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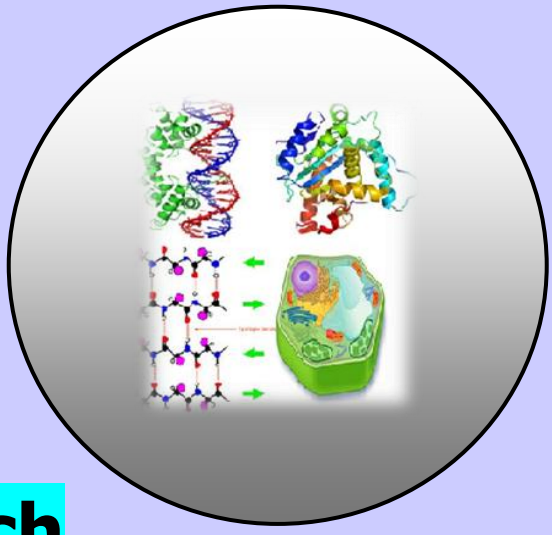

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RESEARCH PAPER

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Correlation of Erythropoietin and Haematocrit in Diabetic Nephropathy Patients in Federal Medical Centre, Umuahia, Nigeria

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ABSTRACT

The study was done to determine the levels of erythropoietin(Epo) and haematocrit in some diabetic nephropathy patients in Federal Medical Centre, Umuahia, Abia State, Nigeria. A total of one hundred and twenty (120) subjects (60 males and 60 females) were recruited for the study. Sixty (60) subjects (30 and 30 females) were those with diabetic nephropathy and sixty (60) subjects (30 and 30 females) were the apparently healthy controls. Venous blood samples were collected from the subjects using aseptic standard method from the antecubital fossa.Serum samples were used for erythropoietin assay and EDTA anticoagualted blood used for Packed Cell Volume tests. Erythropoietin was determined by sandwich ELISA method and PCV was analysed by microhaemtocit method. The results were statistically analysed using student t-test and Pearson Product Moment method and statistical significance set at P<0.05. The result showed significant decrease (P<0.05) in the Erythropoietin and PCV levels of diabetic nephropathy subjects (42.3 ± 21.3 iu/l, 30.8 ± 6.1%) compared to control subjects (48.6 ± 24.0 iu/l, 43.8 ± 4.9%) respectively. The correlation of erythropoietin and PCV a negative (inverse) correlation (relationship) in diabetic nephropathy subjects.
Keywords: Correlation, Erythropoietin, Haematocrit and Diabetic Nephropathy Patients.

INTRODUCTION

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.,* 2007a; Miskowiak *et al.,* 2007b). Rather, it is associated with an increase in hippocampal response and effects on synaptic connectivity, neuronal plasticity, and memory-related neural network (Adamcio *et al.,* 2008). Erythropoietin may have effects on mood (Miskowiak *et al.*, 2007a).

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. Erythropoietin is produced mainly by peritubular capillary lining cells of the renal cortex, which are highly specialized, epithelial-like cells. It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver Regulation is believed to rely on a feedback mechanism measuring blood oxygenation (Jelkmann *et al.*, 2007). Constitutively synthesized transcription factors for erythropoietin, known as hypoxia-inducible factors, are hydroxylated and proteosomally digested in the presence of oxygen.

Erythropoietin is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids (Mocini *et al.,* 2007). Erythropoietin has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues. Its carbohydrate portion contributes 40% of its molecular weight (Mocini *et al.*, 2007). The N-linked polysaccharide side chains appear to be important for the biosynthesis and secretion of erythropoietin, enhance its stability in blood, and limit hepatic clearance, thus facilitating the systemic transit of erythropoietin from kidney to bone marrow (Obeagu, 2015).

The variable nature of the sialic acid content gives rise to erythropoietin isoforms with differences in charge. As the number of sialic acid groups on the carbohydrate portion of erythropoietin increase, so does its serum half-life, whereas receptor-binding capacity decreases (Cartlin *et al.,* 2002; Elliot *et al.*, 2004; Middleton *et al.,* 1999; Weidemann and Johnson, 2009). Clearance, however, appears to have a stronger influence on *in vivo* activity than receptor-binding affinity.

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).

