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Research article

Low CD4⁺ T Cell count among HIV-seronegative Type 2 Diabetes Mellitus patients in Ilorin metropolis

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Abstract

Background and aims: Diabetes Mellitus (DM) is a metabolic disorder that manifests as chronic hyperglycemia accompanied by a dysfunctional metabolism of carbohydrates, lipids, and proteins. Several studies have earlier pointed out several complications associated with the disease and in particular, the sufferer's susceptibility to various infectious diseases. We therefore sought to investigate the adaptive immune status of the condition, as represented by the assessment of CD4⁺ T cell count among DM patients.

Method: Seventy-six type 2 DM patients were recruited for the study. Thirty (30) age and sex-matched, non-diabetic individuals were enrolled as negative controls. Their fasting blood sugar (FBS), HbA1c, and CD4 count were assayed using standardized procedures. The demographic and clinical data of the studied group and controls were compared with respect to age, sex, BMI, FBS, HbA1c, and CD4⁺ T cell counts.

Result: The mean concentration of glucose (7.82 ± 2.58) and the percentage concentration of HBA1c (8.21 ± 2.31) were significantly higher in DM individuals as against the control (3.67 ± 0.66) (p = 0.0001) and (5.20 ± 0.48) (p = 0.0001) respectively. The CD4⁺ cell count was also significantly lower in DM subjects (843.58 ± 297.6) when compared with the control (1067.9 ±195.4) (p = 0.035).

Conclusion: A significant reduction in CD4⁺ T cell level was noted among diabetic patients in our study, which could be a contributing factor for aggravating some of the associated complications in DM, especially those that involve susceptibility to infectious diseases. We found out that having Hb-AA is associated with normal or elevated CD4⁺ T cells in DM patients; whereas having the Hb-AS variant increases the chance of a low CD4⁺ T cell count. Assessment of CD4⁺ T cell count should be included as part of periodic investigations in DM patients, especially for those with unresolved complications, in spite of treatment.

Introduction

Diabetes Mellitus (DM) is a metabolic disorder that manifests as chronic hyperglycemia accompanied by a dysfunctional metabolism of carbohydrates, lipids, and proteins. The onset and course of DM differ greatly in individuals but always include defects in either insulin secretion or response, or both at some point in the progression of the disease. The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result of this, blood glucose concentration increases, cell utilization of glucose falls increasingly lower and utilization of fats and proteins increases [1]. Approximately 5% of all diabetes is type 1, 90% are type 2, and 5% other subtypes [2,3]. Both type 1 and type 2 diabetes may occur in both the adults and children age groups. Type 2 Diabetes mellitus (T2DM) is widely diagnosed in adults, although its frequency among children has been on the rise in the last two decades, ranging between 8% and 45 % of all new cases of diabetes, depending on the population being studied [4]. Children diagnosed with type 1 diabetes usually manifest the symptoms of polyuria/polydipsia and

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about one-third of sufferers present with Diabetic Ketoacidosis (DKA) [5]. In the case of Type 2 DM, metabolic abnormalities such as dyslipidemia, impaired insulin secretion or insulin resistance, and obesity are key players in the induction and progression of this disorder (T2DM) [6]. T2DM risk factors are multiple and may include a combination of genetic, metabolic, and environmental factors that interact with one another in contributing to its prevalence [7]. The primary events are believed to be an initial deficit in insulin secretion whenever there is beta-cell dysfunction, limiting the body's capacity to maintain normal physiological glucose levels, whereas insulin resistance contributes to increased glucose production in the liver and decreased glucose uptake both in the muscle, liver and adipose tissues. Hyperglycaemia is thus enhanced in many patients in whom the condition of insulin deficiency is coupled with peripheral insulin resistance [7].

