academicJournals

Vol. 9(5), pp. 47-50, May 2017
DOI: 10.5897/IJMMS2016.1275
Article Number: 69A182B64162
ISSN 2006-9723

http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Correlation of lymphocyte count with serum calcium level and neutrophil-to-lymphocyte ratio in end stage renal disease patients undergoing hemodialysis in Adamawa State, Nigeria

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Received 29 October, 2016: Accepted 28 November, 2016

Low lymphocyte count in end stage renal disease (ESRD) patients receiving maintenance hemodialysis was documented. This study aims to correlate the levels of lymphocyte count with serum calcium level, and neutrophil-to-lymphocyte ratio (NLR) in ESRD patient receiving hemodialysis in Northeast Nigeria. Seventy eight (78) patients receiving hemodialysis and 75 clinically healthy subjects (control) participated in the present study. Full blood count and serum calcium level were determined by using a Sysmex XP-300 Hematology Analyzer and a Selectra Pro-S Chemistry Analyzer, respectively. The mean levels of lymphocyte count, packed cell volume, hemoglobin and serum calcium level were significantly lower (p<0.001), while neutrophil, and NLR values were significantly higher (p<0.001) in ESRD patients undergoing hemodialysis as compared to the healthy control subjects. Lymphocyte count was negatively correlated with NLR value (r=-0.341; 0.05) but positively with serum calcium (r=0.904; p<0.001). In end stage renal disease (ESRD) patient receiving hemodialysis, lymphocyte count was negatively correlated with NLR value and positively with serum calcium level.

Key words: Lymphocyte count, serum calcium level, neutrophil to lymphocyte ratio (NLR), hemodialysis.

INTRODUCTION

The routine medical choice for a patient with insufficient renal function (10 -15%) is maintenance hemodialysis (Block et al., 2004; Kuwae et al., 2005). Maintenance hemodialysis has been the most common method used to treat advanced and permanent kidney failure since the 1960s (Kultigin et al., 2013). Human beings have an immune system that depends on different types of white blood cells for the protection against diseases (Johnson

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et al., 2005; Donato et al., 1992). These white blood cells include neutrophils, lymphocytes, monocytes, basophils and eosinophils. While neutrophils protect us from bacterial infections and respond to inflammation (Manal and Shaimaa, 2015; Azab et al., 2012), lymphocytes that include natural killer cells, T cells, and B cells protect us against viruses and cancer development (Templeton et al., 2014). Total peripheral blood lymphocyte count is reduced in ESRD patients undergoing hemodialysis, a finding that has been well documented since the 1980s (Manal and Shaimaa, 2015; Kuwae et al., 2005) on the other hand, neutrophile count is high in the peripheral blood of ESRD due to inflammatory processes (Turkmen et al., 2012). Cellular immunity level reflected as total lymphocyte count are reduced in ESRD patient (Johnson et al., 2005; Sakato et al., 2015), a fact that explains the frequent occurrence of infection which is the second leading cause of death in end stage renal disease patients (Egbi et al., 2014; Block et al., 2004). As it has been demonstrated, this reduction is more intense in ESRD patients undergoing maintenance hemodialysis (Block et al., 2004).

Chronic renal disease (CKD), and end-stage renal disease (ESRD) in particular, are associated with high mortality rates due to complications caused by cardiovascular diseases (CVDs) (Binnetoğlu et al., 2014), infections (Petrie et al., 2007; Reddan et al., 2003) and other inflammatory processes due to hypodynamic forces (Lavin-Gomez et al., 2011; Okyay et al., 2013).