Many factors have been suggested as the reason for the ealier onset of anaemia in patients with diabetes, including severe sympathetic denervation of the kidney and loss of appropriate erythropoietin production; damage to the renal interstitium, systemic inflammation ;and inhibition of erythropoietin release. It has also been shown that a normochromic,normocyttic anaemia can occur before evidence of renal impairment is present (Kathrine *et al.,* 2005).

Aim

The aim of this study is to estimate erythropoietin and haematocrit levels in diabetic nephropathy Patients in Federal Medical Centre, Umuahia.

MATERIAL AND METHODS

Study area

The study was conducted at the Federal Medical Centre,Umuahia.Umuahia is the capital of Abia State,with a population of 264,662 and covers a land area of about 245KM^2.It lies on Latitude 5.52627 (decimal degree) North and Longitude 7.48959 (decimal degree) East with an elevated alttitude of 152 meters (NPC, 2006).

Umuahia is inhabited by the three major tribes of Nigeria but dominated by the Igbo speaking people.They are more of traders,Technical workers,civil servants and farmers.About two Universities are located there.They have few government hospitals which the most respected is Federal Medical Centre,Umuahia.They patronise drug dealers without consulting medical doctors,take a lot of NSAIDS,use herbal medicines,high prevalence of UTI and have few markets to buy better food stuffs.

Advocacy, Pre-survey contacts and ethical considerations

With a letter of introduction from the Department,the secretary,Health Research and Ethical Committee of the Health Institution was met with a well detailed research proposal, after which an ethical approval was obtained for the research work.

Study population and enrolments

The study group (patients) were recruited from the Urology Department of Federal Medical Centre, Umuahia by the help of some nurses who helped in retreiving the patients' folders after they gave their consents. A total of one hundred and twenty (120) subjects were recruited for the study whose age ranges from 35-71 years. Sixty (60) subjects (30 males and 30 females) were diabetic mellitus patients(diabetic nephropathic patients) and sixty (60) subjects (30 males and 30 females) were the controls who were apparently healthy individuals.

Sample Collection

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

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.

Principle of Erythropoietin (Sandwich-Elisa Method) of Elabscience

The ELISA kit uses sandwich-ELISA as the method. The micro ElISA plate provided in this kit was coated with an antibody specific to Erythropoietin.Standard or samples are then added to the appropriate micro ELISA plate wells and combined to the specific antibody. Then a biotinylated detection antibody specific for Erythropoietin and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Erythropoietin, biotinylated detection antibody and Avidin-HRP conjugate will appear bluein colour. The enzyme-subtrate reaction is terminated by the addition of a sulphuric acid solution and the colour turns yellow. The optical density (OD) is proportional to the concentration of Erythropoietin. You can calculate the concentration of Erythropoietin in the sample by comparing the OD of the sample to the standard curve.

Assay procedure

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

1. **Add sample:** 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.

2. Biotinylated detection antibody: The liquid of each well was removed. 100 µL 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.

3. Wash: Each well was apirated 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.

4. HRP Conjugate: 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.

5. Wash: The wash process was repeated 5 times as in step 3.

6. Substrate: 90 µL of substrate solution was added to each well an was and covered with a new plate sealer and was incubated for about 15 minutes at 37^0C.

7. Stop: 50 µLof stop solution was added to each well and colour turned to yellow immediately.

8. OD Measurement: The optical density (OD) of each well was determined at once using a microplate reader set to 450nm.

(b) Packed Cell Volume(Microhaematocrit method) was used.

Principle Of Microhaematocrit Method (Cheesbrough, 2004).

The packed cell volume is that proportion of whole blood occupied by red blood cells when it is packed together, expressed as a ratio (Litre/Litre).

Anticoagulated blood in a glass capillary of specified length,bore size and wall-thickness is centrifuged in a microhaematocrit centrifuge at RCF 12000-15000 Xg for 3-5 minutes to obtain constant packing of red cells. A small amount of plasma remains trapped between the packed red cells.The PCV value is read from the scale of a microhaematocrit reader or calculated by dividing the height of the red cell column by the height of total column of blood.

