * = significant at p 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes Mellitus,
The prevalence and association of parathyroid function status among T2DM and controls with normal and abnormal genotypes are shown in table 2. The prevalence of parathyroid function status among the T2DM was (AA and Non AA were 62 (62%) and 31 (31%) normoparathyroidism, 2(2%) and 2 (2%) hypoparathyroidism, 3 (3%) and 0 (0%) hyperparathyroidism respectively) while the prevalence among the controls were 76 (73.1%) and 20 (19.2%) normoparathyroid, 7(6.73%) and 0 (0%) hyperparathyroid and 1 (0.96%) and 0 (0%) hypoparathyroid in AA and Non AA respectively. The associations between parathyroid function in T2DM and controls with normal and abnormal haemoglobin genotypes were not significantly different (p>0.05).

| Parathyroid Function | T2DM (n = 100) | Control (n = 104) |
|----------------------|----------------|------------------|
|                      | AA             | Non AA           | Total | X2  | P    | AA       | Non AA   | Total | X2  | p    |
| Normoparathyroidism  | (62.0)         | 31 (31.0)        | 93 (93.0) | 2.005 | 0.367 | 76 (73.1) | 20 (19.2) | 96 (92.3) | 2.063 | 0.356 |
| Hypoparathyroidism   | 2 (2.0)        | 2 (2.0)          | 4 (4.0)  |     |      | 7 (6.73) | 0 (0.0)   | 7 (6.73)  |     |      |
| Hyperparathyroidism  | 3 (3.0)        | 0 (0.0)          | 3 (3.0)  |     |      | 1 (0.96) | 0 (0.0)   | 1 (0.96)  |     |      |
| Total                | 67 (67)        | 33 (33.0)        | 100 (100.0) |     |      | 84 (80.8) | 20 (19.2) | 104 (100.0) |     |      |

Values are in number (n) and (%) = percentage in parentheses, N = total number, X2 = Chi-squared test, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participant, Controls = Apparently healthy participants without Type 2 Diabetes Mellitus, HbAA (AA) = Normal Haemoglobin, Non AA = Abnormal Haemoglobin
Table 3 shows the prevalence and parathyroid function status among T2DM and controls with and without Beta Thalassemia Trait. The prevalence of parathyroid function status among the T2DM was (BTT and NBTT were 5 (5%) and 88 (88%) normoparathyroidism, 0(0%) and 4 (4%) hypoparathyroidism, 0 (0%) and 3 (3%) hyperthyroidism respectively) while the prevalence among the controls were 6 (5.8%) and 90 (86.5%) normoparathyroid, 0(0%) and 7 (6.73%) hypoparathyroid and 1 (0.96%) and 0 (0%) hyperparathyroid in BTTand NBTT respectively. The association between parathyroid function in T2DM with and without Beta Thalassamia Trait was not significantly different (p>0.05) but the difference between parathyroid function status in the control group with and without Beta Thalassaemia Trait was significant (p<0.05).

| Parathyroid Function         | T2DM (n = 100) | Control (n = 104) |
|------------------------------|---------------|------------------|
|                              | BTT | NBTT | Total | X2  | P    | BTT | NBTT | Total | X2  | p    |
| Normoparathyroidism          | 5 (5.0) | 88 (88.0) | 93 (93.0) | 0.396 | 0.820 | 6 (5.8) | 90 (86.5) | 96 (92.3) | 14.40 | 0.001* |
| Hypoparathyroidism           | 0 (0.0) | 4 (4.0) | 4 (4.0) | 0 (0.0) | 7 (6.73) | 7 (6.73) | |
| Hyperparathyroidism          | 0 (0.0) | 3 (3.0) | 3 (3.0) | 1 (0.96) | 0 (0.0) | 1 (0.96) | |
| Total                        | 5 (5.0) | 95 (95.0) | 100 (100.0) | 7 (80.8) | 97 (19.2) | 104 (100.0) | |

