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Research article

Plasma and Aqueous Humour Electrolyte Levels, Sodium Pump Activity and Intraoccular Pressure in Glaucoma and Cataract Subjects attending a Nigerian Tertiary Hospital

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ABSTRACT

In this case-controlled study, electrolyte levels in Aqueous Humour (AH) and plasma, sodium/potassium adenosine triphosphatase (Na+/ K+ ATPase) activity as well as intra-occular pressure were measured in normal, cataract and glaucoma participants as possible biomarkers of degenerative diseases of the eye. Three groups of age-matched consenting participants were recruited viz: Non-visually impaired Control, participants with Open-angle glaucoma (cases) and those with cataract (Negative control). AH fluid was surgically collected from glaucoma and cataract participants. Sodium, potassium, chloride and bicarbonate levels were determined in plasma and in the AH fluid using ISE-4000. Red-cell membrane Na+/ K+ ATPase activity and plasma glucose were assayed spectrophotometrically. Blood pressure (BP) and pH were monitored in all participants using standard methods. Data were analysed using ANOVA and significance was at $p<0.05$. All participants were normotensive and normoglycaemic. Mean Intra-occular Pressure (IOP) was significantly higher in glaucoma. Mean plasma and AH Na+, K+ and Cl- levels in the three groups of participants were similar. Mean AH Cl- (121.70±5.4mmol/L) and HCO3- (13.55 ± 3.71mmol/L) levels in glaucoma were significantly higher when compared with cataract and control groups. Mean Na+/K+ ATPase activity in cases was low (22.35 ±1.09µmiP/µgP/min) in comparison to that of cataract and control [23.40±1.09 and 25±2.0µmiP/µgP/min respectively]. The mean pH of AH in cases was 6.7 in comparison to a pH of 7.2 in all participants plasma. Increased levels of Cl- and HCO3- ions were observed in AH in glaucoma and this may be prognostic for glaucoma subjects.

Keywords: *glaucoma; electrolyte levels; Na/K ATPase activity; anion/cation imbalance*

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INTRODUCTION

Glaucoma is a disease of the eye that develops when there is accumulation of aqueous humour fluid leading to pressure build up inside the eye due to obstruction to the outflow of this fluid. It is often called the 'sneak thief of the sight' because intra-ocular pressure (I.O.P) can build up and destroy sight without obvious symptoms. Elevation of intra-ocular pressure may result in blurred vision and if untreated often leads to complete loss of vision. Thus, awareness is very important because this disease when detected early can be successfully treated (Flammer et al, 1999).

Glaucoma occurs in about four different forms. It could be Primary Open-Angle Glaucoma (POAG) also called wide-angle glaucoma characterized by structurally normal eye except for accumulation of fluid in the eye due to obstruction along the trabecular meshwork; this is the commonest type of glaucoma. It could also be Chronic Angle-Closure Glaucoma (CACG) which is also called acute or chronic angle-closure. Characteristically, this type of glaucoma is less common but can cause a sudden buildup of pressure in the eyes due to poor drainage occasioned by a narrowing of the channel of fluid outflow. The third is called Normal-Tension Glaucoma where despite normal I.O.P, there is vision loss. This is common in adult women above 60 years of age. The fourth called Congenital Glaucoma is characterized by cloudy cornea, photophobia, epiphora; it is more common in boys than in girls (Gupta 1997).

Cataract is an opacification of the lens of the eyes leading to loss of transparency and eventually causing loss of vision. Most cataracts are caused by degenerative changes often occurring at a later stage in life (Varon et al., 2014). Glaucoma and cataract involve an abnormal alteration in the biochemical composition of the internal environment of the eyes which essentially includes the watery aqueous and the gel-like vitreous humour. These two debilitating diseases are of serious concern because they affect a very vital organ of the body which when damaged leads to redundancy, dependence and even retrenchment for the working-class citizen.