Procedure for PCV (Cheesbrough, 2004)**.**

1. A plain capillary tubes were filled with well mixed EDTA anticoagulated blood.
2. The unfilled ends were sealed with plasticine.
3. The filled capillary tubes were carefully located in numbered slots of the microhaematocrit rotor with the sealed end against the rim gasket.
4. They were centrifuged for 5 minutes at 15,000 xg.
5. The results were read immediately after centrifuging them.

Statistical analysis

The data were presented as mean values and ± standard variation(± SD) in tables.The data were analysed using t-test and Pearson Product moment method.Statistical significance was set at $P<0.05$

RESULTS

Table 1. Comparism of mean value of Erythropoietin and PCV in Patients with diabetic nephropathy and controls.

Parameters	DN(60)	Control(60)	Level of significance
Epo(iu/l)	42.3±21.3	48.6±24.0	P<0.05
PCV(%)	30.8±6.1	43.8±4.9	P<0.05

DN=Diabetic Nephropathy

Table 1 showed significant decrease ($P<0.05$) in the Erythropoietin and PCV levels of diabetic nephropathy subjects (42.3±21.3 iu/l, 30.8±6.1%) compared to control subjects (48.6±24.0 iu/l, 43.8±4.9%) respectively.

Table 2. Correlation of EPO and PCV values in diabetic nephropathy patients.

		PCV(DN)
EPO	Pearson	-0.0340*
	2 tailed	.2732
	N	60

DN=Diabetic Nephropathy

Table 2 showed correlation of erythropoietin and PCV values in the diabetic nephropathy patients which showed a negative (inverse) relationship (-0.0340) in patients with diabetic nephropathy.

DISCUSSION

The study was done to estimate the levels of erythropoietin and haematocrit in patients with anaemias of diabetic nephropathy at the Federal Medical Centre,Umuahia.Anaemia is one of the clinical and laboratory manifestations of chronic kidney disease.Relatively little is known about the development and progression of anaemia in patients with chronic kidney disease.As kidney function declines and in patients with more advanced chronic kidney disease stages,the incidence and prevalence of anemia increased.There is an exponential relationship between glomerular filteration and anaemia. Table 1 showed significant decrease ($P<0.05$) in the Erythropoietin and PCV levels of diabetic nephropathy subjects (42.3±21.3 iu/l, 30.8±6.1%) compared to control subjects (48.6±24.0 iu/l, 43.8±4.9%) respectively. In patients with type 2 diabetes mellitus,anaemia is common and appears in ealier stages of chronic kidney disease (CKD) than in nondiabetic patients (Wanger *et al.,* 2011). This may be as a result of tissue damage or the the set point for erythropoietin production is lowered in relation to tissue oxygenation (Fehr *et al*., 2004). Anaemia further increases the risk for cardiovascular disease (CVD) and death. Renal production of erythropoietin (Epo), the most important hormone of haemoglobin regulation might be impaired in diabetic nephropathy (DN), resulting in absolute erythropoietin deficiency, that is erythropoietin levels as the cause of decreasing haemoglobin levels (National Kidney Foudation, 2006). However, because anaemia can frequently be observed even in the early impairment of kidney function is the sole underlying mechanism (Thomas, 2007). Normochromic normocytic anaemia can occur in various chronic diseases, and it appears that dysregulation of iron homoeostasis and inflammatory processes acta s the main mediators (Ezekowitz *et al.,* 2003; Weiss and Goodnough, 2005). Althogh, anaemia in diabetic chronic kidney disease is likely multifactorial,involving erythropoietin deficiency as well as iron dysregulation and inflammation (Menon *et al.,* 2004; Soriano *et al.,* 2007). Absolute erythropoietin deficiency can be caused by diminished erythropoietin production as well as by erythropoietin-sensing errors (McGill and Bell, 2006). The study did no find support for absolute erythropoietin deficiency,because erythropoietin levels stayed within a considerably normal range even though significantly lower ($P<0.05$) than the control subjects. Table 2 showed the correlation of erythropoietin and PCV values in diabetic nephropathy patients.The table showed that there was a negative (inverse) relationship as in healthy subjects. This may be the reason the erythropoietin and PCV levels were not low in diabetic nephropathy patients because their kidneys might not have been seriously damaged.

CONCLUSION

The study showed that there was decrease in Epo and PCV levels in the diabetic nephropathy patients. The diabetic nephropathy subjects maintained negative correlation when Epo and PCV were correlated in the subjects

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