Values are in number (n) and (%) = percentage in parentheses, N = total number, X2 = Chi-squared test, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes Mellitus, BTT = Beta Thalassaemia Trait, NBTT = Individuals without BTT.
The association of parathyroid function status among T2DM and controls with various haemoglobin genotypes is shown in table 4. The prevalence of parathyroid function status among the T2DM with haemoglobin genotype AA, AC, AS and CC were 62 (62%), 4 (4%), 26 (26%) and 1 (1%) normoparathyroid, 2 (2%), 1 (1%), 1 (1%) and 0 (0%) hypoparathyroid respectively while the prevalence among the controls with haemoglobin genotype AA, AC, AS and SC were 76 (73.1%), 5 (4.81%), 14 (13.5%) and 1 (0.96%) normoparathyroid, 1 (0.96%) and 7 (6.73%) with AA only were hyperparathyroid and hypoparathyroid in controls respectively. There was no significant difference between the function of the parathyroid gland with different haemoglobin genotypes in both T2DM and controls (p>0.05).

### Table 4.0 Prevalence and Association of Parathyroid Function in Individuals with various Haemoglobin Genotypes in Type 2 Diabetes Mellitus and Control Group

| Parathyroid Function | T2DM (n =100) | Control (n =104) |
|----------------------|---------------|-----------------|
|                      | HbAA n (%)    | HbAC n (%)      | HbAS n (%) | HbCC n (%) | Total N (%) | X2 (P) | HbAA n (%) | HbAC n (%) | HbAS n (%) | HbSC n (%) | Total N (%) | X2 (P)  |
| Normoparathyroid     | 62 (62.0)     | 4 (4.0)         | 26 (26.0)   | 1 (1.0)     | 93 (93.0)   | 5.025   | 76 (73.1)  | 5 (4.81)   | 14 (13.5)  | 1 (0.96)   | 96 (92.3)   | 2.063   |
|                      |               |                 |             |             |             | (0.366) |            |            |            |            |            | (0.594) |
| Hyperparathyroid     | 3 (3.0)       | 0 (0.0)         | 0 (0.0)     | 0 (0.0)     | 3 (3.0)     | 1 (0.96) | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 1 (0.96)   | 104 (100)  |          |
|                      | 2 (2.0)       | 1 (1.0)         | 1 (1.0)     | 0 (0.0)     | 4 (4.0)     | 7 (6.73) | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 7 (6.73)   |            |          |
| Total                | 67 (67)       | 5 (5.0)         | 27 (27)     | 1 (1.0)     | 100 (100.0) | 84 (80.8)| 5 (4.81)   | 14 (13.5)  | 1 (0.96)   | 104 (100)  |            |          |

Values are in number (n) and (%) = percentage in parentheses, N = total number, X2 = Chi-squared test, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes Mellitus, Haemoglobin AA = HbAA, Haemoglobin AC = HbAC, Haemoglobin AS = HbAS, Haemoglobin CC= HbCC and Haemoglobin SC = HbSC
Table 5 shows serum levels of calcium, phosphate, albumin and PTH in T2DM and controls. As shown in Table 4, significant increase in phosphate (p< 0.001) and decrease in albumin concentrations were observed (p< 0.001) in T2DM when compared with controls respectively. Moreover, Calcium was lower while PTH was higher when compared with the controls but the difference were not significant (P> 0.05).

| Parameters     | T2DM (n = 100) | Controls (n = 104) | t value | p value |
|----------------|----------------|--------------------|---------|---------|
| Calcium (mg/dL)| 9.03 (0.17)    | 9.12 (0.66)        | -0.123  | 0.902   |
| Phosphate (mg/dL) | 4.39 (0.14) | 3.81 (0.09)        | 3.49    | <0.001* |
| Albumin (g/dL)  | 4.38 (0.43)    | 4.73 (0.07)        | -4.224  | <0.001* |
| PTH (pg/mL)     | 31.61 (1.93)   | 28.81 (1.83)       | 1.053   | 0.294   |

Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total number SEM = Standard Error of Mean, N = total number, t = t tests, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes, Parathyroid Hormone = PTH

Correlation of PTH with calcium, phosphate and Albumin in T2DM and controls is shown in Table 6. There was a negative correlation of PTH with Calcium and albumin in both groups and controls respectively, phosphate was positively correlated in T2DM and controls but no association was found between PTH and all the biochemical parameters (p> 0.05).

