Review Article

Tear Electrolytes Concentration in Hospital-derived African Subjects

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Abstract

A framework for research on the interactions between macro-elements of the lachrymal functional unit and the ocular surface in health and disease requires data on their physiologic values. This study measured the concentration of sodium, potassium and chloride in tear and serum of apparently healthy hospital subjects.

The measurement was from 25 female and 25 male subjects with a mean age of 52.34 ± 13.17 year. The mean concentrations of sodium, potassium and chloride in tear and serum in mMol/L were 131.06 ± 6.39, 21.40 ± 1.57 and 122.86 ± 7.12 respectively and 135.56 ± 3.85, 4.14 ± 0.70 and 101.58 ± 3.25 respectively. Potassium and chloride were found to have a statistically significant higher in concentration in tear than in serum ($p < 0.001$ and $p = 0.001$ respectively). The converse was true for sodium concentration but this was not statistically significant ($p = 0.250$). Age and sex had no statistically significant effect on tear electrolytes but there appeared to be a relatively good fit between age and tear potassium concentration of inverse nature ($R^2 = 0.57$). The strongest correlation in the two body fluids was found for sodium concentration (Adjusted $R^2 = 0.019$, F = 0.107, $p = 0.745$).

High potassium concentration in tear is likely protective against extraneous proximal and distal ocular tissue injury and other yet-to-be identified role(s) for electrolytes in ocular health will await further research. There is however need to standardize instruments for electrolyte analysis and a method that minimizes reflex tearing to make measurements readily reproducible.

Keywords

Tear, Serum, Electrolytes, Potassium

Introduction

Tear as an extracellular fluid is of critical importance in the maintenance of ocular surface integrity and achieving optimal vision. The tear film over the ocular surface exists in a 3-layer sub-structure known as the precornea tear film. The outermost lipid layer retards tear evaporation, prevents tear overflow and minimizes friction during eye blinking. An aqueousmid-layer accounts for about 90% of tear film volume and retains the capacity for further volumetric expansion in response to any form of ocular irritation. Beyond its lavage function, the aqueous component is an immunologically-competent medium, antagonizing pathogenic microbes and neutralizing toxins with its array of immuno-modulators [1,2]. The innermost mucin layer ensures an even spread of the aqueous component over the ocular surface. Refraction of light over the ocular surface is most efficient with a uniformly-spread precornea tear film. At least 70% of light refraction within the optical system of the eye is achieved at the air-precornea tear film interphase. In the absence this, the eye will be significantly under-powered with an attendant high hypermetropia [3].

Tear secretion is controlled by the lacrimal functional unit (LFU) which is an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands) and lids, and the sensory and motor nerves that connect them [4]. Tear fluid contains water, electrolytes, proteins, carbohydrates, lipids and a complex combination

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of the afore-mentioned macromolecules. The multi-lobular lachrymal gland is the major source of tear and is populated by acinar, ductal, and myoepithelial cells. The acinar cells are the predominant cell type and are responsible for primary tear production. The ductal cells modify the primary tear by absorbing or secreting water and electrolytes as it flows though the lachrymal duct to the outflow orifices. The final electrolyte concentration in tear is therefore determined by ductal cells [5-7].

'The major electrolytes found in tear are sodium, potassium, calcium and magnesium and the corresponding anions chloride and bicarbonate. Tear is not necessarily an ultrafiltrate of blood because of the extensive modification by the lachrymal cells. Studies have shown that tear have higher potassium and chloride concentration in comparison to serum while its protein and sugar content, like other extracellular fluids, falls short of the concentration found in blood [8-11]. As essential components of tear fluid, electrolytes regulate tear pH, stabilize the tear film and contribute to the maintenance of cornea epithelial thickness. The bicarbonate ion (HCO_3^-) buffers tear to keep the pH within physiologic limits needed for optimal ocular comfort [12].

