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Packed Cell Volume, Reticulocyte Count and Index among Patients with Chronic Kidney Disease in Sokoto, North Western, Nigeria.

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Research Article

ABSTRACT

Chronic kidney disease (CKD) is a global public health problem which can progress to end stage renal failure. This study was carried out to investigate the Packed Cell Volume (PCV), reticulocyte count and reticulocyte index in patients with CKD in Sokoto, North Western, Nigeria. The PCV, reticulocyte count and reticulocyte index of 96 subjects made up of 60 male (62.5%) and 36 females (37.5%), aged 22-60 years and mean age of 34.6 ± 12.8 with pre-dialysis CKD in Sokoto were studied. Forty six age and gender matched healthy non-renal individuals were monitored as controls. The PCV was determined using the Hawksley microhaematocrit centrifuge (Hawksley, UK) while the reticulocyte count was carried out manually using a supravital stain (New Methylene Blue). The mean PCV, reticulocyte and reticulocyte index among the CKD patients were; 33.32 ± 7.97, 3.85 ± 1.18 and 2.82 ± 0.95 compared to 35.11 ± 5.14, 4.03 ± 0.78 and 3.17 ± 0.74 respectively. The differences between the PCV, reticulocyte count and reticulocyte index of subjects and controls was statistically significant (p= 0.001 and 0.010 and 0.033 respectively). There was no statistically significant differences in the PCV, reticulocyte count and reticulocyte index based on the gender of subjects (p= 0.970, 0.66 and 0.73) respectively. Haematocrit correlated inversely with the duration of kidney disease (r = -0.35, p= 0.05). The PCV, reticulocyte and reticulocyte index was significantly lower among subjects with long standing history and severity of the kidney disease. In this present study, we have observed that severe anaemia associated with a low PCV, reticulocyte count and reticulocyte index is a common feature among patients with CKD in Sokoto, North Western Nigeria. The PCV, reticulocyte count and reticulocyte index seems to worsen with increasing severity of kidney disease. There is need for the provision of erythropoietin for the evidenced-based management of anaemia in renal patients in Nigeria. There is need for the regular monitoring of the biological indices, including PCV, reticulocyte and reticulocyte index in renal patients in Sokoto in particular and Nigeria in general.

INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem which may progress to end stage kidney failure [1]. Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive loss in renal function over a period of months or years. CKD exist in five stages, with stage 1 being the mildest and usually causing few symptoms and stage 5 is associated with severe illness with poor life expectancy if untreated. Stage 5 CKD is also called end stage renal disease (ESRD), chronic kidney failure (CKF), end stage renal failure (ESRF) or chronic renal failure (CRF). Severe CKD requires renal replacement therapy, which may involve a form of dialysis or a kidney transplant [1].

Anaemia is a common finding in patients with CKD. Iron deficiency is an important clinical concern in CKD, giving rise to iron-deficiency anemia and impaired cellular function [2]. Anaemia in CKD is due primarily to reduced production of erythropoietin in the kidney and secondarily to shortened red cell survival. Erythropoeitin (EPO) is produced by peritubular cells in the kidneys of the adult and in hepatocytes in the foetus. The effective investigation of renal anaemia requires the assessment of a variety of biological indices; FBC including a PCV, reticulocyte count, reticulocyte index, vitamin B12, folate, ferritin levels and the saturation of transferrin [3].

Reticulocytes develop and mature in the red bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. The reticulocyte count is used to estimate the degree of effective erythropoiesis [4]. It can be reported as absolute reticulocyte count or as a reticulocyte percentage. The reference range of the reticulocyte percentage in adults is 0.5%-1.5% [5]. There appear to be a paucity of information on the reticulocyte and packed cell volume among CKD patients requiring haemodialysis in Sokoto, North Western Nigeria. The aim of this study was to determine the impact of CKD on the PCV, reticulocyte count and index of patients with pre-haemodialytic renal disease in Sokoto, Nigeria.

Subjects

Subjects for this case-control study included 96 patients with CKD in Sokoto, Nigeria. Forty six age and gender-matched non-renal individuals were monitored as controls. Subjects included 60 male (62.5%) and 36 females (37.5%). The mean age and range of subjects were 34.6 ± 12.8 and 22-60 years respectively.