The human ciliary epithelium is the site of aqueous humour secretion in the mammalian eye through the process of secretion by ciliary epithelium and active filtration across the ciliary membrane (Millar and Kaufman, 1995), this function is largely regulated by the sodium pump. Normally, aqueous humour fluid produced flows through the trabeculae and is drained via the ciliary veins of the epithelia into blood vessels. Transportation of the fluid after production is said to be mediated by ATPase and carbonic anhydrase (Goel et al, 2010). The observed IOP is determined by the rate of aqueous humour formation by the ciliary body and the resistance to its outflow or drainage through its various routes outlined above (Edward and Hugh, 2018). While the ATPase hydrolyses ATP to produce the energy needed for the transportation, active transport across the epithelia is said to be madiated by carbonic anhydrase through the conversion of CO2 to bicarbonate. Hence, bicarbonate level is linked to the regulation of the pH of the milieu and directly affects the Na/K pump in the production of energy needed for the transportation (Goel et al, 2010).

Sodium-Potassium Adenosine Triphosphatase (Na+/K+-ATPase), also known as the Na+/K+ pump, (sodium-potassium pump, or sodium pump, for short) is an enzyme located in the plasma membrane. It is an electrogenic transmembrane ATPase in all animals. Na-pump is a pivotal ion pump in the formation and transport of aqueous humor fluid in the ciliary epithelium which when dysfunction as in open angle glaucoma, could lead to an elevation of intraocular pressure in the eye. Reports on lens ion homeostasis has highlighted the role of the sodium pump in the regulation of ionic movement across the epithelia membrane and consequently that of IOP. This function is mediated through functional muscarinic and purinergic receptors at the surface of the lens and was suggested to be involved in short-term regulation of Na+/K+-ATPase in the ciliary epithelium (Duncan; 1969, 1970). There has been limited exploration of the functions or relevance of this transmembrane pump in the secretion and control of the AH fluid especially in this part of the world. Hence, determination of this enzyme activity in the red cell ghost membrane of glaucoma patients compared with electrolyte concentration in both plasma and aqueous humour fluid could explain the pathophysiology of increasing IOP in glaucoma. This is the focus of this work..

MATERIALS AND METHODS

Participants:

20 patients, clinically diagnosed for open angle glaucoma by a consultant Ophthalmologist (by measurement of IOP and Tonometry), 20 clinically diagnosed patients with cataract (as negative control) and 20 subjects with neither cataract nor glaucoma as blind control all attending Ophthalmology clinic of the University College Hospital, Ibadan, Nigeria were recruited. Ethical approval was obtained for this study from the UI/UCH Ethical Review Committee

Sample Collection:

10mL blood was collected from participants in the three groups; the blood was divided into three to be used for the determination of ATPase activity, plasma electrolytes and plasma glucose levels. 0.2mL of aqueous fluid was also collected at surgery from cataract and glaucoma participants for measurement of Aqueous Humour (AH) electrolytes using ion-selective electrode.

Analysis:

Na+/K+ ATPase activity was estimated using the method of Jarret and Penniston (1978) where centrifugation, washing and haemolysis of the red cell were done prior to hydrolysis of ATP to release inorganic phosphate which was then estimated spectrophotometrically.

Plasma from the remaining blood was used for the measurement of Fasting Plasma Glucose (FPG) and 2hour post prandial glucose (2HR PPG) based on the principle of oxidase /peroxidase enzymatic method (Trinders, 1969). Electrolyte levels (Na^+, K^+, Cl^- and HCO_3^-) were determined in the remaining portion using automated principle of ion selective electrode (Sarl-Benganton, 2009) adapted in ISE 4000 auto-analyzer. pH was measured in the aqueous fluid using a pH meter (Mettler pH meter)

Statistical Analysis

Statistical analysis of the results was done using Analysis of Variance (ANOVA). Pearson correlation was used to determine the correlation between the variables. Value of $p \leq 0.05$ was considered significant..

RESULTS

Gender distribution:

The number of participants (control, cataract and glaucoma) recruited for this study was sixty (60) consisting of 29 females (48.3%) and 31 male (51.7%). Their mean age was 56.4years (range 40 -73years), the ages were matched and were not statistically different. The age bracket of participants recruited was the typical age group when cataract and other ocular degenerative diseases ordinarily set in.