| Parameters | T2DM | Controls |
|------------|------|----------|
| Calcium (mg/dL) | -0.092 | 0.364 |
| Phosphate (mg/dL) | 0.122 | 0.225 |
| Albumin (g/dL) | 0.119 | 0.237 |

Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total number SEM = Standard Error of Mean, N = total number, t = t tests, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes
Table 7 shows the comparison of serum calcium, phosphate, albumin and PTH levels with normal and abnormal haemoglobin genotype in T2DM and controls. ANOVA compared the variables among the normal and abnormal haemoglobin genotypes. There were significant differences in all the biochemical parameters except calcium in T2DM when compared with the control group. Calcium was significantly lower in the T2DM with AA haemoglobin genotype when compared with NON AA of controls (p<0.05). The mean phosphate concentration of T2DM with AA and Non AA were significantly higher than AA of controls (p<0.05). Serum albumin was significantly lower in T2DM with AA and Non AA compared with the corresponding values of controls with AA and NON AA. The mean concentration of PTH in Type 2 diabetics with AA genotype was significantly higher as compared with NON AA in control individuals (P<0.05).

| Parameters | T2DM (n = 100) | CONTROLS (n = 104) | ANOVA Test | Post Hoc Analysis |
|------------|---------------|--------------------|------------|------------------|
|            | Mean (SEM)    | Mean (SEM)         |            |                  |
| Calcium (mg/dL) | 9.029 (0.196) | 9.035 (0.344) | 0.121      | 0.548            |
| Phosphate (mg/dL) | 4.36 (0.172) | 4.5 (0.23)        | 0.007*     | 0.005*           |
| Albumin (g/dL) | 4.37 (0.06)   | 4.39 (0.06)       | 0.001*     | <0.001*          |
| PTH (pg/mL)   | 34.57 (2.62)  | 25.59 (2.07)      | 0.008*     | 0.237            |

Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total number SEM = Standard Error of Mean, N = total number, p = probability, * = significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparency healthy participants without Type 2 Diabetes, Parathyroid Hormone = PTH, P = values obtained from ANOVA, P1 = Comparison between AA of T2DM and AA of Controls, P2 = Comparison between AA of T2DM and NON AA of controls, P3 = Comparison between NON AA of T2DM AND AA of Controls, P4 = Comparison between NON AA of T2DM and NON AA of Controls.
Table 8 shows the comparison of serum calcium, phosphate, albumin and PTH levels with and without BTT. There were significant differences in phosphate and albumin while calcium and PTH were comparable when compared with the controls. The mean phosphate and albumin concentrations of T2DM without BTT were significantly higher and lower than controls without BTT respectively (p < 0.05).

### Table 8 Comparison of Serum PTH, Total Calcium, Phosphate and Albumin in Individuals with Type 2 Diabetes Mellitus and Controls with and without BTT

| PARAMETERS       | T2DM (n = 100) | CONTROLS (n = 104) | ANOVA Test | Post Hoc Analysis |
|------------------|----------------|-------------------|------------|-------------------|
|                  | Mean (SEM)    | Mean (SEM)        |            |                   |
| Calcium (mg/dL)  | 8.42 (0.97)   | 9.06 (0.18)       | 9.23 (0.66) | 9.11 (0.71)       | 0.992 0.782 0.764 0.933 0.952 |
| Phosphate (mg/dL)| 4.65 (0.39)   | 4.38 (0.14)       | 4.26 (0.49) | 3.78 (0.09)       | <0.004* 0.570 0.107 0.803 0.001* |
| Albumin (g/dL)   | 4.49 (0.17)   | 4.37 (0.44)       | 4.54 (0.19) | 4.75 (0.76)       | <0.001* 0.888 0.357 0.472 <0.001* |
| PTH (pg/mL)      | 26.47 (5.62)  | 31.88 (2.01)      | 33.48 (13.07)| 28.48 (1.75)     | 0.586 0.53 0.818 0.830 0.216 |

Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total significant at p < 0.05, T2DM = Type 2 Diabetes Mellitus participants, Controls = Apparently healthy participants without Type 2 Diabetes, Parathyroid Hormone = PTH, P = values obtained from ANOVA, P1 = Comparison between BTT of T2DM and BTT of Controls, P2 = Comparison between BTT of T2DM and NBTT of Controls, P3 = Comparison between NBTT of T2DM AND BTT of Controls, P4 = Comparison between NBTT of T2DM and NBTT of Controls.
There were significant differences in phosphate and albumin while calcium and PTH were not statistically significant in T2DM when compared with the control group. Phosphate was significantly higher in T2DM with AA and AC haemoglobin genotype when compared with AA and AC of controls respectively (p<0.05). Serum albumin was significantly lower in T2DM with AA and AC genotypes when compared with the corresponding values of controls with AA and AC respectively. The mean concentration of PTH in Type 2 diabetics with AC genotype was significantly higher (P<0.05) as compared to AC in control individuals (Table 9).

| Thyroid Hormones | T2DM (n= 100) Mean (SEM) | Control (n =104) Mean (SEM) | t | P1 | P2 | P3 | P4 | P5 | P6 |
|------------------|--------------------------|----------------------------|---|----|----|----|----|----|----|
| Calcium (mg/dL)  | 9.029 (0.196)            | 8.63 (0.75)                | 1.71 | 0.089 | 0.207 | 0.08 | 0.52 | 0.31 | 0.27 |
| Phosphate (mg/dL) | 4.36 (0.172)             | 4.3 (0.30)                 | 2.88 | 0.005* | 0.140 | 0.34 | 0.48 | 0.04* | 0.25 |
| Albumin (g/dL)   | 4.37 (0.06)              | 4.41 (0.14)                | 3.40 | 0.001* | 0.001* | 0.047 | 0.93 | 0.015* | 0.07 |
| PTH (pg/mL)      | 34.57 (2.62)             | 20.01 (9.44)               | 1.02 | 0.31 | 0.046* | 0.036 | 0.49 | 0.33 | 0.17 |

Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total number SEM = Standard Error of Mean, N = total number, t= t tests, p = probability, * = significant at p < 0.05, Haemoglobin AA = HbAA, Haemoglobin AC = HbAC, Haemoglobin AS = HbAS, Haemoglobin CC= HbCC and Haemoglobin SC = HbSC P1 =Comparison between AA of T2DM
Table 10 shows the comparison of PTH, calcium, phosphate and albumin levels according to parathyroid function status in T2DM and controls. There were significant differences in phosphate and albumin while calcium and PTH were not significantly different in T2DM and controls. Serum phosphate and albumin were significantly higher and lower in the normoparathyroid group with T2DM and controls respectively (p<0.05).

|          | T2DM (n = 100) | CONTROL (n= 104) | t value | p value |
|----------|----------------|------------------|---------|---------|
|          | Mean (SEM)     | Mean (SEM)       | Mean (SEM) | Mean (SEM) |       |       |
| Calcium  | (mg/dL)        |                   |          |          |       |       |
| NPT      | 9.12 (1.68)    | 8.80 (1.46)      | 6.69 (2.61) | 9.15 (7.04) | -0.041 | 0.968 |
| HPT      | 8.78 (1.52)    | 8.43 (0.0)       |          |          |       | 0.985 |
| HPPT     | 6.69 (2.61)    | 8.43 (0.0)       |          |          |       | 0.623 |
|          | (mg/dL)        |                   |          |          |       |       |
| Phosphate| 4.36 (1.34)    | 4.28 (1.29)      | 5.54 (2.66) | 3.80 (0.88) | 3.403  | 0.001* |
|          | 3.76 (1.21)    | 5.83 (0.0)       |          |          |       | 0.518 |
|          | (mg/dL)        |                   |          |          |       |       |
| Albumin  | 4.37 (0.42)    | 4.37 (0.36)      | 4.54 (0.83) | 4.75 (0.76) | -4.204 | <0.001*|
|          | 4.60 (3.67)    | 4.33 (0.0)       |          |          |       | 0.331 |
|          | (g/dL)         |                   |          |          |       | 0.848 |
| PTH      | 30.22 (14.10)  | 10.28 (0.11)     | 103.13 (23.50) | 29.30 (16.73) | -0.408 | 0.684 |
|          | 10.44 (0.63)   | 110.99 (0.0)     |          |          |       | 0.636 |