The incidence of infections has been shown to increase among persons with Diabetes Mellitus (DM), with the infections having a more complicated course in diabetic than in non-diabetic patients. The risk of infection is common to both type 1 and type 2 DM patients [8]. For instance, respiratory infections, skin and soft tissue infections, and gastrointestinal and genitourinary infections are commonly occurring phenomena in DM patients [8]. The cause has been linked to a decline in the immune system of these ill persons. In early studies that dealt with the immunological regulation of diabetes, greater attention was focused on the innate immune aspect of DM than the adaptive. Induction of immune response caused essentially by activation of myeloid line cells of the innate immune system (macrophages and neutrophils) results in chronic inflammation [9]. Macrophages are known to be the main effector cells during the metabolic process of T2DM [10]. A study on T2DM patients showed that elevated numbers of circulating leukocytes with expression of high levels of inflammatory gene products were found in their blood, but the achievement of glycemic control was able to bring about a reversal of the situation [11]. There is decreased neutrophil recruitment in the uncontrolled diabetic while both neutrophil and macrophage phagocytic activity and thus bactericidal activity of these immune cells become impaired [8]. It was only recently that greater knowledge about the important role the adaptive immune system, and majorly the T lymphocyte, plays in the pathogenesis of diabetes, especially that of T2DM came to light [12]. It is now known that both the innate and adaptive immune systems are involved in the dysregulation of the immune system in diabetes mellitus. Additionally, it has been discovered that abnormal immune cell activation with consequent inflammation is the key factor in T2DM and its related complications [13]. Naive $CD4^+$ T cells were reported to have declined in patients with T2DM, a phenomenon suggested to be associated with adaptive immune activation and chronic inflammation during the pathogenesis of T2DM [14].

T-lymphocytes which mediate cellular immunity in humans consist mainly of $CD4^+$ T cells along with $CD8^+$ T cells. $CD4^+$ T cells are essential players in adaptive immunity as they drive the process of establishing and coordinating protective adaptive immune responses, by providing help to the other subsets of lymphocytes. They are thus called T helper cells [15]. The $CD4^+$ T cells carry out multiple functions, ranging from activation of the cells of the innate immune system, B-lymphocytes, cytotoxic T cells, as well as non-immune cells, in addition to its role in the suppression of immune reaction when appropriate. The activation and differentiation of $CD4^+$ T cells into distinct effector subtypes is the factor majorly responsible for the secretion of key cytokines that mediate the immune response [16].

Some workers have found that obesity induces MHC class II expression in adipocytes. This in turn induces adipose tissue inflammation once $CD4^+$ T cells are activated [17], implying that $CD4^+$ T cells play a vital role in inducing obesity and its associated complications. During the progression of the disease, red blood cells become glycated, while activated Endothelial Cells (ECs) synthesize elevated levels of adhesion molecules and chemokines that facilitate monocyte recruitment, adhesion, and transmigration across the endothelium toward the subendothelial region. Monocytes are then differentiated into macrophages and eventually, by excess lipid uptake, generate foam cells [8]. The presence of foam cells induces the release of pro-inflammatory cytokines that increase the recruitment of additional monocytes, T cells, and neutrophils. This recurrent state of inflammation damages the pancreatic beta cells and leads to insufficient insulin production, thereby resulting in hyperglycemia, a condition known to cause dysfunction of the immune response, specifically by altering the cytokine profile of the affected individual. Thus in many infections, it has been found that diabetes mellitus predisposes to susceptibility and the immune system is unable to control the spread of invading pathogens in affected persons [8,18].

Diabetes is one of the most common health problems globally, particularly in Nigeria; and the disease is associated with high morbidity and mortality in many parts of the world, including even the developed countries. Until now, the $CD4^+$ T cell count of HIV-seronegative diabetic individuals had not been documented in the population under study. This study was therefore initiated to assess the $CD4^+$ T cell count of individuals with Type2 DM, in the Ilorin metropolis. Assessment of the $CD4^+$ T cell count among people with this condition, being a key aspect of the adaptive immune system, hopefully, should further aid the profiling of the health status of the DM patient in our study population.

Materials and methods

The study was carried out among diabetic patients attending the general outpatient clinic at the General Hospital Ilorin, Kwara State. Ethical approval was obtained via a letter from the Research Ethical Review Committee, referenced MOH/KS/EU/777/343. Kwara State Ministry of Health, Ilorin. All subjects consented to the use of their samples for the study. Only diabetic patients who were free from any underlying diseases such as viral diseases - HIV, viral hepatitis B and C were included in the study. Importantly, diabetic patients with homozygous Haemoglobin S, any form of anemia, chronic cardiovascular diseases, etc. that could interfere with the result were excluded, while diabetic patients without these

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complications and who agreed to participate in the research were recruited. The minimum sample size of 69 was calculated for the study using a standard formula. However, 78 persons were eventually recruited as study subjects, out of which two individuals were found to have type 1 DM. The samples of these 2 persons were not investigated further.