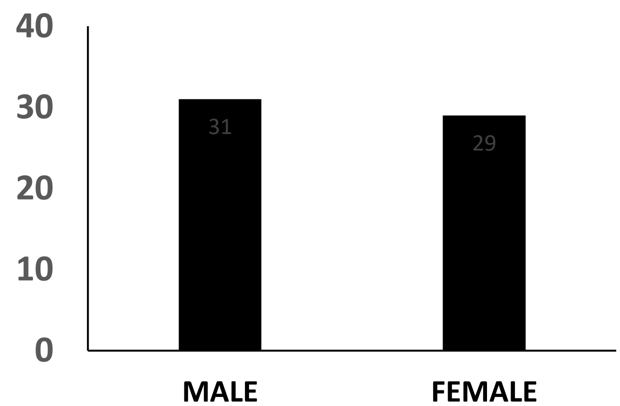

Figure 1
Gender distribution of participants

Blood Pressure

The blood pressure profiles of the participants are shown in Fig. 2. The mean diastolic blood pressure was 113±11mmHg while mean systolic blood pressure was 77±11mmHg in glaucoma participants. Mean diastolic and systolic blood pressure in cataract participants were 114± 11mmHg and 79± 9mmHg respectively while mean diastolic and systolic blood pressure in controls were 106±10mmHg and 72 ±7mmHg respectively. The differences in blood pressure were not significant across the three groups of participants. Overt hypertension was therefore excluded in participants recruited for this study.

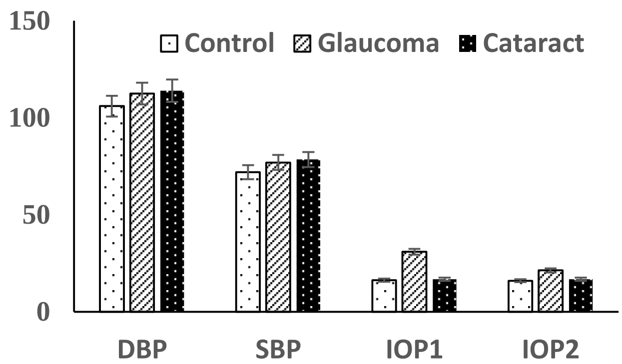

Figure 2

Blood pressure and Intra Ocular Pressure in the two eyes of the participants Studied (Glaucoma).

IOP1= Intra ocular pressure in the right eye; IOP2= Intra ocular pressure in the left eye; DBP= Diastolic blood pressure, SBP= Systolic blood pressure

Intra-ocullar Pressure

The Intra Ocular pressure in the two eyes were 31/21 ±11/6, 17/17±2/3 and 16/16±2/3 for glaucoma, cataract and control participants respectively. The mean IOP in glaucoma participants was significantly different from that of the other two groups ($P \leq 0.05$).

Biochemical parameters

While there was no significant difference in mean blood pressure in the three groups of participants studied, mean plasma glucose levels of 81.80±9.80 mg/100ml, 81.40±14.50 mg/100ml and 86.30±13.60mg/100ml were observed in the three groups respectively. Also, mean plasma electrolyte levels were 136.95±3.07, 3.89±0.39, 103.15±4.00 and 21.50±2.63 for Na+, K+ , Cl- and HCO3- respectively in glaucoma participants; 136.80±3.38, 3.99±0.45, 103.35±3.18 and 20.95±2.31 for Na+, K+ , Cl- and HCO3- respectively in cataract participants while values of 137.65±3.80, 3.67±0.28, 105.40±4.64 and 22.45±1.85 were obtained for Na^+, K^+ , Cl- and HCO3- respectively in controls (Figs 3-5). The differences were non-significant. Mean AH concentrations of Cl- were 122±5.0 mmol/L and 91±4.0 mmol/L while mean AH HCO_3- were 14±4mmol/L and 10±2.0mmol/L in glaucoma and cataract participants respectively; the differences were significant. Although Cl- level in AH of glaucoma participants was similar to those of normal controls, plasma Cl^- levels in cataract participants was very low compared to those in controls. However, glaucoma participants showed hyperchlorydria in comparison to the concentration of Cl^- in AH of cataract participants. Cl- concentration was more than double in AH of glaucoma participants compared to what was obtained in AH of cataract participants.

