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STUDIES ON SERUM ERYTHROPOIETIN AND RED CELL PARAMETERS OF PATIENTS WITH TYPE 2 DIABETES MELLITUS IN SOUTHEAST, NIGERIA

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ABSTRACT

Diabetes mellitus especially the type 2 is a serious global public health threat to the existence of human beings on earth. The study was done to determine the levels of erythropoietin and red cell parameters in patients with type 2 diabetes mellitus in Southeast, Nigeria. The study was done in a tertiary health institution. A total of 200 subjects were recruited for the study comprising 100 subjects each for Patients with type 2 DM (50 subjects were Males, 50 were Females) and 100 subjects for apparently healthy subjects (Control) (50 subjects were Males, 50 were Females) drawn from the Health institution. About 6ml of venous blood was aseptically collected from the antecubital vein of each subject by standard technique. About 4.5ml was dispensed into plain tubes for Erythropoietin assay and the remaining was dispensed into an EDTA bottle for haematological parameters determination. All reagents and kits were commercially purchased from reputable company whose standard operating procedures were strictly followed. Human EPO (Erythropoietin) ELISA kit was purchased from Elabscience with catalog No**:** E-EL-H0066c. The haematological parameters were determined using Mindray BC-5300. The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at $P<0.05$. The results showed decrease in RBC (3.42±0.31 $X10^{12}$/L, 5.01±0.45 $X10^{12}$/L, P=0.0000), haemoglobin (10.25±0.92g/dl, 15.04±1.34g/dl, P=0.000), PCV (30.75±2.76%,45.134.02%, P=0.000), increase in erythropoietin (93.45±22.93iu/l, 21.06±6.59iu/l, P=0.000) and no significant difference in MCV (89.95±0.07fl, 90.00±0.06fl, P=0.100), MCH (29.99±0.02pg, 30.00±0.02pg, P=0.170) and MCHC (333.38±0.10g/l, 333.32±0.06g/l, P=0.227) of patients with DM relative to control. The study revealed decrease in red blood cells, haemoglobin, packed cell volume and increase in erythropoietin of patients with diabetes mellitus relative to apparently healthy individuals. Other parameters showed no significant difference when compared between the patients with diabetes mellitus relative to apparently healthy individuals.

INTRODUCTION

Diabetes mellitus especially the type 2 is a serious global public health threat to the existence of human beings on earth. It has been a major cause of deaths in this part of the world and has continued to elude treatment and the prevalence has continued to rise steadily (Jaman *et al.*, 2019).

Erythropoietin (EPO) is a glycoprotein hormone that regulates erythropoiesis. It is a cytokine for erythrocyte precursors in the bone marrow. Human erythropoietin has a molecular weight of 30.4 kDa (Siren *et al.*, 2001). It is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury (Siren *et al.*, 2001). EPO is also involved in the wound healing process (Haroon *et al.*, 2003).

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin (Ashby *et al.,* 2010). Multiple studies have suggested that erythropoietin improves memory. This effect is independent of its effect on hematocrit (Miskowiak *et al.,* 2007; Miskowiak *et al.,* 2007; Adamcio *et al.,* 2008).

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR)(Livnah *et al.*,1998;Middleton *et al.*,1999). Erythropoietin levels in blood are quite low in the absence of anemia,averaging at around 10 mU/ml. However, in hypoxic stress, erythropoietin production may increase 1000-fold, reaching 10,000 mU/ml of blood. Regulation is believed to rely on a feedback mechanism measuring blood oxygenation (Jelkam *et al.*,2007;, Obeagu,2015). Each erythropoietin molecule has two erythropoietin receptor (EpoR) binding sites. There are two affinities of the EpoR for erythropoietin in solution: one of high and one of low affinity (needs 1,000 times the concentration of erythropoietin for activation) (Weidemann and Johnson, 2009).

This study becomes imperative as many patients that present in the different hospitals and laboratory are faced with the challenges of multsystemic damage as a result of hyperglycaemia.. Haeamtological parameters especially red cell indices are good indicators of health and disease states (Obeagu *et al.,* 2017, Obeagu *et al.,* 2018).

The study was done to determine the levels of erythropoietin and red cell indices in patients with type 2 diabetes mellitus in Southeast, Nigeria.

MATERIALS AND METHODS

Study Area
The study was done in Parklane Hospital, Enugu, Nigeria.

Subjects
A total of 200 subjects were recruited for the study comprising 100 subjects each for Patients with type 2 DM (50 subjects were Males, 50 were Females) and 100 subjects for apparently healthy subjects (Control) (50 subjects were Males, 50 were Females) drawn from the Health institution.