Sample collection

Five (5) ml of fasting whole blood specimens from diabetic patients were collected by venipuncture and dispensed into fluoride oxalate (2mls) and EDTA sample bottle (3 mls). The specimen in the fluoride oxalate bottle was used for glucose estimation while the specimen in EDTA was used for hemoglobin electrophoresis, HIV screening test, glycated hemoglobin, and CD4 count. The samples were labeled and given serial numbers.

HIV screening

Plasma was obtained from an aliquot of blood from the EDTA bottle after it had been centrifuged for 10 minutes at 12,000 rpm. Screening was carried out by the immunochromatographic (Determine HIV1 and 2 kit) method.

Haemoglobin genotype investigation

Haemoglobin variants have different electrical charges and therefore separate when it is subjected to an electric field at a voltage of 250 and 5 - 10 mAmp. Two milliliters of distilled water were added to a drop (0.5 ml) of EDTA anticoagulated whole blood sample to haemolyse the red blood cells. Known and validated AA, AS, and AC blood samples were treated in a similar manner and run in parallel with test samples as controls. One hundred (100) ml of the Tris-EDTA borate buffer (pH 8.4) was poured into each outer section of the electrophoresis chamber. A strip of cellulose acetate membrane was soaked in the TRIS buffer in the electrophoresis tank for five minutes and blotted with Whatman filter paper before the haemolysed sample was applied on the cellulose acetate membrane on a straight line, with the aid of a multi-channel applicator, while the membrane was not yet dried. The loaded cellulose acetate membrane was immediately placed on the electrophoresis tank which is powered at the voltage of 240 Volts and 50 mAmp and left for 25 minutes. The result was read visually, based on the migration pattern and the appearance of separation for the known control samples.

Fasting Blood glucose estimation

Glucose present in the plasma is oxidized by the enzyme Glucose Oxidase (GOD) to glucuronic acid with the liberation of hydrogen peroxide. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a pink or red color quinoneimine dye complex whose absorbance can be measured at 520 nm [19].

Glucose + O_2 oxidase > Glucuronic acid + H_2O_2

$2H_2O_2$ + 4- aminoantipyrine + phenol peroxidase > quinoneimine + $4H_2O$

Standard concentration in mmol/l = 5.55 mmol/l

Absorbance of glucose in serum or plasma/absorbance of Standard × concentration of Standard in mmol/l (5.55) mmol/l = glucose concentration in mmol/l

Glycated hemoglobin estimation by Nephelometry method

Approximately 10 ul of the whole blood was lysed using 0.5 ml haemolysing reagent. The haemolysed whole blood containing HbA1c with other hemoglobins compete to adsorb to the unsensitised latex particles in Reagent1. A mouse anti-human HbA1c monoclonal antibody is added to the reaction that specifically binds to the human HbA1c molecules to form a latex HbA1c–mouse anti-human HbA1c antibody complex. Another antibody, goat anti-mouse polyclonal antibody was added that reacted with the formed complex to give agglutination. The degree of agglutination is proportional to the amount of HbA1c adsorbed onto the surface of latex particles [20].

Test procedure

Stage A

0.5 ml of haemolysing reagent (R3) was pipetted into a test tube

10 ul of well-mixed whole EDTA anticoagulated blood was added and mixed thoroughly

It was allowed to stand for 5 minutes to allow for complete hemolysis

Stage B

The card reader was inserted into the card reader slot in the Mispa–i_2 machine and this was prompted to display: add R1 reagent + sample.

180 ul of R1 was pipetted into the cuvette

5 ul of the lysed sample was added to it, then mixed and placed in the cuvette holder.

After proper incubation, the machine was again prompted to display: add R2 reagent.

60 ul of R2 reagent was then added using the attached sensor pipette to the cuvette.

The result was shown in the display interface and printed out.

Calculation

Estimated average glucose (eAG) = (28.7 x HbA1C) - 46.7

CD4 count estimation

An EDTA whole blood sample was mixed with the antibody conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer was added and the sample was ready for analysis on the Counterflow cytometer. The light source excites the fluorescent dye linked with the stained cell

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and the emitted light is detected while a certain volume of blood sample is running through the instrument. The concentration of the dedicated cell population is calculated by the integrated software.