Tear osmolality is the number of solutes in one liter or kilogram of tear. Electrolytes in tear essentially determine tear osmolality because they are found in much higher concentrations than other dissolved macromolecules such as proteins and sugars. Tear film stability is related to tear osmolality [13]. Abnormalities in tear osmolality therefore results in tear film instability and precedes the development of dry eye disease (DES), one of the commonest ocular conditions affecting up to 5-50% of the global population [14].

Potassium ion has been shown to play an important role in maintaining cornea epithelial thickness [15,16]. Cornea epithelial cells are the primary cells that absorb Ultraviolet light in the wavelength range of 290-315 nM (UVB) [17]. UVB radiation is a component of solar radiation and has been assigned a role in the etiopathogenesis of a wide range of ocular conditions spanning from the anterior to the posterior segments. These disease conditions include pterigium, pingueculum, climatic droplet keratopathy, ocular surface squamous neoplasia (OSSN), cataract and glaucoma through both trabecular meshwork injury and via direct retinal ganglion cell toxicity [18-22].

Tear as a source of biomarker in ocular and systemic conditions is currently increasingly being researched given that it can be accessed in a non-invasive manner. Hagan et al did an excellent review of current pursuits in the field of proteomics as a means of unraveling the presence of biomarker proteins in tear in various disease states [23]. Brain-derived neurotrophic factor (BDNF) have been shown to be low in tear of glaucoma patients [24]. Hypertonicity of tear as indicated by its osmolality is the most consistent objective observation seen in patients with DED [25,26]. Recent studies have suggested that measurement of certain tear electrolytes could be relevant in the assessment of diabetic patients [27,28]. The major cations in tear are sodium and potassium while chloride is the most abundant anion. In this study, these ions will be measured as an initial step in an effort to generate baseline data for their concentration in this important fluid in an African population where apparently none currently exist. For purposes of comparison and because tear is partially ultra-filtrated from blood, the serum electrolytes of the subjects will also be measured.

Study Design

This was an observational cross-sectional study of consecutive and eligible adult subjects aged 30 years and above who presented to the eye clinic of Enugu State University teaching hospital, Parklane, Enugu from December 1st 2015 to February 27th 2016. Enugu is a cosmopolitan city located on latitude 6° 27' 35.8704'' N and longitude 7° 32' 56.2164'' E south-eastern Nigeria with about 722,664 inhabitants [29].

Subjects

A total of 50 patients who fulfilled our inclusion criteria were recruited for the study. The subjects were recruited on the basis of presenting with symptoms of refractive error and/or presbyopia only.

Subjects aged less than 30 years, presence of inflammatory or ocular surface disease, use of potassium supplement, metabolic or chronic systemic disease and pregnancy were exclusion criteria [30,31]. Absence of diabetes mellitus was further excluded with a fasting or random blood sugar using Accu-ChekR Active (Roche) glucometer. Subjects with fasting blood sugar of 120 mg% and above or random blood sugar of 180 mg% or above were excluded [32]. Subjects with glaucoma were equally excluded because they are subject of a related study by the same authors.

Demographic and medical histories of participants were filled using a standardized questionnaire (Appendix 1). Comprehensive ocular examination was carried out on all subjects after documenting their visual acuity and examination with a slit lamp biomicroscope (Haag Streit BM900). Intraocular pressure (IOP was) measured with Haag Streit Goldmann R-type applanation tonometer. The posterior pole with emphasis on the optic nerve head was examined with a +78D super field aspheric lens by Volk R (U.S.A) to exclude glaucoma.

