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Packed cell volume and Vitamin D levels in visceral leishmaniasis and diabetes mellitus co-morbidity among adults in Osun State, Southwestern Nigeria

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ABSTRACT

This study evaluated the association between the comorbidity of visceral leishmaniasis (VL) and diabetes mellitus (DM), by the evaluation of the packed cell volume (PCV), the renal markers (urea and creatinine) and liver enzymes (ALT and AST) in affected adults in Osun State, Nigeria. Samples were collected from patients across Osun State after obtaining ethical clearance and informed written consent. ELISA method was used for VL and Vitamin D determination in the serum. Liver and kidney function assays were done spectrophotometrically using Randox® kits. A total of 146 unrelated subjects (72 cases and 74 controls) were randomly selected for this study. Mean age and the male: female ratio of case and control were calculated. A comparison of frequencies of tested parameters among the groups was done with the chi-square and p- values below 0.05 were considered significant. The VL positive control patients had significantly higher ALT and AST levels compared to the negative control group. Increases in serum creatinine and aminotransferases (ALT and AST) were observed in VL, although only significantly so ($p<0.05$) in the latter, while increases in serum urea and creatinine, although not significantly so ($p<0.05$), were only observed in DM. Although Vitamin D levels were significantly low in the patients with only VL and only DM, it was observed not to have been significantly reduced in patients with both DM and VL. This study affirms the effects of latent VL infection on liver and kidney functions as well as on Vitamin D levels in the DM patients in the presence of latent VL infection. We suggest a more elaborate study involving a randomized controlled trial (RCT) of vitamin D supplementation for which serum 25-hydroxyvitamin D concentrations and other clinically relevant glycemic indices may be measured in patients on pentavalent antimonial medications for VL.

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1. Introduction

Visceral leishmaniasis (VL) is a disease that is potentially able to cause death. It becomes aggravated by factors such as under-nutrition, low immunity, poor housing, and poverty. Well-fed individuals have a good immune function and as such can fully recover from VL following appropriate treatment (Yang et al., 2013). In terms of a few tools available for control, Leishmaniasis is one of the most neglected tropical diseases (WHO, 2006), and a current significant health problem in North and West Africa, for which drugs are expensive and treatment is cumbersome (Kimutai et al., 2009; Uranw et al., 2013). As many as 12 million people are affected worldwide, with 900,000 to 1.3 million new cases each year, but it is not extensively studied in Nigeria and West Africa (Kone et al., 2019). Recently, this global incidence of leishmaniasis has shown an increasing trend in recent years as a result of increased international travels related to leisure and military activities, human interaction with vector habitats as a result of urbanization, construction, deforestation, and of course other health factors that increase susceptibility, including the human immunodeficiency virus infection, malnutrition, and poverty (Skenderi et al., 2018). However, its diagnosis in travelers in non-endemic countries remains a big challenge since there may not be adequate tools, also because this rare disease mimics more common hematological, viral, or even autoimmune diseases.

Mortality in patients with the disease is reduced to approximately 5% with early therapy and supportive care; without which the mortality is high (75-95%) (Skenderi et al., 2018). Mortality often arises from malnutrition and secondary infection, such as bacterial pneumonia, septicemia, dysentery, tuberculosis, cancrum oris, and uncontrolled hemorrhage or its sequelae (Ryabokon et al., 2016). There have been reports of cases of pentavalent antimonial resistance causing increased mortality rates in some endemic regions (Sundar and Singh, 2016; Torres-Guerrero et al., 2017). Symptoms of VL include fever, loss of appetite, weight loss, lymphadenopathy; and these may be aggravated by pancytopenia, hepatomegaly, splenomegaly, and elevated liver enzymes (Mansueto et al., 2014; Georgiadou et al., 2015).

Vitamin D is an essential fat-soluble vitamin in humans, and it is closely linked to human health. Deficiency in vitamin D can cause many health challenges such as osteoporosis in adulthood (Smith et al., 2017). It has been reported that vitamin D might play its therapeutic role effectively through immune-modulatory properties in type 1 diabetes mellitus. Meanwhile, vitamin D also plays an essential role in protecting pancreatic β-cell to remain functional (Harinarayan, 2014).