Procedure

The blood sample in the EDTA tube was inverted gently a few times to mix. Then 0.02 ml of EDTA whole blood sample was pipetted straight to the bottom of the tube to avoid blood from the pipette tip being smeared on the tube wall. Exactly 0.02 ml of CD4 antibody was added directly to the blood and mixed gently. The mixture was incubated for 15 minutes at room temperature in the dark. Then 0.8 ml of no lyse buffer was added after which the tube was mixed gently. The script for CD4 measurement was loaded and the sample tube with the prepared blood sample was inserted into the sample port. Lastly, the stained cells were analysed with a Counterflow cytometer.

Statistical analysis

Results obtained were subjected to statistical analysis using the statistical package for social science (SPSS) 22.0 version. Comparison of proportion was done using the T-test, chi-square test, and regression analysis, while P values less than 0.05 were considered significant

Results

The basic characteristics of enrolled participants are presented in Table 1. All participants were of black ethnicity. Based on the established criteria, the participants were classified into two distinct groups: i.e., only two were type 1 DM while type 2 DM were seventy-six, (n =76). Data for the type 1 DM were discountenanced. Thirty (30) age and sex-matched, non-diabetic individuals were enrolled as negative controls.

The mean age for the two groups was approximately 36 years, and most subjects were between 20 and 50 years. The male-to-female ratios were similar and comparable for both the control and pathological groups, that is (2.3) and (1.9) respectively.

Also, the Body Mass Index (BMI) of controls and cases was computed and found to be (22.12 ± 0.93) kg/m^2 for controls and (25.65 ± 1.4) kg/m^2 for the cases; (p = 0.001).

Table 2 outlines the CD4$^+$ T cell number computed by age groups. The result shows that mean CD4$^+$ T cell count decreased with advancing age except in the age range > 65 years in which only 2 people were involved. Females had a higher CD4$^+$T cell count than males, with a mean value of 1163.2 ± 178.3 compared to 803.2 ± 103.9 for the males. Duration of disease did not show any great impact as there were only minor differences in the count among those that have had the disease for <5 years (920.0 ± 098.3), 5 - 10 years (926.8 ± 154.2), and >15 years (973.1 ± 123.3).

As for the hemoglobin variants, the mean CD4$^+$ T cell count in the AS group (698.9 ± 085.4) was significantly lower than were found in both the AA (833.0 ± 201.4) and AC groups (817.4 ± 086.2). The mean count in the AC group is very similar to that of the AA group, although the latter had a wider range.

CD4$^+$ T cell assay result is shown in Table 3. As shown in this table, the mean concentration of glucose (7.82 ± 2.58) and the percentage concentration of HBA1c (8.21 ± 2.31) were significantly higher in DM individuals as against the control (3.67 ± 0.66) (p = 0.0001) and (5.20 ± 0.48) (p = 0.0001) respectively. The CD4 count was also significantly lower in DM subjects (843.58 ± 297.6) when compared with the control (1067.9 ± 195.4) (p = 0.035).

The odds ratio of low CD4$^+$ was calculated between the test and control groups and the odds ratio value was found to be 3.6 (Table 4).

Table 1: Characteristics of the study groups.

Characteristic	Control	Diabetes mellitus	p - value
Age	58.50 ± 10.14	61.39 ± 13.50	0.077
Sex (M/F)	21/9	50/26	ND
BMI	23.06 ± 1.85	25.02 ± 2.17	0.003[a]
HIV status	Negative	Negative	NA
HB genotype (AA/AS)	18/12	34/42	NA
Glucose	3.67 ± 0.66	7.82 ± 2.58	0.000[b]

The values are mean ± SD, while the p-value was determined by the Student's t-test as appropriate; any value with p < 0.05 was considered significantly different, **a:** when there is an intergroup difference.
(NA = Not Applicable, ND = Not Determined, BMI = Body Mass Index).

Table 2: Distribution of CD4$^+$ cells in DM in relation to age, gender, DM duration, and Hb-genotype.

		Mean ± SD (CD4$^+$)	Odds ratio	p - value	95% CI	
					Lower	Upper
Age	35 - 45	963.6 ±125.4	2.34	>0.05	1.54	3.14
	46 - 55	864.1 ± 187.8	2.46	>0.05	1.24	3.68
	56 - 65	754.8 ± 288.3	2.89	>0.05	1.01	4.77
	>65	904.2 ± 176.0	3.43	>0.05	1.54	5.32
Sex	M	803.2 ± 103.9	1.93	>0.05	1.04	2.82
	F	1163.2 ± 178.3	2.11	>0.05	1.12	3.13
DM duration	<5	920.0 ± 098.3	NA	-	-	-
	5 - 10	926.8 ± 154.2	NA	-	-	-
	>10	973.1 ± 123.3	NA	-	-	-
Hb-genotype	AA	833.0 ± 201.4	2.23	>0.05	1.55	2.91
	AS	698.9 ± 085.4	0.94	<0.05	0.83	1.53
	AC	817.4 ± 086.2	1.82	-	-	

Table 3: Comparison of DM profile and CD4 between control and DM.