Sample Collection and Analyses

All samples were taken between 10 am and 12 pm to minimize the effect of diurnal variation in electrolyte composition [33]. In order to ensure uniformity, all the participants were subjected to the same instruments, in the same room by the same examiner during the clinical data acquisition all through the study. Four milliliters of venous blood sample was collected from the antecubital vein with a sterile 5 ml hypodermic needle and syringe and transferred to a sterile plane glass tube. The blood was allowed to stand for 30 minutes to clot and was centrifuged with Techmel TM centrifuge (USA) at a speed of 5000 revolutions/minute to yield a supernatant serum. 200 ul of tear sample was collected from the inferior lateral tear meniscus of the ocular surface with a sterile 50 ul plane capillary tube over four collections and emptied into a sterile plane glass test tube [34]. Basal

tear collection is difficult in practice, even from anesthetized cornea. However, the difference in basal and reflex tearing is appears to be mostly in its protein content [35]. Tilting the head facilitates tear collection from the lateral canthal area. Both serum and tear samples were analyzed as native samples using ST- 100 B electrolyte analyzer by Sensa Core R, Hyderabad India, an automated electrolyte analyzer which operates on the principle of direct ion selective electrode (ISE). All results from the electrolyte analyzer were included and no measurement was removed as an outlier during analysis.

Data Management

The measured variables were presented in tables, chart and prose. Data analysis was done with SPSS version 16 (SPSS, Inc, Chicago, USA). Bivariate analysis was conducted using cross- tabulations and 2-tailed t-test was used to evaluate associations between means of tested variables. A linear regression was done to investigate the trend within categorical variables. A p-value < 0.05 was considered statistically significant. Diagnostic accuracy test was performed to determine sensitivity and specificity of the test.

The tenets of the Helsinki Declaration regarding the conduct of research involving human Subjects were observed[36]. Informed verbal consent was duly obtained from all the participants [36] (Appendix 2).

Ethical clearance was obtained from Health Research Ethics Committee of Enugu State University Teaching Hospital, Park lane, Enugu (Appendix 3).

Results

Measurements from 25 females and 25 males were taken. The mean age of the subjects was 52.34 ± 13.17 year. The mean concentrations of sodium, potassium and chloride in tear and serum in mMol/L were 131.06 ± 6.39, 21.40 ± 1.57 and 122.86 ±7.12 respectively and 135.56 ± 3.85, 4.14 ± 0.70 and 101.58 ± 3.25 respectively. These values are depicted in Table 1. A comparison in difference of mean between electrolytes in the two body fluids showed that potassium and chloride were significantly of higher concentration in tear than serum (p < 0.001 and p = 0.001 respectively) while a higher concentration of sodium in serum was observed but this was not statistically significant (p = 0.250)- Table 2. These are depicted in the bar chart shown in Figure 1.

Age as a predictor of electrolyte concentration was not statistically significant (p > 0.05) but there is a relatively good fit between age and tear potassium concentration of inverse nature (R^2 = 0.57) - Table 3. Sodium and chloride concentrations in both serum ([Na$^+$] = 136.42, [Cl$^-$] = 101.81) and tear (([Na+] = 132.38, [Cl$^-$] = 124.08) were higher in female subjects compared to males in both fluids ((([Na$^+$] = 134.63, [Cl$^-$] = 101.33 and [Na$^+$] = 129.63, [Cl-] = 121.54 respectively) - Table 4. However, males had higher concentrations of potassium in serum ([K$^+$] = 4.21 versus [K$^+$] = 4.07]) and tear ([K$^+$] = 21.80 versus [K$^+$] = 21.04). None of these observed differences was statistically significant- Table 4. In view of the relationship between tear production and circulating blood, a linear correlation using serum ions concentration to predict tear equivalents was done and the strongest correlation was found for sodium (Adjusted R^2 = 0.019, F = 0.107, p = 0.745) which is shown in Table 5. Table 6 is a comparison between this study and studies done in other regions in Nigeria.

