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Research Article

Association of Red- Cell Membrane Na^+/K^+ ATPase Activity with Anaemia in Occupational Lead (Pb) Toxicity in an African Population

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ABSTRACT
Incidence of Anaemia and its pathophysiology as a complicating feature of lead (Pb) poisoning in occupationally exposed subject has been severally reported. The effect of lead poisoning on Na^+/K^+ ATpase activity as a basis for the reported anaemia was the focus of this work. Haematocrit value, blood haemoglobin along with blood lead concentrations and red cell ghost membrane Na^+/K^+ Atpase activities were determined in adult humans occupationally exposed to lead(LES), Chronic renal failure patients (CRF) and in subjects not exposed to lead and not suffering from any kidney disease (Control). The mean haematocrit and haemoglobin values were found to be 43%, 37%, 29% and 13.4g/l, 11.0g/l and 9.7g/l for the control, the LES and CRF patients respectively. The mean blood lead concentrations (in µg/100ml) were found to be 23, 32 and 90 and the mean Na^+/K^+ ATpase activity (in µmiP/µgP/min) were 67.0, 77.0 and 29.0 for the Control, the LES and CRF subjects respectively. The differences in the values obtained for the controls when compared with those obtained for the LES and CRF subjects were found to be statistically significant while the haematocrit and haemoglobin values were also found to correlate with the blood lead concentration and Na^+/K^+ ATpase activities of the ghost membrane. The need to consider membrane enzyme concentration derangement in some unexplained anaemia especially in patients with history of occupational lead exposure was highlighted in this work.
Key Words: *Na^+/K^+ ATpase, Anaemia, Pathophysiology, lead toxicity, haematocrit.*

INTRODUCTION

Na^+/K^+ ATPase is the largest protein complex in the family of P-type ATPases expressed in all living organisms. It is essential for the generation and maintenance of Na^+ and K^+ gradients between the intracellular and extracellular milieu necessary for cellular homeostasis and functions of specialized tissues. Na^+/K^+-ATPase is an oligomeric transmembrane protein consisting of two noncovalently linked obligatory α- and β- subunits. The α-subunit is the catalytic protein subunit while the β-subunit is a regulatory glycoprotein. The molecule also has a third non-obligatory proteolytic component. It catalyzes an ATP-dependent transport of three sodium ions out and two potassium ions into the cell per pump cycle, thereby generating a transmembrane sodium gradient across the plasma membrane. The sodium gradient generated by the enzyme provides the primary energy for α uptake and extrusion of a variety of solutes by epithelial cells and is crucial for efficient functioning of other Na^+ coupled transport systems Woo *et al* [1]. The Na^+/K^+-ATPase is localized to the basolateral plasma membrane in most epithelial cells and has been widely used as a marker for epithelial polarity.

Anaemia has been summarily described as a disorder characterized by a decrease in haemoglobin in the blood and a decrease in the red cell population Waldron, [2]. It has been severally reported as one of the complications associated with Lead (Pb) poisoning Waldron [3]; Hernberg and Hansan [4]. Essentially, the anaemia of lead poisoning has been attributed to the combined effects of the inhibition of certain enzymes in heamoglobin synthesis, and the shortened lifespan of circulating erythrocytes. There is a decrease in heam synthesis resulting from the inhibition of three major enzymes involved in its pathway; these are ALA-dehydratase, delta-aminolaevulinic acid (δ-ALAS) and the enzyme Ferrochelatase catalyzing the incorporation of iron into the porphyrin ring. Although the mechanism for shortened erythrocyte survival is not well understood, the degree of

shortening correlates with the level of anaemia, coproporphrinuria, and blood lead concentration. Some characteristics of the erythrocyte membrane are also altered during exposure to lead Hammond . Increased resistances to hypo-osmotic lysis and increased mechanical fragility have also been reported as the basis of the erythrocyte shortened life span Waldron [3]; Hansan and Hernberg [4].

The inhibition of erythrocyte membrane Na^+/K^+-ATPase with increased loss of intracellular potassium have been reported to occur in people with only moderately elevated lead exposure Hernberg and Nikkernen [5]. This potassium leakage would eventually lead to an increased fragility of the red blood cells and consequent lysis. A number of theories have been advanced for the observed leakage in red cell membrane in lead poisoning; White and Selhi [6] proposed that conformational changes in the membrane caused by lead would alter the environment of the enzyme and result in enzyme inhibition as well as in great loss of potassium ions from the cell, possibly by creating a potassium gate.

