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Studies on Serum Erythropoietin and Red Cell Indices of Patients with Urinary Tract Infection in Southeast, Nigeria

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Abstract

A total of 200 subjects were recruited for the study comprising 100 subjects each for Patients with UTI (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 erythropoietin was bought from Elabscience Biotechnology Co.Ltd, Wuhan. 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 (3.75±1.40 $X10^{12}$/L; 5.16±0.34 $X10^{12}$/L, P=0.004), Haemoglobin (11.24±4.21g/dl, 15.48±1.03g/dl, P=0.004), PCV (33.73±12.62%, 46.45±3.08, P=0.004), increase in EPO (87.29±7.66 iu/l, 19.35±5.75 iu/l) and no significant difference in MCV (90.02±0.08fl, 90.00±0.05fl), MCH (30.00±0.03pg, 30.00±0.02pg) and MCHC (330.59±9.04g/l, 333.33±0.06g/l) of patients with UTI compared to control respectively. The results showed increase in EPO (64.23±9.19 iu/l, 17.10±6.86 iu/l, P=0.002) and no significant difference in RBC (4.94±0.99 $X10^{12}$/L; 5.26±0.17 $X10^{12}$/L, P=0.613), Haemoglobin (14.83±2.99g/dl, 15.78±0.51g/dl, P=0.611), PCV (44.50±8.96%, 47.33±1.53%, P=0.611), MCV (90.01±0.06fl, 90.04±0.03fl, P=0.393), MCH (30.00±0.02pg, 30.01±0.01pg, P=0.389) and MCHC (333.30±0.07g/l, 333.31±0.08g/l, P=0.901) of patients with UTI compared to control respectively. The results showed decrease in EPO (21.12±6.23 iu/l, 87.68±8.89 iu/l, P=0.003) and no significant difference in RBC (5.31±0.28 $X10^{12}$/L; 4.69±1.20 $X10^{12}$/L, P=0.242), Haemoglobin (15.94±0.83g/dl, 14.07±3.60g/dl, P=0.243), PCV (47.83±2.48%, 42.20±10.80%, P=0.242), MCV (90.03±o.04fl, 90.01±0.07fl, P=0.638), MCH (30.01±0.02pg, 30.00±0.03pg, P=0.635) and MCHC (333.31±0.05g/l, 333.31±0.09g/l, P=0.982) of patients with UTI compared to control respectively. The study maintained positive relationship of the red blood cell, haemoglobin and packed cell volume to erythropoietin. Erythropoietin level should be monitored in the patients to avoid anaemia that may be normocytic normochromic blood picture.

Keywords: Serum erythropoietin, red cell indices, patients with urinary tract infection.

INTRODUCTION

A urinary tract infection (UTI) is an infection that affects part of the urinary tract [1] which can occur in the lower urinary tract and the upper urinary tract it [2]. It has been that urinary tract infections are the most common bacterial infection in women [3]. They happen most commonly from the ages of 16 and 35 years, with 10% of women getting an infection annually and more than 40–60% were having an infection at some point in their lives [4, 5]. Urinary tract infections occur four times more frequently in females than males [4].

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 [6]. 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 [6]. EPO is also involved in the wound healing process [7].

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiUogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin [8]. Multiple studies have suggested that erythropoietin improves memory. This effect is independent of its effect on hematocrit [9-12]. Erythropoietin may have effects on mood [9].

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR) [13, 14]. 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 [15, 16]. 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) [17].

Urinary tract infection is a highly prevalent infection in this part of the world in both sexes and all ages but may be more pronounced in aging patients and all immunocompromised persons. Urinary tract infections may have adverse effects on the kidney where erythropoietin is majorly produced and regulate the level of erythropoiesis and also contribute to anaemia in UTI patients. This study becomes imperative as many patients that present in the different hospitals and laboratory are faced with the challenges of UTI. Haeamtological parameters especially red cell indices are good indicators of health and disease states [18, 19].

The study was done to determine the levels of erythropoietin and red cell indices in patients with UTI 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 UTI (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 Uti and Control

Parameters	UTI	CONTROL	P-Value
RBC($X10^{12}$/L)	3.75±1.40	5.16±0.34	0.004*
Haemoglobin(g/dl)	11.24±4.21	15.48±1.03	0.004*
PCV(%)	33.73±12.62	46.45±3.08	0.004*
MCV(fl)	90.02±0.08	90.00±0.05	0.505NS
MCH(pg)	30.00±0.03	30.00±0.02	0.624NS
MCHC(g/l)	330.59±9.04	333.33±0.06	0.328NS
EPO(iu/l)	87.29±7.66	19.35±5.75	0.006*

The results showed decrease in RBC (3.75±1.40 $X10^{12}$/L; 5.16±0.34 $X10^{12}$/L, P=0.004), Haemoglobin (11.24±4.21g/dl, 15.48±1.03g/dl, P=0.004), PCV (33.73±12.62%, 46.45±3.08, P=0.004), increase in EPO (87.29±7.66 iu/l, 19.35±5.75 iu/l) and no significant difference in MCV (90.02±0.08fl, 90.00±0.05fl), MCH (30.00±0.03pg, 30.00±0.02pg) and MCHC (330.59±9.04g/l, 333.33±0.06g/l) of patients with UTI compared to control respectively.