A novel strategy for diabetes treatment include targeting the seemingly ubiquitous presence of vitamin D receptors on pancreatic β cells (Bland et al., 2004) and in the skeletal muscle (Maestro et al., 2002), the expression of 1-α hydroxylase in pancreatic β cells (Zittermann, 2006), the presence in the insulin gene of vitamin D response element (Maestro et al., 2003) and the ability of 1,25(OH)D to increase the transcription of insulin receptor genes (Maestro et al., 2002), while at the same time suppressing the renin gene and thus reducing the hyperglycemic-induced increases in renin levels in pancreatic β cells and blockade of renin-angiotensin activity (Cheng et al., 2011).

Vitamin D has a protective effect on diabetes and this is due to calcium, phosphorus, and insulin receptor gene regulation; and its anti-inflammatory potentials (Maestro et al., 2002). It seems that vitamin D increases the calcium content of the cells, which in turn increases the transport of glucose into the muscle (Ojuka, 2004). Vitamin D also regulates the nuclear peroxisome proliferative activated receptor (PPAR) that has an important role in insulin sensitivity (Cohen-Lahav et al., 2007). Therefore, vitamin D deficiency leads to inflammatory diseases. Since vitamin D attenuates the expression of proinflammatory cytokines involved in insulin resistance such as interleukins, IL-1, IL-6, TNF-a, and down-regulates NF-Kb (Nuclear factor) activity (Cohen-Lahav et al., 2007), its deficiency predisposes to inflammatory responses.

As diabetes mellitus is a possible predisposing factor for the development of VL (Joshi et al., 1999), being an immunocompromising disease (Fletcher et al., 2015), it potentially impacts on T lymphocyte function (Clement et al., 2004; Trevelin et al., 2016) which are critical in controlling Leishmania protozoa

(Georgiadou et al., 2015). It may be quite difficult to diagnose VL in travelers in non-endemic countries because its symptoms mimic the more common hematological, viral, or even autoimmune diseases.

Diabetes mellitus (DM) describes a metabolic disorder that arises from defects in insulin secretion, insulin action, or both (Ozougwu et al., 2013). Type 2 diabetes is more common in individuals who have insulin resistance (IR) and usually relative insulin deficiency (American Diabetes Association, 2009; Von Hurst et al., 2013). It is characterized by hyperglycemia, the chronicity of which is associated with long-term damage and failure of various organ systems mainly affecting the eyes, nerves, kidneys, and the heart as well as wound healing in different parts of the body (Standards of Medical Care in Diabetes, 2016). A biochemical basis underlies how DM microvascular and macrovascular complications lead to delayed wound healing (Orasanu and Plutzky, 2009; Chawla et al., 2016). Studies have shown that patients benefit from early glycemic control as the future risk of microvascular and cardiovascular complications is reduced. This benefit is found to be sustained as metabolic memory, beyond the period of the control (Nathan et al., 2005; Holman et al., 2008).

Annually in August, some tourists travel to the Osun Osogbo groove for the festival of the "Yemoja" diety. When Leishmaniasis patients travel to countries where the disease is endemic, an increase in infections with Leishmania in non-endemic countries may be expected (Weisser et al., 2007; Pavli and Maltezou, 2010; Wall et al., 2012). Therefore, it is possible to have infected individuals come into the State, increase the possibility of the incidence of the diseases as the vectors are present at the groove. it can also be left undetected due to the long incubation period which can be up to several years, usually 10 years between exposure and the start of symptoms (Weisser et al., 2007; Yangzom et al., 2012).

Owing to the involvement of vitamin D in inflammation and wound healing, we suggest its role in diabetes mellitus and leishmaniasis co-morbidity. Cross-sectional, longitudinal, observational and intervention studies have shown that vitamin D is a potential diabetes risk modifier (Mitri et al., 2011; Soderstrom et al., 2012; Mitri and Pittas, 2013). Vitamin D insufficiency or deficiency is also detrimental to insulin synthesis and secretion, and it is now recognized as a pandemic (Elsammak et al., 2010; Al-Shoumer and Al-Essa, 2015). Therefore in this study, we sought to measure packed cell volume and vitamin D levels in visceral leishmaniasis and diabetes mellitus co-morbidity among adults in Osun State, Southwestern Nigeria.