Discussion

The concentration of major cations and anion namely sodium [Na$^+$], potassium [k$^+$] and chloride [Cl$^-$] found in tear and serum of 50 of subjects aged 30 years and above who
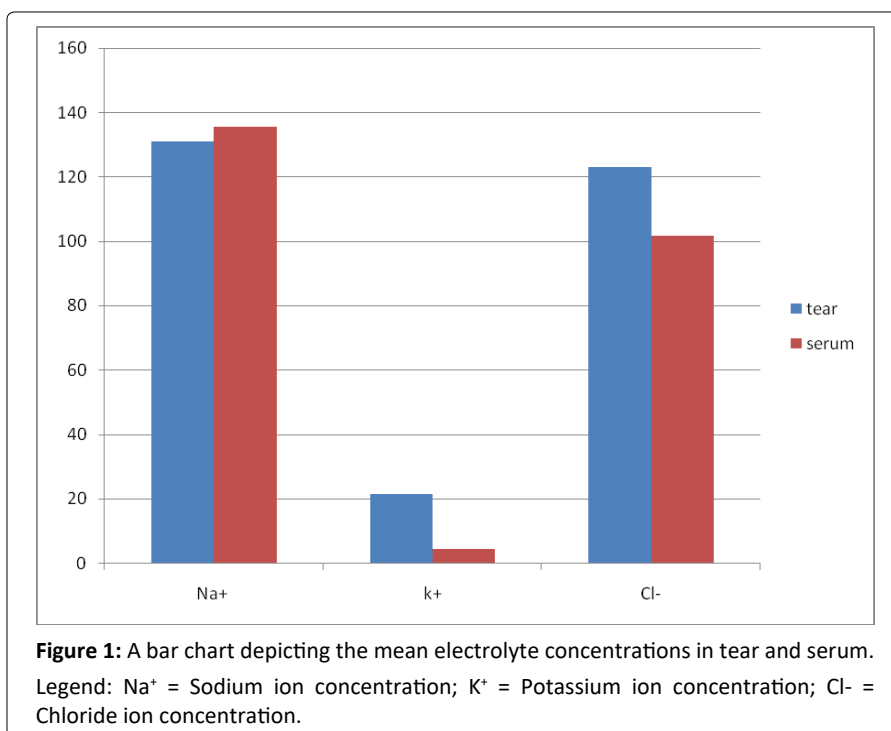

Figure 1: A bar chart depicting the mean electrolyte concentrations in tear and serum.

Legend: Na$^+$ = Sodium ion concentration; K$^+$ = Potassium ion concentration; Cl- = Chloride ion concentration.

Table 1: The major demographic attributes of subjects and the mean electrolyte concentrations.

	Tear	Serum
Age	52.24 ± 13.172	52.24 ± 13.172
Sex		
Male	25	25
Female	25	25
Mean Na$^+$	131.06 ± 6.39	135.56 ± 3.85
Mean K$^+$	21.40 ± 1.57	4.14 ± 0.70
Mean Cl-	122.86 ± 7.12	101.58 ± 3.25

Table 2: A comparison of means of tear and serum electrolytes.

	Mean	t -value	P - value	Df (n-1)
Na$^+$ Tear	131.06 ± 6.39	-4.265	0.250	98
Na$^+$ Serum	135.56 ± 3.84			
K$^+$ Tear	21.40 ± 1.57	71.065	< 0.001*	98
K$^+$ Serum	4.14 ± 0.69			
Cl$^-$ Tear	122.86 ± 7.12	19.224	0.001*	98
Cl$^-$ Serum	101.58 ± 3.25			

Table 3: A linear regression of the influence of age on observed electrolyte concentrations in tear and serum.

	F- Value	R- Squared	t-Value	P-value
Age & Na$^+$ T_C	0.350	0.080	52.716	0.586
Age &K$^+$T_C	0.016	0.004	33.838	0.906
Age &Cl$^-$ T_C	0.670	0.144	26.467	0.459
Age & Na+ S_C	2.183	0.353	47.800	0.214
Age & K$^+$ S_C	5.325	0.571	26.404	0.082
Age &Cl$^-$S_C	0.71	0.017	88.900	0.803

The table shows that potassium concentration in tears had a relatively good fit (R^2 = 0.571) in predicting a lower tear potassium concentration with increasing age.

Legend: Na$^+$ T_C = Sodium ion concentration in tear; K$^+$T_C = Potassium ion concentration in tear; Cl$^-$ T_C = Chloride ion concentration in tear; Na+ S_C = Sodium ion concentration in serum; K$^+$ S_C = Potassium ion concentration in serum; Cl$^-$S_C = Chloride ion concentration in serum.