Inclusion and exclusion criteria

Inclusion criteria included age ≥ 18 years, history of CKD, pre-dialysis and willingness to give a written informed consent after counselling. Exclusion criteria included; age <18 years, non- renal pathology, patients on dialysis and failure to give written informed to be part of the study.

Informed consent and ethical clearance

Written informed consent was obtained from all participants recruited into this study (controls and subjects). Ethical clearance was obtained from the ethical committee in the Department of Haematology in the Faculty of Medical Laboratory Science in Usmanu Danfodiyo University Sokoto, Nigeria.

Study area

This present research work was carried out at the Haematology Department in the Faculty of Medical Laboratory Science of Usmanu Danfodiyo University in collaboration with the Haematology Department of Usmanu Danfodiyo University Teaching Hospital in Sokoto in the North West geo-political zone of Nigeria. Sokoto State is located in the extreme North Western part of Nigeria near to the confluence of the Sokoto River and the Rima River. With an annual average temperature of 28.3 °C (82.9 °F), Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40 °C (104.0 °F). The warmest months are February to April when daytime temperatures can exceed 45 °C (113.0 °F). The rainy season is from June to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission respectively. Report from the 2007 National Population Commission indicated that the state had a population of 3.6 million [6].

Statistical Analysis

Statistical analyses were conducted using SPSS (version 11) software. Comparisons were assessed using mean and chi-square test. A p-value of ≤ 0.05 was considered statistically significant in all statistical comparison. Correlation was compared using a version of linear regression analysis.

METHODS

Three millilitres of blood sample was drawn aseptically with Monovette vacutainer blood collection system (Sarstedt, Germany) from the median antecubital vein for all the subjects into dipotassium ethylenediamine tetracetic acid (K_2EDTA) anticoagulated blood containers. The EDTA anticoagulated sample was used for the determination of PCV using a microhaematocrit centrifuge (Hawksley, UK). Despite the recent technical development of scientific laboratories and the development of automated blood counter, the microhaematocrit method remains the most common method used for determination of PCV particularly in resource-limited settings around the world. Standard methods described by Dacie and Lewis [7] was used for PCV analysis. The supravital staining technique described by Brecher [8] in 1949 and Koepke [9] in 1986 was used for the enumeration reticulocyte. In this technique, a few drops of the supravital dye solution (1.0% w/v of new methylene blue or Brilliant Cresyl Blue) are mixed with an equal volume of EDTA-anticoagulated peripheral blood and incubated for 10

minutes. A thin blood film was made on a microscope slide. A Wright counterstain was applied and the slide was examined by light microscopy. An adequate number of erythrocytes (usually 1000) in a well-stained area was examined, and the proportion of reticulocytes was determined. Reticulocytes are recognized by a blue intracytoplasmic precipitate, which can vary from individual small blue granules to a network of blue reticular material. The reticulocyte count was reported as a percentage (number of reticulocytes per total red blood cells examined). Reticulocyte Production Index was calculated as follows:

Reticulocyte Index = Reticulocyte Count X Haematocrit/Normal Haematocrit

A value of 45 is usually used as a normal hematocrit. The idea of the RPI is to assess whether the bone marrow is producing an appropriate response to an anaemic state.

RESULTS

The mean PCV, reticulocyte and reticulocyte index among the CKD patients were; 33.32 ± 7.97, 3.85 ± 1.18 and 2.82 ± 0.95 respectively compared to 35.11 ± 5.14, 4.03 ± 0.78 and 3.17 ± 0.74 respectively. Table 1 show the mean packed Cell Volume (PCV), reticulocyte count and reticulocyte index of CKD patients and non-renal controls. The differences between the PCV and reticulocyte count and reticulocyte index of subjects and controls was statistically significant ($p= 0.001$ and 0.010 and 0.033 respectively). There was no statistically significant differences in the PCV, reticulocyte count and reticulocyte index based on the gender of subjects ($p= 0.970, 0.66$ and 0.73) respectively. Table 2 show the mean packed PCV, reticulocyte count and reticulocyte index based on gender. Haematocrit correlated inversely with the duration of renal disease ($r = -0.35, p= 0.05$). There were no statistically significant differences in the PCV, reticulocyte and reticulocyte index of subjects with chronic kidney disease and acute kidney injury. Table 3 show the mean PCV, reticulocyte count and reticulocyte Index of tests based on duration of kidney dysfunction. The PCV, reticulocyte and reticulocyte index was significantly lower among subjects with long standing history and severity of the renal disease.