*Values are in mean with SEM = Standard Error of Mean (SEM) in parentheses, N = total number SEM = Standard Error of Mean, N = total number, t= t tests, p = probability, * = significant at p < 0.05, Parathyroid Hormone = PTH, NPT = Normoparathyroidism, HPT = Hypoparathyroidism, HPPT = Hyperparathyroidism P1 = Comparison between NPT of T2DM and NTP of Controls, P2 = Comparison between HPT of T2DM and HPTT of controls, P3 = Comparison between HPPT of T2DM and HPTT of controls.
4.0 DISCUSSION

Elevated levels of Parathyroid Hormone (PTH) has been associated with insulin resistance, beta cell dysfunction, abnormal glucose levels with eventual development of diabetes in recent studies (Kramer et al., 2014). However, increased frequency of functional hypoparathyroidism in patients with type 2 diabetes mellitus with impaired blood sugar regulation have also been reported (Seddek et al., 2016). Parathyroid hormone has some regulating effects on osteoblasts and any discrepancy in its concentration could result in bone loss and increased risk of fracture (Wang et al., 2005 & Motyl et al., 2012). Bone disease denotes a perceptible cause of morbidity in persons with thalassaemia and other haemoglobin disorders (Voskaridou et al., 2013).

In our study, normal parathyroid function, hyperparathyroidism and hypoparathyroidism were present in 93% vs 96%, 3% vs 0.96% and 4% vs 6.73% in T2DM and controls respectively. No significant association of parathyroid function in the T2DM and controls was observed (p<0.05). The parathyroid function status among T2DM and controls with AA and Non AA who had normoparathyroid, hyperparathyroid and hypoparathyroid were 62% vs 31%, 3% vs 0% and 2% vs 2% respectively (p<0.05). The association between parathyroid function in T2DM and controls with normal and abnormal haemoglobin genotypes was also not significantly different (p<0.05). Parathyroid dysfunction was not found in T2DM with BTT while 0.96% of the control group with BTT had hypoparathyroidism. The association between parathyroid function in T2DM with and without Beta Thalassaemia Trait was not significantly different (p<0.05) but the difference between parathyroid function status in the control group with and without Beta Thalassaemia Trait was significant (p<0.05). No significant difference between the function of the parathyroid gland with different haemoglobin genotypes in both T2DM and controls (p<0.05) was observed. Hypoparathyroidism was present in T2DM and controls with Haemoglobin genotype AA (2%), AC (1%) and AS (1%) vs 6.73 in controls respectively while hyperparathyroidism was present in T2DM and controls with only Haemoglobin genotype AA (3%) and (0.96%) respectively. There was no significant difference between the function of the parathyroid gland with different haemoglobin genotypes in both T2DM and controls (p<0.05).

The low prevalence of hyperparathyroidism of in our study in the case and control groups is consistent with the study of Taylor et al., (1997) who reported 0.99% in diabetic patients and increased by three times more when compared with the general population. Taylor in 1991, also observed the frequency of 7.8% in 205 diabetic patients with primary hyperparathyroidism and 3.0% among 200 successive patients without hyperparathyroidism attending the same medical clinic. Hyperparathyroidism may possibly be the long-term status of hypercalcaemia and hypophosphatemia which triggers insulin resistance and hyperinsulinemia, and reduces the number of insulin receptors (Kumar et al., 1994). Several other studies also reported low prevalence rates of hypoparathyroidism ranging from 0.5 to 7.6% in thalassaemic patients (Shamshirsaz et al., 2003; Gamberini et al., 2008 and Canatan, 2013). In this study, we observed low prevalence of hyperparathyroidism (0.96%) in controls with BTT. However, hypoparathyroidism was not detected in T2DM and controls with BTT. This parathyroid dysfunction in controls with BTT in this study may likely be due to iron deposition in the parathyroid gland as reported by Habe et al., (2013) in beta thalassaemia patients.