Conventionally, markers of anaemia have always been the haematocrit and blood haemoglobin estimation, this therefore makes blood haemoglobin measurement of particular importance as an index of lead effects in-vivo; this has been particularly employed in this work.

Occupations like automobile engineering, Battery-repairing, Welding, Vulcanizing, Vehicle painting, and Lead factory works have been known to expose their practitioners to excessive lead ingestion leading to a number of systemic disorders, one of these disorders is mild anaemia especially at the latent stage. In spite of advances made in medical diagnosis, the actual etiology of the anaemia associated with chronic lead exposure is still not clear, thus the role of lead (and other toxic metals) in precipitating this disorder especially in the face of abundant evidences that lead interferes with a number of SH-containing enzymes like the ATPases is still uncertain.

The aim of this work was therefore to investigate the effect of chronic exposure to lead on the activity of Na^+/K^+ ATPase in the above subjects towards establishing the pathogenesis of the anaemic state reportedly associated with excessive lead exposure.

MATERIALS AND METHODS

Materials

Three categories of subjects were recruited for this study; these were *the Control, the Experimental and the chronic renal failure subjects.* In recruiting subjects for this study, our Institution guidelines governing such was applied. This involved obtaining ethical clearance and consent of the subjects before administration of questionnaire for the purpose of the experiment.

The Control Subjects

This group consisted of fifty healthy adults (men and women aged between 20years and 50years) who were students and civil servants studying and working respectively in the University College Hospital, Ibadan. They had no known previous occupational exposure to lead and had no history or clinical symptoms of anaemia or renal dysfunction as deducted from the administered questionnaire (appendix 1).

Experimental Subjects (occupationally exposed)

125 subjects aged between 23yrs and 47yrs consisting of workers in a lead smelting and battery manufacturing plant (Associated Battery Manufacturing Company (ABM) and ABM Metal Recovery Division based in Ikeja, Lagos), automobile-mechanics, battery-repairers, welders, vulcanizers, and vehicle-painters were chosen for this group. They have worked for some periods of time ranging from 1 to 16yrs both as factory workers when they were smelting lead and in packaging of manufactured batteries and as artisans in the routine practice of their trade.

Chronic Renal failure (CRF) patients

Twenty five CRF patients clinically diagnosed at the medical out-patient department of the University College Hospital, Ibadan were chosen for this group of subjects. Twenty of these patients were already slated for renal dialysis. After the administration of appropriate questionnaire to determine their suitability or otherwise for the study, about 10mls of their blood was collected through venepuncture with minimal stasis from the occipital vein of the forearm into both lithium heparin and EDTA bottles. The specimen in EDTA bottles were analyzed for haematological parameters- PCV and Haemoglobin- while blood in lithium heparin bottle was divided into two

portions; One portion of the blood was used for lead analysis and the second was used for Na^+/K^+ATPase assay.

Methods of Analysis

(i)Determination of Packed Cell Volume (PCV)

The method used in this study was the modified method of Pearson et al (71982) using micro glass capillary tubes (from Vitrex Medical and Associates, Denmark). The method was based on separation of blood particulate matter based on their molecular size and shape under centrifugal force.

(ii) Estimation of Blood Haemoglobin Concentration

The modified colorimetric method of Clegg and King (81942) was employed in the measurement of blood haemoglobin concentration. The method was based on the conversion of haemoglobin into cyanmethaemoglobin. Aculute pellets which were commercially prepared pellets containing a mixture of potassium cyanide and ferricyanide with sodium bicarbonate incorporated were appropriately diluted as directed by the manufacturers and used in oxidizing the haemoglobin molecule. Appropriate quality control was included in the analysis.

(iii) Blood Lead Assay

Blood-lead concentrations were determined using Atomic Absorption Spectrophotometry (AAS) based on the modified method of Hessel [9]. The chemically digested blood samples were read off in a Buck Atomic Absorption Spectrophotometer (Buck Scientific, USA). Appropriate quality control samples were also included in the analysis.

(iv) Red Cell Na^+/K^+ ATPase Activity

Red cell ghost membrane Na^+/K^+ ATPase activity was estimated in the control, the occupationally exposed and CRF subjects using the method of Olorunsogo *et al* [10]. Principally, the method was based on the hydrolysis of the γ-phospho-diester bond of ATP to liberate inorganic phosphate by the enzyme; the concentration of inorganic phosphate liberated per hour in a given mass of the red cell ghost membrane was then quantified being proportional to the enzyme concentration. The modified method of Jarret & Penniston [11] was used to prepare haemoglobin-free red cell ghost membrane.