Parameters	Control	Diabetes Mellitus	p - value
Glucose (mmol/L)	3.67 ± 0.66	7.82 ± 2.58	0.000[a]
HBA1c (%)	5.20 ± 0.48	8.21 ± 2.31	0.000[a]
CD4+ (cell/mm^2)	1067.9 ± 195.4	843.58 ± 297.6	0.035[a]

The values are presented as mean ± SD; the p - value was determined by the Student's t-test as appropriate;
p < 0.05 was considered significantly different, a: when there is an intergroup difference.
Key: HBA1c = Glycated haemoglobin, CD4$^+$ = T lymphocyte subset with a cluster of differentiation four.

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Table 4: Evaluation of Odds ratio of low CD4 between DM and non-DM control subjects.

Parameters	Number with normal CD4+	Number with low CD4+	Odds ratio
Control	25 (83.4 %)	05 (16.6 %)	3.6
DM subjects	44 (57.9 %)	32 (42.1 %)	

Values shown above are the numbers and the percentage (%) of persons with normal or low $CD4^+$ T cells. The odds ratio was calculated in order to compare DM participants with normal control subjects. Exposure to DM increases the odds of low $CD4^+$ T cells to > 1.

Discussion

The study population consisted of 76 adult Nigerians with Type 2 diabetes mellitus (aged 35 years - 57 years) and a control group which consisted of 30 apparently healthy individuals matched for age and sex. All the participants are residents of the Ilorin metropolis. Ilorin is located in the North-central part of Nigeria. Yoruba, Fulani, and partial Hausa ethnic descendants dominate the community. A standard questionnaire and interview method were used to gather relevant profile information from the participants after informed consent had been sought and given. The WHO consent template was slightly modified and used for this study [21]. Questionnaires have the advantages of easy administration and gathering of enormous information. This study is a test case in assessing immune regulation among diabetics.

A total of seventy-eight (78) individuals were recruited for this study. Two of them did not fit into the classification of type 2 DM and were excluded, leaving us with 76 type 2 DM subjects. Their fasting blood sugar (FBS), HbA1c, and CD4 count were assayed using standardized procedures. The demographic and clinical data of the studied group and controls were compared with respect to age, sex, BMI, FBS, HbA1c, and $CD4^+$ T cell counts. The ages of control subjects were comparable with that of the pathology group ($p > 0.05$). Earlier studies had reported that the age of onset of type 2 DM varies from one locality to another. Environmental factors and lifestyle are part of the conditions that determine the age of onset [22].

The Body Mass Index (BMI) was compared between DM test subjects and the control group. Although the pre-morbid weights of the study subjects were not known, it was observed that the DM group had a significantly higher mean BMI compared to the control group. The mechanism/environmental factors that could be responsible for the changes in BMI are not fully understood. However, it could be attributed to the associated dyslipidemia that is common in DM patients [6].

Duration of disease did not show any great impact as there were only minor differences in the count among those who have had the disease for more than 5 years and those who were recently diagnosed. Most workers agree that a prolonged duration of DM is associated with complications. In the work of Ramanathan [23], DM was associated with diabetic nephropathy while Ghouse, et al. [24] found that strict glycaemic control was associated with an increased risk of death among individuals with long (≥ 5 years) diabetes duration, whereas, among those with short diabetes duration, strict glycaemic control was associated with the lowest risk of death. The mean $CD4^+$ T cell count decreased with advancing age except in the age range > 65 years in which only 2 people were involved. Aging naturally weakens the immune system because there is diminished proliferation of $CD4^+$ cells [25,26]; and the additional co-morbidity caused by DM in this case makes it a double-barrel attack that would significantly deplete the immune cells. Females had a higher $CD4^+$ T cell count than males. The reason for this is not known. However, most males have the responsibility of providing for the needs of their household, despite their debilitating condition caused by DM. This puts an added stress on their immune system. Additionally, because males are away from home for a greater part of the day, they hardly have access to nutritious meals that should build up their immune cells.