2. Materials and Methods

Five millimeters of blood was collected aseptically from the participant by venipuncture. ELISA kit from Diagnostic Automation Inc. was used for the (PCV) hematocrit evaluation, VL and Vitamin D Quantitative determination in human serum. The Leishmania ELISA kit was used for the screening of serum antibodies, primarily IgG, for visceral leishmania and assay and biochemical assessment for Liver and Kidney function tests were carried out spectrophotometrically using Randox commercial kit from Randox, Antrim, UK.

Study Site and sampling

The study was conducted in Osun State, South-western Nigeria, located in the forest and derived savannah zone. The State is politically structured into three senatorial districts namely; Osun Central, Osun East, and Osun West senatorial districts of Osun State, Nigeria. The samples were collected from four selected centers spread across the three senatorial districts, viz: Ladoke Akintola University of Technology Hospital Osogbo, Obafemi Awolowo University Teaching hospital Ile-Ife, Group Diagnostic Laboratory Osogbo, and State General Hospital Ede, Osun State Nigeria. Consented participants were selected randomly from a population of donors attending each of the centers, following ethical approval (LTH/EC/2017/11/338). Five millimeters of intravenous blood was drawn from each individual after obtaining informed written consent from all subjects. The samples were collected from patients across Osun State. A total of 146 unrelated subjects (72 cases and 74 controls) were selected for this study; the mean age of cases and control was derived as well as the male: female ratio in both cases and controls. This study is designed as an

ethnically matched case-control study (age group, sex, and geographical location). A questionnaire was interviewer-administered in either English or Yoruba language depending on the educational level of the patients. Also, ethical clearance was obtained from the Ladoke Akintola University of Technology Teaching Hospital review committee.

Inclusion Criteria

Adult type 2 diabetic subjects with type 2 DM who consent to participate in this study and healthy subjects without the condition as the control.

Exclusion Criteria

Minors, pregnant and lactating women, smokers, and any type 2 DM patient who do not consent to partake in this study.

Standard Operating Procedure

Enzyme-linked Immunosorbent Assay (ELISA) techniques for VL diagnosis and Vitamin D Quantitation in human serum was carried out in flat-bottom 96-well microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark). The plates were coated with 5 μg of acetone-treated antigen/ml (100 μl/well) in a buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated overnight at 4 °C. After being blocked with casein buffer (1% casein in 0.05 M Tris-HCl buffer with 0.15 M NaCl, pH 7.6) for 2 hours at room temperature, the wells were loaded with 100 μl of serum (1:10 dilution in casein buffer) or serum (1:4,000 dilution in casein buffer) and incubated at 37°C for 1 h. The plates were washed four times with PBS containing 0.05% Tween 20 (pH 7.4) and incubated with peroxidase-conjugated anti-human IgG (Tago, Camarillo, Calif.), IgA (Zymed, South San Francisco, Calif.), or IgM (Tago) (1:4,000 dilution in casein buffer) at 37°C for 1 h.

After being washed four times, the plates were incubated with substrate ABTS (KPL Inc., Gaithersburg, Md.) for 1 hour at room temperature, and the optical density was measured at 415 nm. Each sample was assayed in duplicate. Antibody levels were expressed as units based on a standard curve.

Biochemical Assessment

Liver (Alanine aminotransferase and Aspartate aminotransferase) and Kidney (Urea and creatinine) function tests were carried out spectrophotometrically using Randox commercial kit from Randox, Antrim, UK.

Statistical Data Analysis

All data were analyzed using SPSS version 20.0. Frequencies of mutations and haplotypes among the groups the significant difference among the parameters measured were analyzed using chi-square. $P < 0.05$ was considered statistically significant.

3. Results

In the socio-demographic result (Table 1 below), the male participants have a higher percentage than the female gender, the participants with age group 11-20 years were the lowest age group while the age group 41-50 years have the highest percentage of participants. Also, the single (unmarried) participants have the highest percentage while those married have the lowest percentage. The highest percentage of participants possess tertiary education while those with primary education had the lowest.