Statistical Analysis

ANOVA was used to analyze the data obtained.

RESULTS

Haematocrit and Blood Haemoglobin levels

From the results, the mean PCV values obtained were 43%, 37% and 29% with standard deviation values of ±2.0, ±2.2 and ±1.9 while the mean blood haemoglobin concentrations obtained were 13.4g/100ml, 11.0g/100ml and 9.7g/100ml with standard deviations of ±0.6, ±0.7 and ±0.4 for the control, the occupationally exposed and the CRF patients groups respectively. Out of the occupationally exposed subjects investigated for their PCV and blood haemoglobin levels, 45% showed a mild/moderate degree of anaemia (using an haemoglobin bench mark level of ≤ 11.0g/100ml). Only 25% showed a reduction in their PCV levels using a PCV bench mark ≤ 30%. When the observed PCV and blood haemoglobin concentrations on one hand were compared with the blood lead concentrations, there was a significant correlation between the values obtained. There was also a significant variation between the values obtained for the control in comparison to the values for the occupationally exposed and the CRF patients group. ($P \leq 0.05$, $P \leq 0.01$respectively) (Tables 1 and 2).

TABLE 1: Haemoglobin Concentration/Values (g/100ml) in Lead Poisoning Hb (g/100ml)

Group	No of Subjects	Mean±SD
Control	**21**	**13.4±0.6**
LES	**117**	**11±0.7**
CRF	**21**	**9.7±0.4**
P-value	**Control Vs LES** **Control Vs CRF**	**P<0.05** **P<0.01**

KEY:

LES= Lead Exposed Subjects

CRF= Chronic Renal Failure

TABLE 2: Values of Packed Cell Volume (PCV) in Lead poisoning PCV (%)

Groups	No of subjects	mean±SD
Control	**21**	**43**±2.1
LES	**117**	**37**±2.18
CRF	**21**	**29**±1.9
P-Value	**Control Vs LES** **Control Vs CRF**	**P<0.05** **P<0.01**

KEY:
LES= Lead Exposed Subjects
CRF= Chronic Renal Failure Subjects

Blood Lead (Pb) concentration

The mean blood lead concentrations obtained were 23µg/100ml, 32 µg/100ml and 90 µg/100ml for the control, the occupationally exposed and the CRF patients groups respectively. There was a statistically significant difference between the mean blood lead concentration obtained for the occupationally exposed and the CRF patients groups when compared with the mean blood lead concentrations obtained for the control group ($p \leq 0.05$). There was also a positive correlation between the mean blood lead concentration and the degree of anaemia (as depicted by their haemoglobin concentrations) when compared laterally with the controls and directly with the others in the experimental group.

From the results, about 17.5% showed blood lead concentrations ≥ 40µg/100ml – the non-conventional adult reference limit. Those with the highest results of 82µg/100ml; 88µg/100ml; and 90µg/100ml were amongst those with the lowest concentration of blood haemoglobin and haematocrit values and hence with the highest degree of anaemia as previously stated.

It could be affirmed that about 18% of the occupationally exposed subjects had excessive level of lead in their blood. However, most of them had some degree of anaemia as shown by their haemoglobin values (Table 3).

TABLE 3: Blood Lead (Pb) Levels in Lead Poisoning Blood (Pb) Levels (µg/100ml)

Group	No of Subjects	Mean ±SD
Control	**56**	**23±19.7**
LES	**123**	**32±20.8**
CRF	**8**	**90±13.9**
P-Values	**Control Vs LES** **Control Vs CRF**	**P<0.05** **P<0.05**

KEY:
LES= Lead Exposed Subjects
CRF= Chronic Renal Failure Subjects

Na^+/K^+ATPase Activity

The results of Na^+/K^+ATPase assay showed mean values of 67.0µmiP/µgP/min, 77.24 µmiP/µgP/min and 28.83 µmiP/µgP/min for the control, the occupationally exposed and CRF subject groups respectively (Table 4).

On statistical analysis, there was a statistically significant variation between values obtained for the CRF patients in comparison to the other two groups ($P \leq 0.05$).

From the results, it became apparent that though the Na^+/K^+ ATPase activity in both control and experimental subjects were insignificantly different, statistically, however, a positive correlation was later found between the Na^+/K^+ ATPase activity on one hand, and the blood haemoglobin concentration and packed cell volume, (PCV) on the other.