Inflammation contributes to the development of ESRD by triggering both the release of cytokines and the increased production and activity of adhesion molecules (Memoli et al., 2000; Panichi et al., 1998), which together result in lymphocyte adhesion and movement into the interstitium (Johnson et al., 2005; Roberto et al., 2003). In ESRD, the vascular endothelium is injured by numerous potential insults, including hemodynamic forces (Hatice et al., 2012; Sakato et al., 2015). Although, circulating leukocytes do not adhere to the healthy vascular endothelium (Turkmen et al., 2012), the injured endothelium expresses different classes of adhesion molecules that selectively bind to leukocytes (Sakato et al., 2015; Templeton et al., 2014) and as a result, their presence is reduced at the site where they are needed. The changes in the hematological parameters and the correlation of lymphocyte count with serum calcium level and NLR value in ESRD patients in Northeast Nigeria have not yet been fully studied and this fact was the impetus for the present study.

MATERIALS AND METHODS

This study was carried out at the Federal Medical Center of Yola, in Adamawa State, in Northeast Nigeria. 153 subjects took part in the study, 78 of them were ESRD patients receiving hemodialysis (35 men and 43 women, with an average age of 43±14 years). While the rest 75 were clinically healthy individuals used as controls (46 men and 29 women, with an average age of 35±7 years). Blood sample from the ESRD patients receiving regular maintenance hemodialysis were collected prior to their routine hemodialysis session. Blood samples of clinically healthy individuals were also collected at similar days.

Full blood count and serum calcium level were determined using a Sysmex XP-300 Hematology Analyser and a Selectra Pro-S Chemistry Analyser respectively. All analyses were performed according to the standard operational procedures.

All subjects gave their informed consent prior to their inclusion in the study. Inclusion criteria: Only ESRD patients receiving routine maintenance hemodialysis were used as test group. Exclusion criteria: ESRD patients that were not receiving routine maintenance hemodialysis and subject above 70 years old were excluded from this study.

Sample collection

Three milliliters of blood were aseptically collected through the antecubital vein of the subjects and put in both EDTA and plain vacutainer, that were further labeled with the patient number, sex and age. The full blood count for each subject was performed in the sample put in the EDTA vacutainer within one hour of collection, while the serum obtained from plain vacutainer, was centrifuged and was used for serum calcium level determination.

Sample analyses

Full blood count

Using the Sysmex XP 300 machine, the procedure for blood cell (full blood count) determination was performed as follows: EDTA samples were placed in a hematology blood mixer for five minutes and the blood cells were automatically counted through a probe fitted in the Sysmex XP 300 machine. After four minutes, the results of the blood cell count were displayed on the color LCD screen of the machine.

Calcium level measurement

The Selectra pro-S machine and direct colorimetric and complexometric methods were used. The exact procedure is described below:

1) Selectra pro-S machine was switch on and allow to boot and acclimatize for 30 min.
2) The calcium (arsenazo) reagent was automatically dispensed into the corresponding compartment of the machine.
3) Calibrator, control and test sample were introduced into the correspondent compartment.
4) The machine was allowed to calibrate automatically.
5) The machine was allowed to pipette, and incubate the samples automatically
6) The results were automatically displayed on the monitor screen.

Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) measurement

The NLR and PLR values were obtained by dividing the values of neutrophils and platelet by lymphocytes in full blood count, respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for

Table 1. Full blood count, and serum calcium levels in ESRD patients receiving hemadialysis and clinically healthy subjects (controls).

Parameter	ESRD patients (N = 78)	Controls (N=75)	Normal values	P value
Lymphocyte count (%)	18.27±4.17	38.53±10.19	25-45	0.001
Neutrophile count (%)	70.36±7.36	52.84±16.35	40-70	0.005
Platelet count (x 10^3/µl)	229.4±79.54	198.6±53.47	150-300	0.005
Packed cell volume (l/l)	25.64±1.61	43.65±8.17	37-50	0.001
Hemoglobin (g/dl)	8.2±0.51	13.77±3.13	12-16	0.01
Total white cell count (x 10^3 /µl)	9.53±2.79	6.99±1.38	4-10	0.05
Serum calcium level (mmol/l)	1.02±0.33	2.43±0.19	2.2-2.5	0.01
MCH (pg)	25.9±0.99	27.2±2.90	27-31	0.01
MCV (fl)	84.3±3.09	83.44±6.22	83-101	0.01
MCHC (g/dl)	30.99±0.89	32.55±1.23	31-34	0.01
NLR	3.7958	1.2421	1-2.1	0.05

Table 2. Correlation of lymphocyte count with other measured parameters in ESRD patients receiving hemodialysis.