Table 1: Socio-Demographic variables of research participants

Variable	Frequency	Percentage
Age group		
11-20yrs	5	3.4
21-30yrs	28	19.2
31-40yrs	29	19.9
41-50yrs	32	21.9
51-60yrs	28	19.2
Above 60yrs	24	16.4
Marital status		
Single	121	82.9
Married	12	8.2
Widowed	13	8.9
Educational Level		
Primary	2	1.4
Secondary	60	41.1
Tertiary	84	57.5
Gender		
Male	106	72.6
Female	40	27.4

The PCV evaluation among Apparently Healthy and Diabetic Adults

The central tendencies of PCV and biochemical parameters of the participants showed that participants in the control group have the highest PCV value (41.09± 3.06), showing that the participants in the control group had elevated PCV compared to the DM group. The PCV of participants with a wound is lower than that of diabetes, this is statistically significant ($P<0.05$).

Table 2: Haematocrit (PCV) evaluation among Apparently Healthy and Diabetic Adults

	Group	Mean ± SD	t value	P value
PCV (%)	Control	41.09±3.06	8.449	0.000
	DM	36.68±3.82		

The Prevalence of VL among Apparently Healthy and Diabetic Adults

The result shows that 14 of the 74 participants of the control group have VL with a prevalence of 18.92%. Among the total of the 72 participants of adults living with DM 12 of the participants also tested positive for VL with a prevalence of 16.67%. A total of 26 participants tested positive to VL out of 146 participants (of the control and adults with DM groups) with a total prevalence of 17.81%.

Vitamin D Levels in Participants

The vitamin D level result for the participant groups shows that the control group has a P value of 0.043 from the negative and positive results of 16.41±5.61 41ng/mL and 13.00±5.41ng/mL, respectively, this is statistically significant ($p<0.05$) between its negative and positive groups. Mean ±SD of the control groups also reveals that the Vitamin D level in the negative control group is higher when compared to that of the positive group.

The results from the DM participants showed 13.62±6.55 41ng/mL and 15.42±6.52 41ng/mL for the negative and positive VL in adult DM, respectively (p = 0.338), which was not statistically significant. While Vitamin D levels significantly reduced (p<0.05) in patients with only VL and only DM, it was observed not to have significantly reduced in patients with both DM and VL.

Table 3: Comparison of Vitamin D levels of research participants

Diabetics Status	VL	Mean ±SD (ng/mL)	F value	p-value
Control	Negative	16.41±5.61	4.226	0.043
	Positive	13.00±5.41		
DM	Negative	13.62±6.55	0.756	0.388
	Positive	15.42±6.52		

Creatinine and Urea levels among Control Group Participants:

The creatinine result shows the participants tested negative and positive to VL status had 81.72±8.23 mmol/L and 84.43±8.71 mmol/L, respectively. The p-value of 0.276, was not statistically significant (p<0.05). From the urea result, the participants tested negative and positive to VL status had 4.56±0.69 mmol/L and 4.46±0.32 mmol/L, respectively. With a p-value of 0.583, this was not statistically significant (p<0.05). Increases in serum creatinine and aminotransferase (ALT and AST) were observed in VL, although only significantly so (p<0.05) in the latter.

Table 4: Comparison of Renal markers and Liver enzymes of test and control groups

VL Status		Urea (mmol/L)	Creatinine (mmol/L)	ALT(IU/L)	AST (IU/L)
Negative	Mean± SD	4.56±0.69	81.72±8.23	5.52±2.47	7.55±1.74
	N	60	60	60	60
Positive	Mean± SD	4.46±0.32	84.43±8.71	7.86±4.54	9.14±4.47
	N	14	14	14	14
	F value	0.304	1.206	7.167	4.728
	p-value	0.583	0.276	0.009	0.033

Aspartate transaminase (AST) and Alanine Transaminase (ALT) Level Test for Liver Enzymes among Control Group Participants

Aspartate transaminase (AST) result shows that the participants that tested for negative and positive to VL status have Mean± SD of 7.55±1.74 IU/L and 9.14±4.47 IU/L, respectively. The p-value of 0.033, shows it is statistically significant (p<0.05). Alanine transaminase (ALT) result reveals that the participants tested negative and positive to VL status has 5.52±2.47 IU/L and 7.86±4.54 IU/L, respectively. The p-value of 0.009 shows it was statistically significant (p<0.05).