TABLE 4: Na^+/K^+ATPase activities in Lead poisoning ATPase activity in (µmiP/µgP/min)

Groups	Minimum	Mean	Maximum	SD value	P-value	No of Subjects
Control group	13	67±19.65	98	±19.65		7
Occupationally Exposed group (LES)	34	77.24±62.55	200	±62.55		15
CRF patients group	5	28.83±29.45	88	±29.45	P≥0.05	12

P –Value is not significant at P≥0.05

DISCUSSION

As stated earlier, mild anaemia is a common feature of lead poisoning. Presently, the two theories on the pathogenesis of the anaemia associated with plumbism centered on the blockage of porphyrin synthesis at the level of the enzymes dehydratase and ferrochelatase in the haematopoietic pathway. The formation of lead phosphate and hydrochloric acid when lead interacts with the inorganic phosphate abundant in the red cell according to the equation below was the third pathway for the anaemia:

$3Pbcl_2 + 2Na_2HPO_4 = Pb_3(PO_4) + 4Nacl + 2Hcl$

In this study, using a bench mark of 33% as the lower PCV reference limit, 29% of the occupationally exposed subjects (34 out of 117) could be said to be anaemic. This same percentage (29%) was also found to be anaemic based on a minimum blood haemoglobin concentration of 11.0g/100ml. This was the basis for the determination of Na^+/K^+ATPase activity in the red cell ghost membrane of selected blood samples from both the control and occupationally exposed groups. A few numbers of subjects in the three groups were randomly picked for the ATPase assay. This random selection was not only due to the complexity and cumbersomeness of the ATPase method but also since it has been proved that ATPase being an integral membrane enzyme obeys an all or none condition; hence its activity would be demonstrated in a definite and uniform pattern in a given condition Pranay Kathrine, [12]. The objective of demonstrating the activity of this enzyme that regulates ionic movements across cell membranes was therefore to find out whether the observed anaemia in lead exposed subjects could be as a result of an abnormality in its level as a consequence of the excessive blood lead concentration in this vulnerable group. The fact that excessive lead level has been reported to interfere with most -SH- containing body enzymes like Na^+/K^+ATPase Prankerd, [13] lent credence to the choice of the analysis of this membrane bound enzyme; the derangement in the level of this enzyme could therefore precipitate an alteration in the degree of porosity of the red cell membrane with a possible consequent leakage of haemoglobin out of the red cell.

However, from this study, only a mild/moderate degree of anaemia could be ascertained in the occupationally exposed subjects as depicted by the haematological parameters studied. This was similar to findings of other researchers like Barltrop *et al* [14] and Barltrop *et al* [15] who all reported moderate /mild anaemia in subjects chronically exposed to lead. That the anaemia observed could be as a result of the failure of the Na^+/K^+ATPase pump normally responsible for maintaining the environmental milieu of the red cells could not be confirmed from this study. It was observed that there was no statistically significant variation in the Na^+/K^+ ATPase activity in the ghost membrane of both the experimental and control subjects in the sample population studied; although this finding was not totally in consonance with those of others, Jeddi-Hansan *et al* [16]; Hernberg [17], however, there was a positive correlation between the mean PCV, blood haemoglobin levels and the mean blood lead and ATPase concentrations. A positive correlation was also found between the mean blood lead concentration and the mean Na^+/K^+ATPase activity in the control and exposed group of subjects studied (Tables 5 and6). Since the function of the ATPase enzyme in regulating ionic movement across membranes is not in doubt, the need to take this into consideration in the management of complications associated with lead and other known metal poisons should be borne in mind by the physicians handling metal poisoning. This becomes imperative as the enzyme has also been implicated in the tubular exchange activities of the kidney and even in the regular exchange processes of the gut Fernando Magro *et al*, [18]. The fact that this enzyme is membrane bound points to the need to monitor the integrity of the cell membrane

especially in established cases of metal toxicity as in occupational exposure. This could ensure early correction of the abnormality and prevention of the complicating features in most cases of metal toxicity thus narrowing clinical management to the primary cause and effect of the problem. Also, the role and implication of this enzyme especially in occupationally related exposures should also be borne in mind by the haematologist when investigating the basis of cases of unexplained anaemic state.