Sample collection
About 6ml of venous blood was aseptically collected from the antecubital vein of each subject by standard technique. About 4.5ml was dispensed into plain tubes for Erythropoietin assay and the remaining was dispensed into an EDTA bottle for haematological parameters determination. The blood samples for serum were allowed to clot for 2 hours at room temperature before centrifugation for 20 minutes at approximately 1000Xg. EDTA whole blood was used for haematological parameters determination.

Laboratory investigations
All reagents and kits were commercially purchased from reputable company whose standard operating procedures were strictly followed. Human EPO (Erythropoietin) ELISA kit was purchased from Elabscience with catalog No**:** E-EL-H0066c. The erythropoietin was bought from Elabscience Biotechnology Co.Ltd,Wuhan.

Assay procedure
All the reagents were allowed to reach room temperature, mixed thoroughly by gently swirling before pipetting.

100µL of standard, blank, or sample was added per well. The blank well was added with reference standard and sample Diluent .Solutions were added to the bottom of micro ELISA plate well, mixed gently and covered the plate with sealer and incubated for 90 minutes at 37^0C.

The liquid of each well was removed. 100µL of biotinylated Detection Antibody working solution was added immediately to each well and covered with plate sealer. The plate was gently tap to ensure thorough mixing and then incubated for 1 hour at 37^0C. Each well was aspirated and washed 3 times. It was washed by filling each well with wash buffer (approximately 350 µL. At the last wash the remaining wash buffer was removed. The plate was inverted and pat against thick clean absorbent. 100 µL of HRP conjugated working solution was added to each well and covered with the plate sealer and incubated for 30 minutes at 37^0C. The wash process was repeated 5 times as in step 3. 90 µL of substrate solution was added to each well and covered with a new plate sealer and was incubated for about 15 minutes at 37^0C. 50 µLof stop solution was added to each well and colour turned to yellow immediately. The optical density (OD) of each well was determined at once using a microplate reader set to 450nm.

Haematological investigations
The haematological parameters were determined using Mindray BC-5300. The haematological parameters investigated include RBC, Haemoglobin, PCV, MCV, MCH, MCHC and EPO.

Ethical Consideration
The details of the research were explained to the subjects and written consents obtained from them and were assured of joining the study willingly and confidentiality also assured. The subjects who gave their consents were allowed to participate in the study.

Statistical Analysis
The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at $P<0.05$.

RESULTS

Table 1: Mean ± SD of erythropoietin and haematological parameters of patients with DM and control.

Parameters	DM	CONTROL	P-Value
RBC($X10^{12}$/L)	3.42±0.31	5.01±0.45	0.000*
Haemoglobin(g/dl)	10.25±0.92	15.04±1.34	0.000*
PCV (%)	30.75±2.76	45.134.02	0.000*
MCV(fl)	89.95±0.07	90.00±0.06	0.100NS
MCH(pg)	29.99±0.02	30.00±0.02	0.170NS
MCHC(g/l)	333.38±0.10	333.32±0.06	0.227NS
EPO(iu/l)	93.45±22.93	21.06±6.59	0.000*

The results showed decrease in RBC (3.42±0.31 $X10^{12}$/L, 5.01±0.45 $X10^{12}$/L, P=0.0000), haemoglobin (10.25±0.92g/dl, 15.04±1.34g/dl, P=0.000), PCV (30.75±2.76%,45.134.02%, P=0.000), increase in erythropoietin (93.45±22.93iu/l, 21.06±6.59iu/l, P=0.000) and no significant difference in MCV (89.95±0.07fl, 90.00±0.06fl, P=0.100), MCH (29.99±0.02pg, 30.00±0.02pg, P=0.170) and MCHC (333.38±0.10g/l, 333.32±0.06g/l, P=0.227) of patients with DM relative to control.

Table 2: Mean ± SD of erythropoietin and haematological parameters of patients with DM based on sex.