Urea level and Creatinine test for renal markers among adult living with DM group participants: (mmol/L)

Creatinine result shows that the DM group participants that tested for negative and positive to VL status had 77.18±17.31 mmol/L and 82.33±10.33 mmol/L, respectively. With a p-value of 0.325, it is not statistically significant ($p<0.05$).

Urea result shows that the DM group participants that tested for negative and positive to VL status had 4.84±1.37 mmol/L and 5.30±0.95 mmol/L, respectively. The p-value of 0.270 was not statistically significant ($p<0.05$).

Increases in serum urea and creatinine, although not significantly so ($p<0.05$), were observed in DM.

Aspartate transaminase (AST) and Alanine transaminase (ALT) Alanine transaminase (ALT) and level test for liver enzymes among adult living with DM group participants (IU/L)

AST result shows that the DM group participants that tested for negative and positive to VL status had 8.35±2.25 IU/L and 8.25±1.49 IU/L, respectively. The p-value of 0.883, is not statistically significant ($p<0.05$)

ALT result shows that the DM group participants that tested for negative and positive to VL status had 4.95±1.97 IU/L and 4.92±1.44 IU/L, respectively. The p-value of 0.956, is not statistically significant ($p<0.05$).

Table 5: Comparison of renal markers and liver enzymes of patients with DM

DM Status		Urea	Creatinine	ALT	AST
Negative	Mean ± SD	4.84±1.37	77.18±17.31	4.95±1.97	8.35±2.25
	N	60	60	60	60
Positive	Mean ± SD	5.30±0.95	82.33±10.33	4.92±1.44	8.25±1.49
	N	12	12	12	12
	F value	1.235	0.985	0.003	0.002
	p-value	0.270	0.325	0.956	0.883

4. Discussion

In this study, the liver and renal enzymes markers (alanine aminotransferase, aspartate aminotransferase, and urea, creatinine, respectively), as well as the PCV and vitamin D levels were determined to correlate the possible impact of visceral leishmaniasis on the predisposition to and progression of diabetes in the participating adults, leading to co-morbidity of this double burden disease. The PCV of participants with a wound was lower than that of diabetes ($p<0.05$), which can be due to anemia from bleeding, hemorrhage, or certain medications (Penicillin, tetracycline, aspirin) (Thomas, 2009). Studies have shown that patients with wounds frequently have a low level of hemoglobin and haematocrit, and treatment of leishmaniasis with Pentamidine® cause diabetes mellitus, and nephrotoxicity which often lead to death (Radwan et al., 2007; WHO, 2010; Singh et al., 2012; Jain and Jain, 2013).

The roles of liver enzymes and Vitamin D in the development of type 2 diabetes, and possible progression of visceral leishmaniasis were re-affirmed and as possible factors to observe in the comorbidity of diabetes mellitus and visceral leishmaniasis (Anderson et al., 2010). Patients that tested positive for VL had low

levels of vitamin D. Thus, this vitamin is a biomarker to observe and monitor in the comorbidity of diabetes mellitus and visceral leishmaniasis. The same participants who tested positive for *Leishmania* parasite also had elevated levels of ALT and AST. Visceral leishmaniasis predisposes to liver damage and can be fatal thereby leading to chronic hepatotoxicity if not treated on time. Vitamin D deficiency is an important causation factor for the development of many complications and comorbidities with diabetes mellitus. The role liver function and Vitamin D play in type 2 diabetes, development and possible progression of visceral leishmaniasis as observed in this study, show clinical relevant factors to observe in the comorbidity of diabetes mellitus and visceral leishmaniasis.