Generally, the diagnostic criteria for diabetes include fasting plasma glucose, a 2-hour postprandial test, an oral glucose tolerance test, and the use of glycated proteins [27,28]. In addition, some of the glycated proteins such as glycated hemoglobin, glycated albumin, and some other serum proteins collectively known as fructosamine, which are based on thresholds of glycemia that are associated with microvascular disease, can also be used as biomarkers for assessing the level of glycemia in patients with diabetes or pre-diabetes. Among the group of glycated proteins, glycated hemoglobin is the most commonly used in assessing glycemic control [29]; and it is now an assay of choice in the correct diagnosis of DM since it correlates linearly with average glucose concentration in most patients with diabetes because the assay of glycated hemoglobin provides a measure of glycemia that is valid for 2 months - 3 months. In some patients with conditions that may affect the lifespan of the red blood cell, however, the use of glycated hemoglobin may be inappropriate or inadequate for determining the average glucose concentration in such patients [30].

In this study, the mean FBS level was significantly higher in DM participants than in the control. This is in agreement with previous studies. A study by Kumar, et al. [31] showed that FBS levels were consistently higher in DM subjects than in control. The prevalence of DM in Nigeria is 3% [32]. The difference in prevalence from one locality to another may be attributed to variations in genetic make-up, diet, lifestyle pattern, and socioeconomic factors which vary from one locality to another [22]. Indeed, the mean level of fasting glucose among DM patients in our study is more than two-fold of that found in the control subjects. Sequel to insulin resistance/deficiency in DM, the combination of increased hepatic glucose production and reduced peripheral tissue metabolism leads to elevated plasma glucose levels [33], although in some other conditions like Cushing's syndrome, there may be hyperglycemia that is not directly caused by insulin insufficiency. The observed raised fasting plasma glucose in the subjects aligns with the increased Body Mass Index (BMI) in this study. According to Galicia-Garcia, et al. [7], patients with T2DM are mostly characterised by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region. In this condition, adipose tissue promotes insulin resistance through various inflammatory mechanisms, including increased Free Fatty Acid (FFA) release and adipokine deregulation.

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The variants of the subjects' hemoglobin were evaluated in both the test and control groups because abnormal hemoglobin variants such as HbSS and other conditions affecting the lifespan of erythrocytes can affect the levels of glycated hemoglobin [30]. Thus, glycated hemoglobin alone may be inadequate for determining the average glucose concentration in persons with hemoglobinopathy. Therefore, for assessing glycemic state in such patients, other glycated proteins aside from HbA1c are usually employed for predicting diabetes complications likely to develop. The glycated hemoglobin or the fraction of glycated hemoglobin increases in a predictable manner, proportionally to the mean level of plasma glucose. Therefore, it provides the blood sugar level estimate for the past three months, with the recent glucose levels having the greatest influence on its value [34,35]. The outcome of this study revealed that there was a significant difference between the level of glycated hemoglobin in the control and DM participants. Sometimes, a lack of linear correlation might occur between glycated hemoglobin levels and average glucose concentration because numerous factors affect glycated hemoglobin levels, including genetics and hematological factors [27]. However, Niwaha, et al. [29] believe that HbA1c is the optimal test for glycaemic control for DM patients, even in low-income and middle-income countries where other disease conditions that may affect its validity are prevalent. Zhou, et al. [8] have suggested that there is a correlation between glycaemic control and infectious diseases among diabetics, stating that the risk of certain infections was greater among patients with poor glycemic control (HbA1c >8.5%) compared with those with good glycemic control; and that intervening in order to lower glucose levels appears to mitigate the risks. Following this, we found that a comparison of the mean $CD4^+$ T cell level between the control and DM subjects revealed a significantly lower $CD4^+$ T cell count in DM patients. This implies that the low value of the mean $CD4^+$ T cell count observed in DM is associated with the pathophysiology of DM. There is a paucity of information in the literature on the impact of DM on $CD4^+$ T cell count level. Most studies have hitherto linked $CD4^+$ T cell count to HIV infections essentially, without consideration for some other diseases in which the immune status is also diminished. Immunologically, there is some semblance between HIV and type 2 diabetes. For instance, in a similar manner, the immune status is compromised in HIV, so it is with DM, in which susceptibility to all manner of infectious diseases has been observed [8,18]. Earlier studies have focused attention on the innate immune response to DM, ignoring the role of the adaptive immune system in its development. Since poor glycaemic control engenders susceptibility to all manners of infections, and both the innate and the adaptive immune systems are involved in mediating immunity in DM, the low count of $CD4^+$ T cells among diabetic patients in our study depicts an impairment to the adaptive immune system and is a logical prelude to diverse infectious ailments. The lowered $CD4^+$ T cell number in DM patients may partly be responsible for the sufferer's susceptibility to other diseases, in the similitude of what obtains in HIV patients. Indeed, the USA has classified persons with low $CD4^+$ T cell count as persons with disability, since a strong relationship exists between advanced immune impairment and clinical outcomes [36]. $CD4^+$ T cells are essential players in adaptive immunity and may be divided into two subsets - the Treg and the T helper cells [15]. Studies have shown that a decrease in Treg cells in adipose tissue among obese individuals leads to a potent proinflammatory environment and promotes the development of type 2 diabetes [16]. Upon differentiation to Th1, $CD4^+$ cells are able to protect against autoimmune diseases including DM [15,37]. As a result, a decline in the $CD4^+$ T cell number translates to a weakened adaptive immune response with consequent morbid repercussions for the sufferer. Ironically, chronic autoimmune disease conditions also promote memory $CD4^+$ T cell death and inhibit their proliferation and survival [37]. These contrasting roles have been found to be a function of the primary Th subset that initiated the immune response in the first place [37]. It is important to add that the mechanism by which the CD+4 T cells are depleted in HIV/AIDS is different from that of Diabetes mellitus. Girard and Vandiendonck [38] and Berbude, et al. [39] affirmed that diabetic complications are a result of immune system dysregulation present in the patient.