Parameters	Male	Female	P-Value
RBC($X10^{12}$/L)	3.50±0.28	3.34±0.35	0.486NS
Haemoglobin(g/dl)	110.50±0.84	10.00±1.05	0.488NS
PCV (%)	31.50±2.52	30.00±3.16	0.486NS
MCV(fl)	89.94±0.07	89.96±0.07	0. 702NS
MCH(pg)	29.98±0.02	29.99±0.03	0.401NS
MCHC(g/l)	333.33±0.13	333.42±0.06	0.269NS
EPO(iu/l)	87.50±21.21	99.40±26.13	0.506NS

The results showed no significant difference in RBC (3.50±0.28 $X10^{12}$/L, 3.34±0.35 $X10^{12}$/L, P=0.486), haemoglobin (110.50±0.84g/dl, 10.00±1.05g/dl, P=0.488), PCV (31.50±2.52%,30.00±3.16%, P=0.486), MCV (89.94±0.07fl, 89.96±0.07fl,P=0. 702), MCH (29.98±0.02pg, 29.99±0.03pg, P=0.401), MCHC (333.33±0.13 g/l, 333.42±0.06g/l, P=0.269) and erythropoietin (87.50±21.21iu/l, 99.40±26.13iu/l, P=0.506) of patients with DM relative to control).

Table 3: **Mean ± SD of erythropoietin and haematological parameters of patients with DM based on age ranges.**

Parameters	40-50 YEARS	51-60 YEARS	61-70 YEARS	F-VALUE	P-VALUE
RBC($X10^{12}$/L)	3.67±0.16	3.56±0.00	3.22±0.33	2.303	0.196NS
Haemoglobin(g/dl)	11.00±0.67	10.67±0.00	9.67±0.98	2.285	0.197NS
PCV (%)	33.00±1.41	32.00±0.00	29.00±2.94	2.277	0.198NS
MCV(fl)	89.92±0.04	89.89±0.00	89.99±0.06	3.167	0.129NS
MCH(pg)	29.97±0.00	29.97±0.00	30.00±0.02	2.500	0.177NS
MCHC(g/l)	333.34±0.14	333.44±0.00	333.36±0.12	0.457	0.657NS
EPO(iu/l)	75.00±10.60	82.50±0.00	108.15±24.39	2.352	0.191NS

The results showed no significant difference in RBC (3.67±0.16 $X10^{12}$/L, 3.56±0.00 $X10^{12}$/L, 3.22±0.33 $X10^{12}$/L, P=0.196), haemoglobin (11.00±0.67g/dl, 10.67±0.00g/dl, 9.67±0.98g/dl, P= 0.197), PCV (33.00±1.41%,32.00±0.00%, 29.00±2.94%, P=0.198), MCV (89.92±0.04fl, 89.89±0.00fl, 30.00±0.02fl, P=0.129), MCH (333.34±0.14pg, 29.97±0.00pg, 30.00±0.02pg,P= 0.177), MCHC (333.34±0.14g/l, 333.44±0.00g/l, 333.36±0.12g/l, P= 0.657) and erythropoietin (75.00±10.60iu/l, 82.50±0.00iu/l, 108.15±24.39iu/l, P=0.191) when compared among the age groups of patients with DM.

Table 4: **Mean ± SD of erythropoietin and haematological parameters of patients with DM among 40-50 years and 51-60 years.**

Parameters	40-50 YEARS	51-60 YEARS	P-VALUE
RBC($X10^{12}$/L)	3.67±0.16	3.56±0.00	0.694^{NS}
Haemoglobin(g/dl)	11.00±0.67	10.67±0.00	0.693^{NS}
PCV (%)	33.00±1.41	32.00±0.00	0.690^{NS}
MCV(fl)	89.92±0.04	89.89±0.00	0.589^{NS}
MCH(pg)	29.97±0.00	29.97±0.00	1.000^{NS}
MCHC(g/l)	333.34±0.14	333.44±0.00	0.419^{NS}
EPO(iu/l)	75.00±10.60	82.50±0.00	0.716^{NS}

The results showed no significant difference in RBC (3.67±0.16 $X10^{12}$/L, 3.56±0.00 $X10^{12}$/L, P=0.694), haemoglobin (11.00±0.67g/dl, 10.67±0.00g/dl, P=0.693), PCV (33.00±1.41%,32.00±0.00%, P=0.690), MCV (89.92±0.04fl, 89.89±0.00fl, P=0.589), MCH (333.34±0.14pg, 29.97±0.00pg, P=1.000), MCHC (333.34±0.14g/l, 333.44±0.00g/l, P=0.419) and erythropoietin (75.00±10.60iu/l, 82.50±0.00iu/l, P=0.716) when compared among40-50 years and 51-60 years age groups of patients with DM.

Table 5: **Mean ± SD of erythropoietin and haematological parameters of patients with DM among 40-50 years and 61-70 years**.