This study shows that in Osun State, Southwestern Nigeria, there are adults with visceral leishmaniasis and that some of them are at the same time diabetic. Among the one hundred and forty-six 146 (100%) participants, twenty-six (26) (17.81%) tested positive to Leishmania parasite, (12) twelve (16.67%) of the seventy-two (72) total participants in the case (DM) study group tested positive to both DM and VL, while only (14) fourteen (18.92%) of the total seventy-four (74) participants of the control group tested positive for VL. Most of the participants that tested positive to Leishmania are unemployed. Their poverty level and poor housing conditions are strong risk factors of VL (Quinnell and Courtenay, 2009). This is lesser to the prevalence of VL in other countries such as an annual estimate for the incidence and prevalence of VL (kala-azar) cases worldwide is 0.5 million and 2.5 million, respectively. Of these, 90% of the confirmed cases occurred in India (Bora, 1999; Epco et al., 2012), Nepal (Joshi et al., 2009), Brazil (Lisiane et al., 2017), Sudan (Zijlstra and el-Hassan, 2001), Iraq. This can be as a result of the fact that epidemics of visceral leishmaniasis flourish under conditions of famine and poverty, among other conditions (Boelaert et al., 2009).

Visceral leishmaniasis predisposes to liver damage and can be fatal thereby leading to chronic hepatotoxicity if not treated on time (Ayerden et al., 2008; Long and Dagogo-Jack, 2011). Participants who tested positive for Leishmania parasite in this research have low Vitamin D and elevated ALT and AST levels, which are statistically significant ($P<0.05$). This is similar to the study carried out in Thi Qar, Iraq on the effect of visceral leishmaniasis on some liver enzymes, evaluating 121 confirmed cases of visceral leishmaniasis (Dawood, 2008).

Rodents infected with *Plasmodium berghei* were successfully protected from cerebral malaria, with oral administration of Vitamin D (He et al., 2014). Also, a study on African children of Ethiopia origin revealed the prevalence of vitamin D deficiency (<20 ng/mL) to be greater than 50% in individuals living with VL. However, a relationship could not be established between vitamin D levels and clinical VL, because a healthy control group was not used in the study (Diro et al., 2015). In contrast to this, patients with high levels of anti-inflammatory IL-10 cytokine and dermal leishmaniasis on account of with Kala Azar presented a significant increase in vitamin D levels when compared to healthy controls (Mukhopadhyay et al., 2014).

Leishmania species are obligate intracellular parasites that are eliminated by a strong Th-1 host response. Vitamin D treatment reduces inappropriate Th-1 responses thus reducing or eliminating symptoms of autoimmune diseases. Low vitamin D levels in humans have been associated with a large number of diseases like multiple malignancies, cardiovascular diseases, metabolic disorders, autoimmune disorders, and infectious diseases (Plum and DeLuca, 2010; Lang et al., 2013; Feldman et al., 2014). Gender has also been shown to affect innate responses to Leishmaniasis and other infections (Guerra-Silveira and Abad-Franch, 2013). Also, the male gender is a risk factor for VL, as it is not just a socio-cultural determinant, especially after the age of 10 (Cloots et al., 2020).

Various assessments done to establish associations between vitamin D levels and protozoan infections in rodents yielded inconsistent results in the past (Ehrchen et al., 2007; Rajapakse et al., 2007; Ramos-Martínez et al., 2013). However, studies within a decade ago have shed light on this issue by demonstrating a role for vitamin D in Babesia infections in dogs (Kuleš et al., 2014), as well as in *Plasmodium falciparum* infection in children where vitamin D deficiency was also associated with severe cerebral malaria in Uganda (Cusick et al., 2014).

Vitamin D deficiency in patients could be linked to an excess of vitamin consumption during the inflammatory process, following a similar pattern as for vitamin A during chickenpox infection. One of the explanations that have been rendered to support this hypothesis is the rapid conversion of 25(OH)D to the bioactive 1, 25(OH)2D by inflammatory cytokines that activate the enzyme catalyzing the reaction. Unfortunately, the 1,25(OH)2D levels in the canine population could not be analyzed, as its half-life is very short and the test for its evaluation is not readily done in most laboratories.