Table 4: Mean electrolyte concentrations in male and female subjects.

	Sex		Mean	T test	P value	Df(n-2)
Na$^+$ _ serum	Male	24	134.63	-1.683	0.477	48
	Female	26	136.42			
K$^+$ _ serum	Male	24	4.21	0.682	0.251	48
	Female	26	4.07			
Cl$^-$ _ serum	Male	24	101.33	-0.512	0.897	48
	Female	26	101.81			
Na$^+$ _ Tear	Male	24	129.63	-1.547	0.069	48
	Female	26	132.38			
K$^+$ _ Tear	Male	24	21.80	1.769	0.060	48
	Female	26	21.04			
Cl$^-$_ Tear	Male	24	121.54	-1.265	0.959	48
	Female	26	124.08			

From the above table, there was a higher sodium and chloride concentration in tear and serum of female subjects when compared to male subjects while potassium was higher in tear and serum of male subjects when compared to females. However, these differences were not statistically significant.

Legend: Na+ = Sodium concentration; K+ = Potassium concentration; Cl- = Chloride concentration.

Table 5: Linear Regression analysis of serum ion concentration as a predictor of tear ion concentration.

	Mean	R^2	Adjusted R^2	F	T - Value	p- value
Na⁺ tear	131.06	0.002	-0.019	0.107	-0.327	0.745
Na⁺ serum	135.56					
K⁺ tear	21.404	0.024	0.004	1.193	1.092	0.280
K⁺ serum	4.136					
Cl⁻ tear	122.86	0.011	-0.009	0.555	0.745	0.460
Cl⁻ serum	101.58					

The adjusted R^2 values suggested that sodium ion concentration in tears is more likely related to its concentration in serum more than the other pairs tested.

Table 6: A comparison of findings between the present study and others done in other regions in Nigeria.

Serum electrolytes (mmol/L)	Present study (Enugu)	Nsukka	Minna	Ibadan
Sodium	135 ± 3.85	142.15 ± 15.16	135.59 (125 - 142)	130.0 ± 5.2
Potassium	4.14 ± 0.70	5.17 ± 1.04	3.95 (2.9 - 5.0)	3.5 ± 0.8
Chloride	101 ± 3.25	98.50 ± 11.22	98.97 (90 - 110	92.0 ± 7.8

had no evidence of ocular surface nor inflammatory eye disease were measured. The average age of the subjects was 55.2 years. The subjects were equally matched for sex with 25 males and 25 females (Table 1). The mean concentrations of sodium potassium and chloride in tear (mmMol/L) were 131.06, 21.40 and 122.86 respectively. This is comparable to values obtained by other authors including Rismondo, et al. who reported a mean potassium ion concentration of 17.0 mmMol/L in tear of laboratory rats [7,8,37]. Potassium ion concentration was also found to rise to about fifty percent (50%) above baseline when laboratory rats were subjected to intense pilocarpine-induced stimulation of its lachrymal gland in one study [37]. This increase did not affect sodium and chloride concentrations. Thaysen and Thorn using the same agent in human subjects, failed to demonstrate any significant increase in potassium concentration in tear [7]. A global paucity of data analyzing tear electrolyte behavior under different conditions have not allowed for a consensus position in potassium concentration changes during basal and reflex tearing. There is a need for further research to resolve this lingering research question. Overall, no statistically-significant sex or age-related changes were observed but a modest reduction in potassium tear concentration with increasing age was seen (p = 0.082). Apparently, no equivalent study has been reported in literature to allow for comparison but indirect evidence using tear osmolarity suggests that increasing age as well as female sex are significant risk factors for increased electrolytes concentration leading to hypertonicity and subsequent development of DED [29-34]. The absence of this trend in the present study might be attributed to the stringent exclusion criteria.