Parameters	40-50 YEARS	61-70 YEARS	P-VALUE
RBC($X10^{12}$/L)	3.67±0.16	3.22±0.33	0.107^{NS}
Haemoglobin(g/dl)	11.00±0.67	9.67±0.98	0.108^{NS}
PCV (%)	33.00±1.41	29.00±2.94	0.108^{NS}
MCV(fl)	89.92±0.04	89.99±0.06	0.156^{NS}
MCH(pg)	29.97±0.00	30.00±0.02	0.127^{NS}
MCHC(g/l)	333.34±0.14	333.36±0.12	0.847^{NS}
EPO(iu/l)	75.00±10.60	108.15±24.39	0.107^{NS}

The results showed no significant difference in RBC (3.67±0.16 $X10^{12}$/L, 3.22±0.33 $X10^{12}$/L, P=0.107), haemoglobin (11.00±0.67g/dl, 9.67±0.98g/dl,P=0.108), PCV (33.00±1.41%,29.00±2.94%, P=0.108), MCV (89.92±0.04fl, 30.00±0.02fl, P=0.156), MCH (333.34±0.14pg, 30.00±0.02pg,P=0.127), MCHC (333.34±0.14g/l, 333.36±0.12g/l, P=0.847) and erythropoietin (75.00±10.60iu/l, 108.15±24.39iu/l, P=0.107) when compared among the age groups of 40-50 years and 61-70 years patients with DM.

Table 6: **Mean ± SD of erythropoietin and haematological parameters of patients with DM among 51-60 years and 61-70 years.**

Parameters	51-60 YEARS	61-70 YEARS	P-VALUE
RBC($X10^{12}$/L)	3.56±0.00	3.22±0.33	0.199^{NS}
Haemoglobin(g/dl)	10.67±0.00	9.67±0.98	0.202^{NS}
PCV (%)	32.00±0.00	29.00±2.94	0.203^{NS}
MCV(fl)	89.89±0.00	89.99±0.06	0.067^{NS}
MCH(pg)	29.97±0.00	30.00±0.02	0.127^{NS}
MCHC(g/l)	333.44±0.00	333.36±0.12	0.453^{NS}
EPO(iu/l)	82.50±0.00	108.15±24.39	0.189^{NS}

The results showed no significant difference in RBC (3.56±0.00 $X10^{12}$/L, 3.22±0.33 $X10^{12}$/L, P=0.199), haemoglobin (10.67±0.00g/dl, 9.67±0.98g/dl, P=0.202), PCV (32.00±0.00%, 29.00±2.94%, P=0.203), MCV (89.89±0.00fl, 30.00±0.02fl, P=0.067), MCH (29.97±0.00pg, 30.00±0.02pg,P=0.127), MCHC (333.44±0.00g/l, 333.36±0.12g/l, P= 0.453) and erythropoietin (82.50±0.00iu/l, 108.15±24.39iu/l, P=0.189) when compared among the age groups 51-60 years and 61-70 years of patients with DM.

DISCUSSION

Diabetes mellitus especially the type 2 is a serious global public health threat to the existence human beings on earth. It has been a major cause of deaths in the part of the world and has continued to elude treatment and the prevalence has continued to rise steadily (Jaman *et al.*, 2018). The study revealed decrease in red blood cells, haemoglobin, packed cell volume and increase in erythropoietin of patients with diabetes mellitus relative to apparently healthy individuals. Other parameters showed no significant difference when compared between the patients with diabetes mellitus relative to apparently healthy individuals. This shows that kidney

fibroblasts may be affected causing aneamia in the patients with increased erythropoietin levels. The red cell indices were not changed showing that the anaemia in the diabetes mellitus patients may be normocytic normochromic anaemia. When the parameters were compared among patients with diabetes mellitus based on sex and age groups showed no significant difference. The target in the management in patients with diabetes mellitus should be on red blood cells, haemoglobin, packed cell volume and also erythropoietin levels to ensure that the dangers of anaemia are prevented. Haeamtological parameters especially red cell indices are good indicators of health and disease states (Obeagu *et al.*, 2017, Obeagu *et al.*, 2019).

CONCLUSION

The study revealed decrease in red blood cells, haemoglobin, packed cell volume and increase in erythropoietin of patients with diabetes mellitus relative to apparently healthy individuals. Other parameters showed no significant difference when compared between the patients with diabetes mellitus relative to apparently healthy individuals. The clinicians and all the health workers who are involved in managing patients with diabetes mellitus should monitor red cells, haemoglobin and packed cell volume to prevent anaemia by regulating the levels of erythropoietin in the patients.

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