Parameters	Correlation coefficient (r)	P-value
Lymphocyte count and neutrophil count	-0.201134	0.001
Lymphocyte count and platelet count	0.090885	0.05
Lymphocyte count and packed cell volume	0.179913	0.05
Lymphocyte count and hemoglobin	0.035250	0.005
Lymphocyte count and serum calcium	0.904	0.001
Lymphocyte count and NLR	-0.341	0.005
Lymphocyte count and PLR	-0.401	0.005

Social Sciences (SPSS) 20.0 software (Chicago IL). Descriptive values were given as mean standard error of mean. Categorical variables were expressed as the number of cases and the percentage value. The Student's *t*-test was used to compare the means between ESRD patients and control subjects, while Pearson's correlation coefficient was used to calculate the relationship between lymphocyte count, serum calcium level and NLR values.

RESULTS

The mean levels of lymphocyte count, packed cell volume, hemoglobin and serum calcium levels were significantly lower ($p<0.001$), while neutrophil and NLR values were significantly higher ($p<0.001$) in ESRD patients undergoing hemodialysis as compared to controls as shown in Table 1. In addition, lymphocyte count was negatively correlated with neutrophil count, PLR and NLR value and positively with serum calcium level as shown in Table 2.

DISCUSION

In this study, lymphocyte count was reduced when NLR value increased, and as it was observed, this reduction was proportional to serum calcium levels decrease indicating that calcium may play an important role in

lymphocyte kinetics and functions in ESRD patients. In general, the healthy Kidney turns vitamin D into active hormones called calcitriols (Egbi et al., 2014) that increase calcium absorption from the intestine into the blood (Block et al., 2004). In ESRD patients receiving hemodialysis, calcitriols are deficient due to kidney malfunction (Fung et al., 2002; Lavin-Gomez et al., 2011) and so intestinal adsorption of calcium may be impaired and its concentration in the peripheral blood is reduced. In addition, packed cell volume was decreased in ESRD patients due to failure of the kidneys to release erythropoietin (Fung et al., 2002), which is the hormone that is required for adequate red cell production in humans. NLR value was increase in the 78 ESRD patients undergoing maintenance hemodialysis. It is believed that, the increase of NLR value is a result of the increased neutrophil count observed during inflammatory processes. This inflammation may be caused by hypodynamic forces on vascular epithelial cell (Lavin-Gomez et al., 2011; Roberto et al., 2003). Hypodynamic forces observed in ESRD may be due to changes in plasma protein ratio, oncotic pressure and electrolyte imbalance arising from kidney malfunction (Kato et al.,

2012; Memoli et al., 2000).Low lymphocyte count in patients is a result of Neutrophillia (that was observedamong ESRD patients) and is an inflammatory response that inhibits immune system response by suppressing the activity of immune cells such as lymphocyte, activated T cells, and natural killer cells (Manal and Shaimaa, 2015; Keizman et al., 2012). In addition, lymphocyte adheres to the inflamed vascular epithelial cells (Pecoits-Filho et al., 2002) and their concentration is reduced in the peripheral blood system**.**

Conclusion

In a study conducted in Adamawa State in Northeastern Nigeria, the mean lymphocyte count, packed cell volume, hemoglobin and serum calcium level were significantly lower and values of neutrophil and NLR were significantly higher in ESRD patients undergoing hemodialysis as compared to clinically healthy subjects. Moreover, lymphocyte count was negatively correlated with NLR value and positively with serum calcium level indicating that, calcium may play an important role in lymphocyte kinetics in ESRD patient receiving maintenance hemodialysis in Northern Nigeria.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations: EDTA, Ethylene diamine tetraacetic acid; **NLR,** neutrophil-to-lymphocyte ratio; **ESRD,** end stage renal disease; **PLR,** platelet-to-lymphocyte ratio.

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