Another hypothesis linking vitamin D and leishmaniasis is that dogs with vitamin D insufficiency before infection might be at a higher risk of developing leishmaniasis. Although vitamin D levels have been determined in other canine infectious diseases (Rosa et al., 2013; Kuleš et al., 2014), not many longitudinal studies have been documented so far. The few longitudinal studies conducted in humans reported vitamin D deficiencies before clinical manifestations of multiple sclerosis (Décard et al., 2012), pulmonary exacerbation in children with cystic fibrosis (McCauley et al., 2014), and more severe inflammatory bowel disease (Kabbani et al., 2016).

In a group of patients with malignant neoplasias, colorectal cancer was found to be consistently associated with low pre-diagnostic vitamin D levels (Fedirko et al., 2012). Both low vitamin D-dietary intake and low UV light exposure have been established as the main causes of vitamin D deficiency in humans. Besides, vitamin D also exerts its immunomodulatory role by shaping B cell and T cell responses. Exposing human B cells to 1,25 (OH)2D inhibits their proliferation, IgG secretion, and memory B cell generation, and induces B cell apoptosis (Chen et al., 2007).

Vitamin D deficiency is considered a pandemic and a worldwide problem for human health (Holick and Chen 2008; Riaz et al., 2016; Smith et al., 2017; Sokolovic et al., 2017). The 25-Hydroxyvitamin D which is a biological marker of vitamin D can be measured to monitor the vitamin D level in the human blood sample (Forouhi et al., 2012). A serum level of 25-hydroxyvitamin D more than 75 nmol/L (30 ng/ml), is required to yield vitamin D's beneficial effects for health, and vitamin D deficiency is defined as serum 25(OH) D level less than 20 ng/ml (Holick and Chen 2008; Shehab et al., 2012).

The importance of nutrients in fortifying immune response especially in infection and disease cannot be overemphasized (Maggini et al., 2007; Kau et al., 2011). Therefore, the efficacy of drug therapy can be potentially improved if administered with planned nutrition that serves as immunostimulants towards improving recovery.

It is documented in the literature that there is an indirect relationship between vitamin D level and DM complications. Complications of diabetes mellitus affect patients with DM, a major cause of morbidity, and increased mortality (Argoff et al., 2006). It is important to clarify the potential risk factor, as we all known complications increased with age and diabetic duration. Some studies equally established that vitamin D level makes a tremendous impact on the generation and development of DM and its complications (Penckofer et al., 2008; Pittas et al., 2017).

Conclusion

From the results, a brief but very important conclusion can be drawn as follows: some adults are living with comorbidity of visceral leishmaniasis and diabetes mellitus in Osun State, southwestern Nigeria, whereas, the poverty level, unemployment, and poor housing conditions are strong risk factors of VL. An urgent need to supply vitamin D appropriately to type 2 diabetes is important for preventing the condition from degenerating to more complications of comorbidities with other chronic diseases, including VL. On the other hand, prolonged use of antimonial VL drugs predisposes to the onset of diabetes mellitus. Detailed travel history is crucial; the data which may not be well documented and curated as VL has a very long incubation period of up to several years. Immunocompromised patients with HIV, autoimmune diseases, chronic alcohol abuse, or diabetes mellitus are at a higher risk of VL than immunocompetent individuals and may face lower cure and higher relapse rates.

Recommendations

A healthy life and nation have a pivotal role in the social, economic, political, and scientific implications of the drive towards a green and sustainable development in the utilization of renewable resources. Therefore,

1) there is a need for public health enlightenment on the prevalence of visceral leishmaniasis and how to prevent the same.
2) the government should provide facilities at the health and the diagnostic centers for visceral leishmaniasis diagnosis and treatment; as some patients may have Leishmania parasite and be asymptomatic.
3) the governments should launch housing plans in rural areas because this may bring about positive control as poor housing is a risk factor of VL spread.
4) based on the outcome of this study, it is recommended that further observational studies and randomized controlled trials (RCTs) of vitamin D supplementation be conducted.

Conflict of interest: The authors hereby declare no conflict of interest.

Data access statement: Data can be accessed upon request from authors.

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