Serum concentration of sodium, potassium and chloride (mMol/L) were 135.56, 4.14 and 101.58 respectively. There is little agreement with other studies done within the country where all appeared uniquely different except for the data from Maanan, et al. which appeared similar to the findings in this study (Table 6). These wide variations underscore the need for global standardization of method and instruments employed in the measurement of serum electrolytes. The multiplicity of the testing algorithms also calls into question the validity of reference values in our various centers.

Sodium ion concentration in tear closely resembled its concentration in serum though in a lower concentration, 131.06 and 135.56 respectively (p = 0.42). It does seem that sodium in tear is ultra-filtrated from blood. This is further buttressed by the fact that a binary regression of serum electrolytes as a predictor of tear electrolytes showed that sodium and tear electrolytes had the best fit (adjusted R^2 = 0.019) and a commensurate high p-value (p = 0.75).

The findings for potassium and chloride ion concentrations in tear and serum is remarkably different. Tear have about 5 times higher concentration of potassium than serum, 21.40 and 4.14 mmol/L respectively (p < 0.001). chloride is also significantly higher in tear when compared to serum, 122.86 and 101.58 respectively (p = 0.001). These concentration disparities have been documented in other studies, even with different measuring algorithms [15,16]. It has been suggested that there is an active secretion of K⁺ and Cl⁺ into tear fluid by apical acinar cells of the lachrymal gland [5,6]. Studies have shown that high tear [K⁺] is essential in maintaining a proper cornea epithelial thickness which in turn prevents both proximal and distal UVB-induced cell injury [15-17]. The significance of lower [Na⁺] in tear compared to serum is unclear but it might be a compensatory mechanism to offset the high [K⁺]. Further studies are needed to clarify this relationship.

Strenghts and Limitations of the Study

The native samples were used to avoid any confounding influence of a diluting fluid. All the clinical assessments were done by the same person and all the laboratory tests were also carried out by the same laboratory scientists without sample storage and using the same instrument. However, only single tests were carried out for each patient considering the time needed to collect adequate tear sample. The presence of co-existing morbidity cannot be entirely ruled out. Reflex tearing cannot be totally avoided and its effect on measured variables is unknown.

Conclusion and Recommendations

Data on the concentration of the major electrolytes in an important extracelluar fluid such as tear is important

considering its known and putative roles in ocular health and disease. Such knowledge can form the basis for further studies on the possible role of electrolytes in the etiopathogenesis of several ocular and systemic conditions and may offer alternative therapeutic options. It is however important that instruments and methods for such measurements are standardized to achieve reproducible outcomes. Such approach should minimizing reflex tearing. The physiologic effects of reflex tearing on tear electrolyte concentration deserves further research considering conflicting extant opinions. Data derived from a population-based study using treatment-naïve eyes could provide a better assessment of the tested parameters.

Conflict of Interest

Acknowledgement

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APPENDIX 1

BIODATA

HOSPITAL NUMBER...

AGE (AS AT LAST BIRTHDAY) ....

SEX...

OCCUPATION..

ADDRESS ....

PHONE NUMBER...

PRESENT OCULAR DIAGNOSIS ....

FAMILY HISTORY OF GLAUCOMA (YES/NO)...

PRESENT SYSTEMIC CO – MORBIDITY (IF ANY)..

LAST MENSTRUAL PERIOD (PRE-MENOPAUSALFEMALE SUBJECTS)............................

PRESENT MEDICATION(S) (IF ANY) ....

LAST EYE DROP USED AND TIME (IF ANY)...

APPENDIX 2: Consent proforma form.