Table 1: Mean packed Cell Volume (PCV), Reticulocyte count and Reticulocyte Index of renal failure patients and non-renal controls.

Study Group	PCV (%)	Reticulocyte Count (%)	Reticulocyte Index (%)
Subjects	30.32±7.97	3.85±1.18	2.82±0.95
Controls	35.11±5.14	4.03±0.78	3.17±0.74
t-value	1.594	0.890	2.153
p-value	0.001	0.010	0.033

Table 2: Mean packed Cell Volume (PCV), Reticulocyte count and Reticulocyte Index of Male and Female Renal failure on dialysis patients (Test).

Gender	PCV (%)	Reticulocyte Count (%)	Reticulocyte Index (%)
Male	33.29±7.70	3.91±1.26	2.85±0.98
Female	33.35±8.30	3.80±1.10	2.79±0.92
t-value	0.038	0.439	0.341
p-value	0.970	0.661	0.734

Table 3: Mean packed Cell Volume (PCV), Reticulocyte count and Reticulocyte Index of tests based on duration (months) of renal dysfunction.

Duration of Kidney Dysfunction (Months)	PCV (%)	Reticulocyte Count (%)	Reticulocyte Index (%)
1-6	36.12±1.02	4.10±0.68	3.05±1.09
6-12	32.86±6.58	3.93±1.24	2.81±1.07
>12	31.19±5.66	3.61±1.09	2.70±0.71
f-value	2.582	0.850	0.664
p-value	0.05	0.01	0.05

DISCUSSION

In this current study, we observed a significant difference between the PCV of CKD subjects compared to non-renal controls. We observed a mean PCV of 30.32±7.97 among our cohort of 96 subjects with pre-dialytic CKD. We observed a significant negative correlation between PCV and duration in months of chronic kidney dysfunction. Our finding is consistent with previous report which observed that most patients with CKD present with features of anaemia and low reticulocyte count [10]. Similarly, Akinsola and coworkers [11] observed a mean haematocrit was 24.1 +/- 6.7 and a negative but significant negative correlation between PCV and the degree of renal failure from their study of a cohort of 39 patients with pre-dialytic CRF. The management of iron-deficiency anaemia in patients

with non-dialysis chronic kidney disease (ND-CKD) remains controversial, particularly regarding the use of oral versus intravenous iron supplementation. A previous report suggest that iron supplementation is advisable for all iron-deficient CKD patients receiving erythropoiesis stimulating agents (ESAs), and intravenous iron may be preferable to oral iron [12] .

Anaemia is a cardinal feature of chronic kidney failure and classically it is normochromic normocytic. Iron deficiency is an important clinical concern in CKD [2]. Hypochromic anaemia in these patients is often attributed to iron deficiency [13,14]. Haematocrit correlated inversely with the degree of renal failure as assessed by serum creatinine in CKD [11]. Evidenced –based best practice in the developed world recommend the use erythropoietin in the management of anaemia in patients with CKD [15]. The availability and widespread use of haemopoietic growth factors on a large scale for in vivo and in vitro management of anaemia particularly in CKD patients has opened a new era in transfusion medicine. Many anaemic patients being managed with EPO alone or with some combined strategy of EPO plus red cell replacement have shown that the RBC transfusion requirement is substantially reduced [16,17,18]. Anaemia is frequently diagnosed in patients with cancer. A systematic literature review (1996–2003) to produce evidence-based guidelines on the use of EPO in anaemic patients with cancer shows that RBC transfusion requirements are significantly reduced with EPO protein therapy in patients with chemotherapy-induced anaemia or when used to prevent cancer anaemia. Level I and III evidence indicates that patients with chemotherapy-induced anaemia or anaemia of chronic disease initially classified as non-responders to standard doses proceed to respond to treatment following a dose increase [19]. Similarly a report on treatment with epoetin alpha as a single weekly dose significantly increased haemoglobin levels in patients with cancer who were undergoing radiotherapy. The response was greater in patients treated with radiotherapy alone than in those receiving combined therapy. The duration of EPO treatment was shorter in the group treated with radiotherapy alone than in the combined treatment group [20]. Experience with preoperative single weekly dose of 150 µg/kg of EPO in Ghanaian patients showed that it is effective in raising preoperative haemoglobin [21]. Cost and availability of EPO is a major challenge in Sub- Saharan Africa. Poor use of EPO is more likely in developing countries that have lower annual per capita health care expenditures, lower proportions of privately funded health care, and a national health service [22]. Financial considerations and a haemoglobin level of 10 g/dL appear to influence EPO use in the United States, whereas financial considerations alone determine EPO use elsewhere, particularly in Sub-Saharan Africa. It is believed that the number of renal patients requiring transfusions could be reduced further if there were novel long-acting ESAs that could be used for ND patients [23].