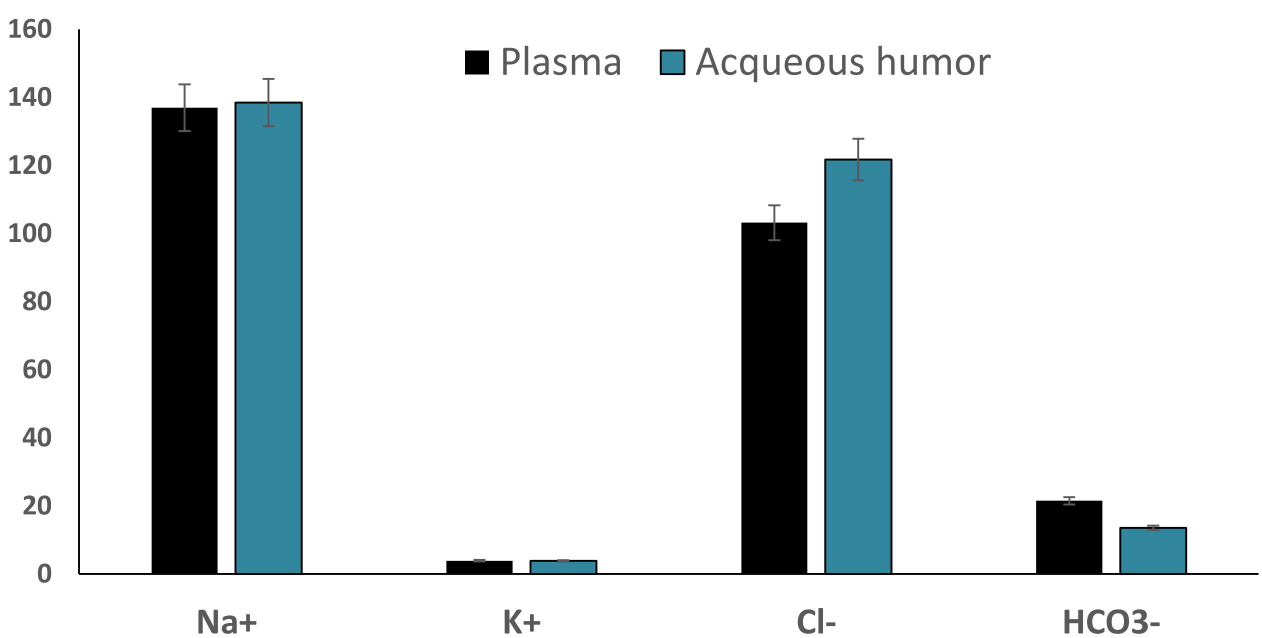

Figure 3:

Plasma and Aqueous Humor Biochemistry in Participants with Glaucoma

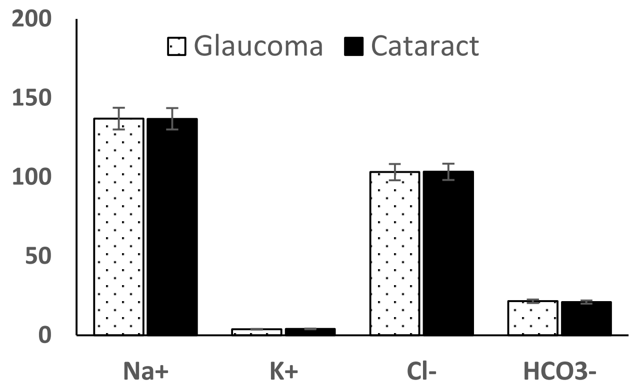

Figure 4:
Disparity in plasma and aqueous humor biochemistry in Participants with Cataract.