This, coupled with detailed investigation of the various enzymes associated with the cell membrane pumps on a larger scale might be able to explain the pathophysiology of the anaemia and other complications usually associated with lead poisoning; such studies should also consider the probability of polymorphism based on racial differences in the enzyme composition. Perhaps future efforts could be geared towards developing membrane markers that could qualitatively identify this enzyme such that a microscopically demonstrable presence or absence of the enzyme could easily be detected and used towards early diagnosis and therefore proper management of complications like anaemia associated with Pb poisoning. The need for such a better marker of anaemia as a complication of lead (Pb) poisoning especially in the less developed world where the use of more sophisticated and sensitive methods of monitoring plumbism and other metal toxicity is not visible cannot be overemphasized.

TABLE 5: SUMMARY OF THE MEAN RESULTS

	CONTROL	**EXPERIMENTAL/OCCUPATIONALLY EXPOSED SUBJECTS**	**CRF SUBJECTS**
Lead (µg/100ml)	23.42	31.69105691	89.875
PCV	43.80	36.86324786	29.4762
HB g/100ml	13.37	11.02905	9.70952
Na^+/K^+ ATPase	68.5	77.24	28.83

Key to Table:
PCV= Packed Cell Volume; HB= Haemoglobin concentration

REFERENCES

1. A. L. Woo., P.F. James., and J.B. Lingrel. (1999). Characterization of the Fourth @ Isoform of the Na, K – ATPase. *J. Memb. Biol*. 169**(1)**, 39-44.
2. Waldron, H.A. (1963). The Anaemia of Lead Poisoning: A Review. *Br. J. Ind. Med*. **3**, 90-100.
3. Waldron H.A. (1964). The Effect of Lead on the Fragility of the Red Cell Incubated *in Vitro*. *J Clin. Pathol.* **17**, 405 (1964).
4. Hernberg S. and Hansan J: Hernberg, S.(1976).Biochemical, Subclinical and Clinical responses of lead and their relation to different exposure levels. Nordberg, NY.
5. White J.M. and H.S. Selhi. (1975). Lead and the Red Cell. *Brit. J Haematology*: **30:** 133-138.
6. Hernberg, S., and Nikkernen, J. (1970). Na^+/K^+ATPase Lancet, **i 63**,
7. Pearson, J.B., Dacie, J.V., Grimes, A.J., and Brain,M.C., (1982).Recent Advances in Haematology pp78-80; (Eds Goldberg, Edinburgh and London; Churchill Livingstone Pub. (1982).
8. Clegg, J.W. and King, E.J. (1942). Estimation of haemoglobin by the alkaline haematin method: *British Medical Journal*, **ii**, 329.
9. Hessel, D.W.,(1968). A simple and rapid quantitative determination of lead in blood,; Atom Absorp. New. **7**, 50-55.
10. Olorunsogo, O.O., Lawal, S.O.A., Falase, A.O., and Okunade, W.G.(1991). Erythrocyte Membrane Ouabain-Sensitive Na^+/K^+ATPase of Hypertensive Nigerians. *Afr. J. Med. Sci.*, **20**, 75-82.
11. Jarret, H.W., and Penniston, J.T.(1978). Purification of Ca^{2+} stimulated ATPases Activators from Human Erythrocytes: Its membership in the Class Ca^{2+}-Binding Modulator Proteins. *J. Biol. Chem*., **253**, 4076-82.
12. Pranay Kathurai, and Paresh Jadav (2003). Lead Nephropathy eMedicine WebMD.
13. Prankerd, J.A. T., Laris, P.C., Ewers, A. and Novinger, G., (1962). A Comparison of the Inhibition of Stromal Adenosinetriphosphatase and Inhibition of Sugar Permeability in Erythrocytes, *J. Cell. Comp. Physiol*. 59, 145-154,
14. Barltrop D.: (1968).Lead poisoning in childhood. Postgrad. Med. J. **44**: 537-546.
15. Barltrop, D., Strehlow, C.D., Thorton, I., and Webb, J.S.,Postgrad.(1975). Med. J., **51**, 801.
16. Jeddi Hassan, Sven Hernberg and Veikko Vikko. (1967). Deficient Red Cell Membrane Na^+/K^+ATPase in lead poisoning. Arch. Environ. Health: **14** 313-318.
17. Hernberg, S. (1976). Biochemical, Subclinical and Clinical responses of lead and their relation to different exposure levels. Nordberg, NY.
18. Fernando Magro, Sónia Fraga, Tomé Ribeiro, and Patrício Soares-da-Silva*:(2004). Intestinal Na^+–K^+–ATPase activity and molecular events downstream of interferon-γ receptor stimulation. B*rit. J. Pharmacol*. 142**(8)**, 1281-1292.