We observed a significantly lower reticulocyte count and reticulocyte index among the CKD patients compared to the non-renal controls. Previous report indicates that anaemia in CKD is normochromic, normocytic and hypo proliferative [24]. Proliferative red cell activity is often monitored by the determination of the reticulocyte count, the reticulocyte index and the reticulocyte production index. The enumeration of peripheral blood reticulocytes is often performed to obtain information about the functional integrity of the bone marrow [25]. Reticulocytosis occurs in anaemic patients with functional bone marrow while anaemic patients with dysfunctional bone marrow tends to have reticulocytopenia. Reticulocytosis (an increased number of circulating reticulocytes) is a normal finding in anaemic patients with functional bone marrows (haemorrhage, haemolytic anaemia, sickle cell anaemia, thalassemia, spherocytosis, glucose-6-phosphate dehydrogenase deficiency, immune haemolytic anaemia and hypersplenism, patients who have been successfully treated for other types of anaemia. However patients with marrow ablative disorders, impaired erythropoiesis, or decreased erythropoietin production may show a normal or decreased reticulocyte count in spite of severe anaemia (renal failure, myelofibrosis, pernicious anaemia, iron deficiency anaemia, folate or vitamin B12 deficiency anaemia, immunologic or drug-induced red cell aplasia, leukemia and metastatic carcinoma [26].

Accurate reticulocyte enumeration is critical for the diagnosis of and monitoring of patients receiving recombinant human erythropoietin (rhEPO) and other hematologic growth factors to stimulate erythropoiesis. Human erythropoietin is often used in conjunction with oral or parenteral iron administration to stimulate erythropoiesis in chronic renal failure [27]. There has been controversy on the accuracy of visual (manual) counting of reticulocytes. However recent reports [28,29] indicates that manual method can be used as a reliable, cost effective and a readily available tool for estimating reticulocytes and efficiently discriminating between high and low reticulocyte ranges particularly in resource-limited countries where there is unavailability of automated haematology analysers as a result of unaffordability [30].

We did not observe a significant difference between the PCV, reticulocyte and reticulocyte index based on the gender of chronic renal disease subjects. Our finding is consistent with previous report by Johannes and colleagues [31] which indicated that there was no gender effects in the reference ranges for reticulocyte parameters. We observed that the PCV, reticulocyte and reticulocyte index were significantly deranged in subjects with longer history of chronic renal dysfunction compared with shorter history of renal dysfunction. This finding is consistent with previous reports [11, 12, 14, 23] which indicates that anaemia parameters (PCV, reticulocyte and reticulocyte index) are often deranged in CKD and tend to worsen with increasing severity of the renal failure.

CONCLUSION

In this present study, we have observed a low PCV, reticulocyte count and reticulocyte index that seems to worsen with increasing severity of renal disease. There is need for the provision of erythropoietin for the evidenced-based management of anaemia in kidney disease patients in Nigeria like their counterparts in the developed world. There is need for the regular monitoring of the biological indices, including PCV, reticulocyte and reticulocyte index of CKD patients in Sokoto in particular and Nigeria in general.

ACKNOWLEDGMENTS

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