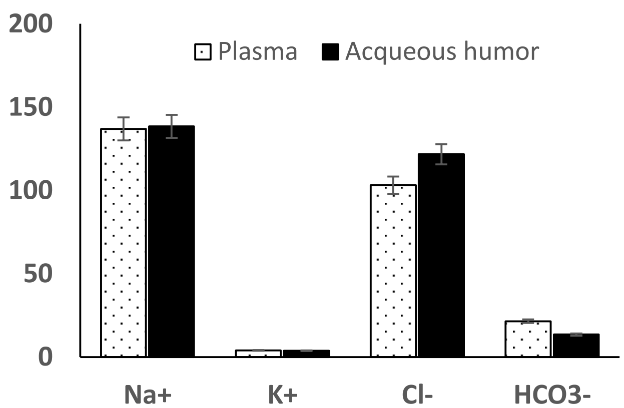

Figure 5:
Plasma and Aqueous Humor Biochemistry In Participants With Glaucoma

In contrast, there was a marked reduction in HCO_3- concentrations in AH fluid of glaucoma and cataract participants. This was clearly different from what was obtained in the plasma as the HCO_3^- level was similar in all the three groups. On further analysis, significant statistical difference was observed in the plasma levels of K^+ and HCO_3^- in control and cataract participants only while reduced Na+ ATPase activity was observed in red cell ghost membrane of glaucoma participants relative to control and cataract participants (Figs 3 – 5). The pH of the pooled AH fluid from glaucoma was 6.7 in comparison to the plasma pH of 7.2 indicating slight acidity in the AH fluid relative to the plasma. Multiple comparative analysis showed that concentrations of Na^+, Cl^- and HCO_3^- in AH were significantly different in glaucoma and cataract participants while comparatively, plasma K and HCO_3^- concentrations in control and cataract were significantly different.

DISCUSSION

Although incidence of glaucoma has been reported to be high, affecting over 6.7million people and ranking second to cataract (19.3 million) as a cause of blindness worldwide (WHO, 1998), the pathophysiology of the disease still remains diffuse. In spite of its different clinical impressions, establishing the aetiology of this condition remains a problem. This work thus investigated the biochemical alterations that may be associated with the pathophysiology of the disease. Since age is an important risk factor in the development of most degenerative diseases of the eye including glaucoma and cataract, anthropometric characteristics of participants in this study with particular reference to age became very important. The age of participants in this study agreed with previous works which reported that cataract, glaucoma and most of the degenerative diseases of the eye usually set in from fifty years (Pelletier, Rojas-Roldan, Coffin (2016); Mimura et al, (2013).

Electrolyte composition and aqueous humour fluid in this condition was also investigated since the latter is an ultra-filtrate of the former. This was to establish the basis of the increase in IOP which is known to be prognostic of this condition. The major ions in the Extracellular fluid (ECF) are sodium, calcium, chloride, bicarbonate and others like magnesium. Potassium is an intracellular cation just as phosphate. These electrolytes are largely associated with the maintenance of ionic balance between the ECF and Intra Cellular Fluid (ICF) and that of oncotic against hydrostatic pressure in the lumen of the blood vessels. It is the balance of the oncotic and hydrostatic pressure that largely contributes to the observed blood pressure in the human system. The ionic concentration is usually aggravated by the presence of hyperglycaemia, hyperlipidaemia and accumulation of some drug metabolites (Lupsa and Inzucchi, 2014). Ionic Concentration has always been associated with variations in pressure, this is largely due to their characteristic Brownian movement. Aside from ions, some metabolites have also been known to contribute to the ionic pressure within the human system. Hence, hyperglycaemia, hyperlipidaemia and even metabolites of some common drugs when present in excess have been documented as agents of increased ionic concentration and consequently pressure in the human system (Lupsa and Inzucchi, 2014, Iris et al., 2012). For example, in hypertensives, progression or reduction in the blood pressure has always been synergistic with the concentration of sodium chloride which constitutes the largest ionic group in the ECF. Hypertension has also been known to be aggravated by the presence of hyperglycaemia, hyperlipidaemia and excessive accumulation of metabolites of common drugs like analgesics. In this study, all three groups of participants recruited were normotensive and normoglycaemic; this may largely indicate normal ionic movement and pressure in the three groups. Composition of aqueous humour fluid was investigated in our search for the cause of increased IOP in glaucoma patients. The AH is a transparent, watery fluid similar to plasma, but containing low protein concentrations. Unlike the plasma electrolyte composition of the three groups of participants investigated in this study which was found to be similar, elevated Cl^- and $HCO3^-$ concentrations were observed in the AH of glaucoma participants. The increased Cl^- and HCO_3- were not concurrently followed by corresponding increase in the Na+ and K+ ions in the AH fluid of glaucoma participants clearly indicating a preponderance of negatively charged ions relative to the positively charged cations. These anions were found to be higher in glaucoma than in cataract participants. However biochemically, Cl- and HCO3- ions are known to usually associate with Na+ and K+ respectively in almost a definite manner within the milieu of the human system (Curtis et al., 2006). Under normal physiological conditions, the body tends to maintain ionic neutrality by either passive or active movement of ions across the membrane barrier to ensure equilibrium, homeostatic and ionic balance in the ECF and ICF. This known association was disorganised in the group of glaucoma patients studied. This disruption in the relationship may be the basis of the increased IOP in glaucoma. However, for electrical neutrality to be maintained, there may be substitution of the two known cations (Na and K) with other cations of equivalent charges while that of Cl and HCO3 gradually rises within the AH fluid.