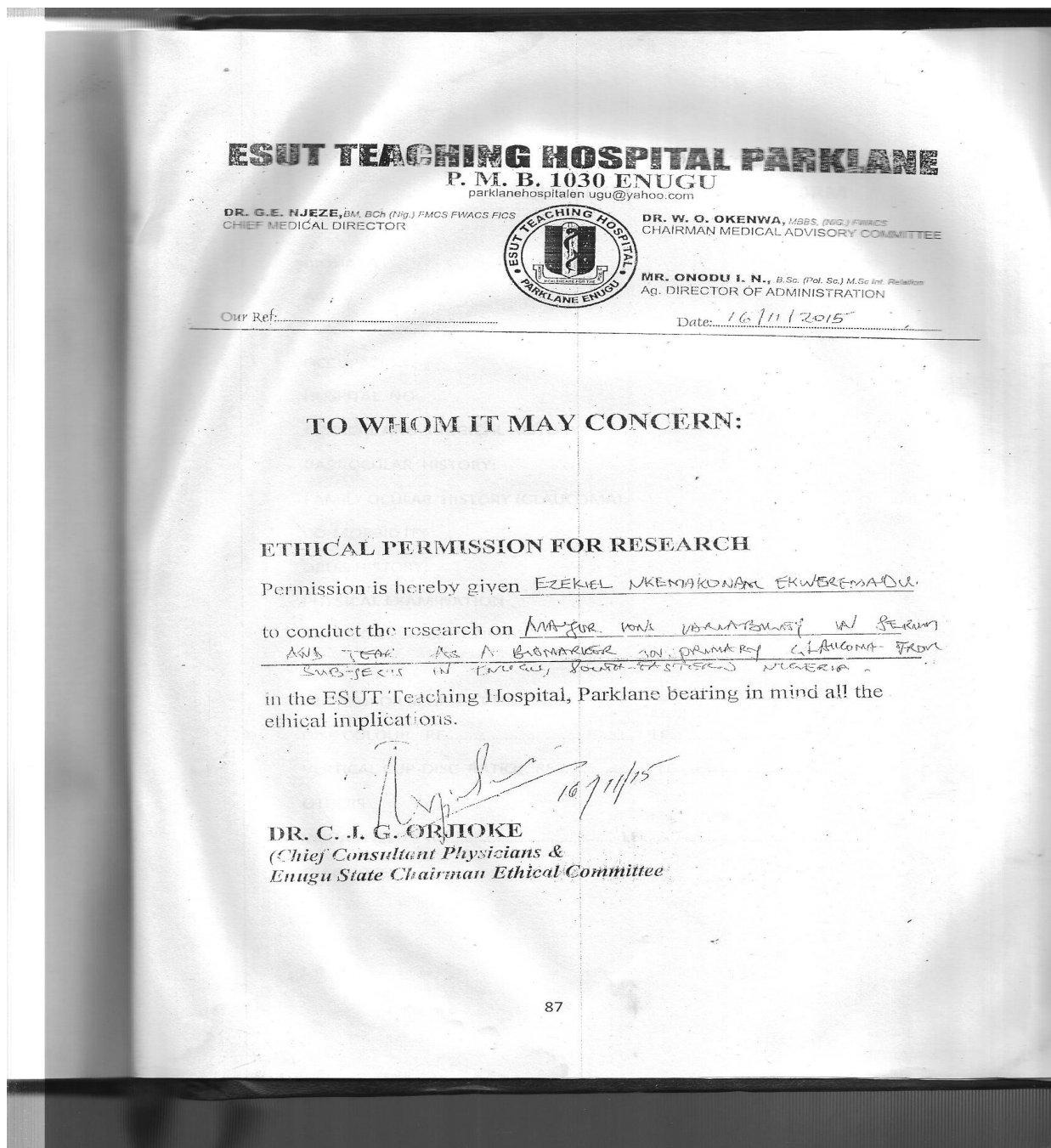I,...…hereby consent to participate in this study titled 'Major Ion Variability As a Biomarker in Serum and Tear of Primary Glaucoma Subjects in Enugu, South-Eastern Nigeria'. Theextent of my participation in the Study was explained to me and I also understood that I can exit from the study whenever I want and that will not result in any form of penalization as a patient of the Hospital. The result of the tests will be disclosed to me in a confidential manner and I understood that the findings of this study will also be disseminated in the scientific community in a manner that will not result in the violation of my privacy.

…………………………..   …………………………………

Signature of patient   Signature of witness to patient

Appendix 3: Ethical Permission for Research.

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