The odds ratio was computed for Hb-AA and Hb-AS variants, different age groups as well as the gender of DM patients. From the results obtained in this study, age, and gender are not associated with the alteration in the $CD4^+$ T cell count. However, the odds ratio for the Hb variants was 1.55 and 0.89 respectively for Hb-AA and Hb-AS. This finding implies that having Hb-AA is associated with normal or elevated $CD4^+$ T cells in DM patients; whereas having the Hb-AS variant increases the chance of a low $CD4^+$ T cell count, with a consequence that the adaptive immune status of the AS individual is lowered, in comparison to normal AA individuals. This is in consonance with widely held views in some studies that the AS variant predisposes to some level of morbidity [40,41], but not to the extent seen in SS individuals. Nevertheless, while the $CD4^+$ T cell count of the AS group is lower than that of the AA group, the count in the AS group in our study is not sufficiently low to be considered as being immune-deficient.

To our knowledge, this study is the first to evaluate the $CD4^+$ T cell count of individuals with diabetes mellitus, although other studies have endeavoured to elucidate the adaptive mechanism involved in this disease condition. However, it is limited by the low number of recruited subjects, necessitated by limited funds, since the study was self-funded.

Conclusion

Diabetic patients usually have significantly poorer glycaemic control with a higher prevalence of complications than their non-DM counterparts. A significant reduction in $CD4^+$ level was noted among diabetic patients in our study, which could be a contributing factor for aggravating some of the associated complications in DM, especially those that involve susceptibility to infectious diseases.

Recommendation

$CD4^+$ T cell count should be included as part of a periodic investigation in DM patients, especially among those with unresolved complications, in spite of treatment. Investigation

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of other immune indices among DM patients is recommended. It is being proposed that intervention to boost the adaptive cellular immunity in addition to conventional treatment, could ameliorate the morbidity associated with Type 2 DM, especially those that involve infectious agents.

Ethical approval

Ethical approval was obtained via a letter from the Research Ethical Review Committee, referenced MOH/KS/EU/777/343. Kwara State Ministry of Health, Ilorin.

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Informed consent

The patients' informed consents for the use of their samples for research were obtained at the point of sample collection.

Authors contribution

Conception or design: A.O.I., T.A.A. Acquisition, analysis and interpretation of data: A.O.I., T.A.A., R.I., T.J.O., W.A.A. Drafting the work or revising: A.O.I., T.J.O, W.A.A. Final Approval of the manuscript: A.O.I., T.A.A., R.I., T.J.O., W.A.A.

Data availability

The authors declare that data supporting the findings of this study are available within the article.

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