The preferential alteration in the ionic composition (i.e. anion > cation) informed the need to investigate the sodium/potassium pump known to regulate this process. The selective retention of anions (Cl^- and HCO_3-) may have been facilitated by a special "isomer" of the enzyme Na/K ATPase responsible for this action or it may be attributed to a modification of the molecular type of the enzyme (Na/K ATPase) in the AH of glaucoma patients that allows exclusive accumulation of the anions without a concurrent increase in the concentration of the cations in glaucoma cases. Although the observed level of Na/K ATPase activity in the three groups of subjects could not confirm this phenomenon, the significantly reduced difference in the ATPase level in glaucoma patients may be an explanation for the observed alteration in ionic concentration. Further studies on possible isolation and characterization of the type of ATPase enzyme in the AH of glaucoma patients in comparison to those normally present in most cell membranes may be able to clarify this. The observed imbalance in anion:cation gap in glaucoma may also be due to a defect in the homeostatic system that normally should have balanced up the difference in the ionic concentrations on the two sides of the cell. A complete electrothermal analysis of both ECF and ICF fluid from participants with glaucoma may be able to establish this and further explain the observed increase in IOP in these participants. This becomes plausible because concentrations of the known cations and some macromolecules (glucose) that physiologically trigger increase in ionic movements and eventually manifest as increase in either hydrostatic or oncotic pressure are similar in all the groups. This deduction is also confirmed from their measured BPs which were statistically similar in the three groups in spite of the differences in ionic compositions. Earlier works on ionic homeostasis in eye fluid by Duncan and his team also supported this (Duncan et al, 1960). It may then be inferred that continuous sodium-pump-mediated sodium extrusion is imperative in the maintenance of ionic homeostatic balance in the eye fluid. It may equally be stated that without this active sodium extrusion, sodium and calcium ion content in the fluid may increase resulting in lens swelling and deterioration of transparency. Again, the discovery of functional muscarinic and purinergic receptors on the surface of the lens suggests that purinergic receptors might be involved in short-term regulation of Na/K-ATPase in the epithelium (Duncan et al, 1970). Purinergic receptor agonists of ATP and UTP selectively activate certain tyrosine kinases and stimulate Na/K-ATPase activity. This might represent part of a control mechanism capable of adjusting, perhaps fine-tuning lens ion transport machinery. The long-term effect of this imbalance may largely be seen in the alteration in IOP characteristic of glaucoma. It may therefore be inferred that imbalance in the anion/cation gap of electrolytes in AH of glaucoma patients coupled with a reduced pH may be prognostic of glaucoma.

Since this work investigated Open angle glaucoma, it may be necessary to explore and investigate the biochemical picture in other types of glaucoma. Secondly, the sample size may need to be increased to enable a reliable deduction from this suggestion while further studies on the possible molecular isomer of the type of ATPase enzyme in AH fluid of glaucoma and non-visually impaired subjects may need to be determined

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