Recently, Egshatyan (2017) observed magnesium dependent PTH suppression with development of transient hypoparathyroidism and hypocalcaemia in T2DM patients. Hypoparathyroidism briefness with associated hypomagnesemia was confirmed after normalization of the blood magnesium level and that vitamin D deficiency may be the cause of hypomagnesemia and functional hypoparathyroidism. Similarly, previous studies revealed that T2DM patients with hypomagnesemia, inadequately normal or lowered parathyroid hormone (PTH) that resulted from inhibition of its secretion or synthesis may develop PTH resistance with vitamin D deficiency and hypocalcaemia (Hermans et al., 1996; Chase et al., 1974; Rude et al., 1978; Fatemi et al., 1991; Rude et al., 1976; Rude et al., 1985). In our study, the hypoparathyroidism observed in persons with HbAS, HbAC and HbSC in T2DM and HbAA in controls may be due to the lack or excess magnesium playing a role in defective cyclic AMP generation in the parathyroid glands interfering with PTH synthesis and secretion.

Our study revealed a significant increase in phosphate, decrease in albumin and calcium concentrations were observed (p< 0.05) in T2DM compared with controls respectively. Moreover, calcium was lower while PTH was higher in T2DM when compared with the control group but the difference were not significant (P> 0.05). Hussain et al., (2018) reported similar results but the difference in PTH, calcium, and phosphate concentrations between T2DM patients and healthy controls were not significant. In addition, Atmaca et al., (2014) also reported no significant difference in calcium and albumin with statistical difference in phosphorus with PTH below and above 65 ng/mL in diabetic patients with vitamin D levels <30ng/mL. This is in agreement with our findings. Reis et al., (2016) also
reported higher levels of PTH among blacks compared with whites in diabetes and no significant association was observed this is in line with our study outcome. Serum phosphate and albumin were significantly higher and lower in the normoparathyroid group with T2DM and controls respectively (p<0.05). Alterations in calcium metabolism in addition to vitamin D deficiency in Diabetic patients, particularly poorly controlled ones may manifest as reported by Atmaca et al., 2014. Deficient PTH secretion, lower calcium and lower serum magnesium are present in poorly controlled diabetics compared to controls, whereas patients with good metabolic control have normal calcium ion and PTH secretion (Paula et al., 2001). The comparable differences observed in our study group may be due to well controlled Type 2 Diabetes Mellitus.

In this present study, there was negative correlation of PTH with Calcium and albumin while phosphate was positively correlated but no significant correlation found between PTH and all the biochemical parameters in the T2DM and controls (p>0.05). Arora et al., (2018) observed significant negative correlation of serum calcium and positive correlation of phosphate with PTH. This is in agreement with the observation in our study but the difference was not significant. It seems that type 2 diabetics and apparently healthy individuals with Haemoglobin genotype AA are more likely to have hypoparathyroidism and hyperparathyroidism. There is also a probability that apparently healthy individuals with BTT may be considered to have hypoparathyroidism.

5.0 CONCLUSION
This study has shown a high prevalence of hypoparathyroidism among Type 2 diabetes Mellitus and control individuals. Hypoparathyroidism is often associated with complications and comorbidities like neuropsychiatric conditions, kidney dysfunction, kidney stones, extra skeletal calcifications, cataracts and fracture. These findings support the importance of parathyroid disorder and haemoglobin variants screening to detect and reduce its long-term complications. Further studies are required to elucidate the mechanisms that could explore the impact of haemoglobinopathies on parathyroid function in Type 2 Diabetes Mellitus.

LIMITATION OF STUDY
The study limitation was, no measurements of plasma Vitamin D and Magnesium are available for all the participants. Thus, the possibility to determine which individuals were at high risk of primary hyperparathyroidism or secondary hyperparathyroidism due to vitamin D deficiency and magnesium dependent PTH suppression could not be revealed.

CONSENT
Informed consent was administered to all participants after detailed explanation before being recruited into the study.

ETHICAL APPROVAL
Approval to conduct the study was obtained from the University of Ibadan /University College Hospital, Ibadan ethics committee and study executed in agreement with the Declaration of Helsinki.

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COMPETING INTERESTS
Authors have declared no competing interests.

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