Table-2: Mean ± SD of Erythropoietin and Haematological Parameters of Patients with Uti Based On Age Ranges

Parameters	30-50 YEARS	51-70 YEARS	P-Value
RBC($X10^{12}$/L)	4.94±0.99	5.26±0.17	0.613NS
Haemoglobin(g/dl)	14.83±2.99	15.78±0.51	0.611NS
PCV(%)	44.50±8.96	47.33±1.53	0.611NS
MCV(fl)	90.01±0.06	90.04±0.03	0.393NS
MCH(pg)	30.00±0.02	30.01±0.01	0.389NS
MCHC(g/l)	333.30±0.07	333.31±0.08	0.901NS
EPO(iu/l)	64.23±9.19	17.10±6.86	0.002*

The results showed increase in EPO (64.23±9.19 iu/l, 17.10±6.86 iu/l, P=0.002) and no significant difference in RBC (4.94±0.99 $X10^{12}$/L; 5.26±0.17 $X10^{12}$/L, P=0.613), Haemoglobin (14.83±2.99g/dl, 15.78±0.51g/dl, P=0.611), PCV (44.50±8.96%, 47.33±1.53%, P=0.611), MCV (90.01±0.06fl, 90.04±0.03fl, P=0.393), MCH (30.00±0.02pg, 30.01±0.01pg, P=0.389) and MCHC (333.30±0.07g/l, 333.31±0.08g/l, P=0.901) of patients with UTI compared to control respectively.

Table-3: Mean ± SD of Erythropoietin and Haematological Parameters of Patients with Uti Based on Sex

Parameters	Male	Female	P-Value
RBC($X10^{12}$/L)	5.31±0.28	4.69±1.20	0.242NS
Haemoglobin(g/dl)	15.94±0.83	14.07±3.60	0.243NS
PCV(%)	47.83±2.48	42.20±10.80	0.242NS
MCV(fl)	90.03±o.04	90.01±0.07	0.638NS
MCH(pg)	30.01±0.02	30.00±0.03	0.635NS
MCHC(g/l)	333.31±0.05	333.31±0.09	0.982NS
EPO(iu/l)	21.12±6.23	87.68±8.89	0.003*

The results showed decrease in EPO (21.12±6.23 iu/l, 87.68±8.89 iu/l, P=0.003) and no significant difference in RBC (5.31±0.28 $X10^{12}$/L; 4.69±1.20 $X10^{12}$/L, P=0.242), Haemoglobin (15.94±0.83g/dl, 14.07±3.60g/dl, P=0.243), PCV (47.83±2.48%, 42.20±10.80%, P=0.242) , MCV (90.03±o.04fl, 90.01±0.07fl, P=0.638), MCH (30.01±0.02pg, 30.00±0.03pg, P=0.635) and MCHC (333.31±0.05g/l, 333.31±0.09g/l, P=0.982) of patients with UTI compared to control respectively.

DISCUSSION

The results showed decrease that was statistically significant in the RBC, haemoglobin and

packed cell volume of the Urinary tract infection (UTI) subjects relative to control. This decrease in the three parameters above may be due to bone marrow suppression and damaging effect on the kidneys as there was increase with the level of erythropoietin (EPO) of UTI patients compared to the control. This may be the cause of anaemia in the UTI patients depending on the severity of the disease. Urinary tract infection has been increasing rapidly with its resultant effects of high morbidity and mortality [4, 5]. It has been increasing infertility and other social and health problems. The red cell indices showed no variation indicating that the blood picture could be normocytic normochromic anaemia in the UTI patients. The increase in erythropoietin may be due to increased damage in the fibroblasts of the kidneys. A lot of efforts should be done to prevent UTI.

The study showed no changes in all the parameters based on age ranges and decrease in erythropoietin of UTI patients when the male subjects were compared to female patients. The study revealed that the level of EPO of male UTI is lower than the mean values of EPO in female UTI patients. This shows that fibroblasts of the females may be more damaged than the males [3]. Other parameters showed no changes when compared among the UTI patients based on sex. Sex may not be a major factor in the changes associated with red cell indices except the erythropoietin and also based on age ranges [4].

CONCLUSION

Urinary tract infection is more prevalent in females than in male due to their urethra. The study revealed decrease in red blood cell, haemoglobin, packed cell volume and increase in erythropoietin. The study maintained positive relationship of the red blood cell, haemoglobin and packed cell volume to erythropoietin. Erythropoietin level should be monitored in the patients to avoid anaemia that may be normocytic normochromic blood picture.

The alarming increase in the prevalence of UTI should be prevented, more education on UTI and also monitoring of the red blood cell, haemoglobin, packed cell and erythropoietin to avert the dangers of anaemia which could be the main cause of death in UTI patients if not properly treated.

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