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EVALUATION OF GLUTATHIONE, TOTAL ANTIOXIDANT CAPACITY, TOTAL PLASMA PEROXIDES, OXIDATIVE STRESS INDEX IN CATARACT PATIENTS IN CALABAR

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author IIK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AUO and BIE performed sample collection and laboratory analysis and reviewed the manuscript. Author AA performed statistical analysis and managed the analyses of the study. Author ON managed the literature searches and reviewed the manuscript. Authors EKJ and IE performed data collection and laboratory analysis. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Cataract with prevalence of 15.4% is the leading cause of blindness among blinding eye diseases. The cause of cataracts is not fully understood and may be multifactorial, however oxidative damage to the lens proteins and lipids is suggested to be involved in the development of cataracts. This study aimed to determine the serum levels of glutathione (GSH), Total Antioxidant Capacity (TAC), Total Plasma Peroxides (TPP) and Oxidative Stress Index (OSI) in cataract patients in Calabar.

Materials and Methods: One hundred and seventeen subjects which comprise 75 diagnosed cataract patients and 42 controls were recruited. The cataract patients were sub-divided based on WHO criteria as: No visual impairment (n = 25), visually impaired (n = 25) and blind (n = 25). GSH, TAC and TPP were determined using verified colorimetric methods while OSI was calculated. Anthropometric indices, blood pressure and sociodemographic information were obtained using standard methods. Data were analyzed using Student's t-test, analysis of variance (ANOVA), LSD post hoc and Pearson's correlation at $P < .05$.

Results: The TPP and OSI were significantly higher while GSH and TAC were significantly lower ($P < .05$) in cataract patients compared to the control subjects. GSH and TAC were significantly lower ($P < .05$) in cataract patients with blindness and visually impaired compared to those without visual impairment. Oxidative stress index correlated negatively with TAC ($r = -0.607$, $P < .05$) and positively with diastolic blood pressure ($r = 0.296$, $P = .01$) in cataract patients.

Conclusion: It can be concluded that increased oxidative stress may be associated with the formation of cataracts and further depletion of GSH and TAC may cause the progression of cataracts to blindness.

Keywords: Cataract; glutathione; oxidative stress; antioxidants.

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1. INTRODUCTION

"Good eyesight is an important part of well-being and a significant factor in retaining independence and quality of life as one gets older" [1]. Vision can deteriorate for many reasons and major causes of vision loss are eye diseases which include age-related macular degeneration, glaucoma, cataracts, and diabetic retinopathy [2], with cataract being the leading cause of visual loss [3].

"A cataract is responsible for 51% of world blindness which represents about 20 million people" [4]. "A study conducted in Calabar, Nigeria, which consisted of 4,483 patients show cataracts to have a prevalence of 15.4%. A cataract is an eye disease in which the clear lens of the eye becomes cloudy or opaque, causing a decrease in vision" [5]. "Based on the etiology, it can be classified as age-related or senile cataract, congenital cataract and cataract associated with systemic diseases such as diabetes" [6].

"Cataract formation is mostly considered to be a multifactorial disease, and oxidative stress might be one of the leading causes" [7]. "Reactive oxygen species (ROS)-induced damage in the lens cell may consist of oxidation of proteins, DNA damage and/or lipid peroxidation, all of which have been implicated in cataractogenesis" [8].

"Oxidative stress has been implicated in many ocular diseases such as age-related macular degeneration, retinal light damage, cataract" [9], and "retinopathy of prematurity seen in premature infants treated with pure oxygen for a long time" [10]. "The reducing compound glutathione (GSH) is present in high concentration in the lens where it plays a role as an important antioxidant vital for the maintenance of the tissue's transparency, in conjunction with an active glutathione redox cycle present in the lens epithelium and superficial cortex, glutathione detoxifies potential damaging oxidants such as hydrogen peroxides and dehydroascorbic acid; studies have shown an important hydroxyl radical – scavenging function of GSH in lens epithelial cells, independent of the cell's ability to detoxify hydrogen peroxides, if GSH is depleted or the redox cycle is inhibited, low levels of antioxidant will damage lens epithelial targets such as Na/K-ATPase, certain cytoskeletal proteins and proteins associated with normal membrane permeability" [11].

Oxidants including free radicals and other non-radical reactive derivatives have been implicated in the aetiopathogenesis of cataracts, however, in our locality; there is a dearth of scientific information on the relationship between cataract formation and oxidative stress. Therefore, this study aims to evaluate the levels of glutathione (GSH), total antioxidant capacity (TAC), Total Plasma Peroxides (TPP) and oxidative stress index (OSI) in patients diagnosed with cataract and compare these parameters to normal control subjects in Calabar, Cross River State.

2. MATERIALS AND METHODS

2.1 Subject Selection

One hundred and seventeen (117) consented adults of age 20-88years were enrolled in this case-control study, comprising 42 Controls and 75 diagnosed cataract patients. The test subjects were cataract patients attending the Cross River State Eye Care Centre, Ministry of Health, Mary Slessor Avenue, Calabar and Mission for Vision Centre, General Hospital, Calabar. The cataract patients were sub-divided according to the World Health Organization categorization of vision of patients (best corrected visual acuity in the better eye) as: Cataract patients with no visual impairment with visual acuity of 6/6 - 6/12 (n = 25), Cataract patients with Visual impairment with visual acuity of 6/18 - 3/60 (n = 25) and Patients that were blind due to cataract with visual acuity of <3/60 - no light perception (n = 25). The controls were healthy adults who had no other ocular pathology aside from refractive errors. Subjects in the control group also had normal findings on ocular examinations and no personal or family history of cataracts or any other eye problems. Socio-demographic data, family history and brief medical history were obtained from each subject using a well-structured questionnaire. Anthropometric data were obtained using standard methods. The research was carried out by the Ethical Principles for Medical Research Involving Human Subjects as outlined in the Helsinki Declaration in 1975.

2.2 Sample Collection

Sterile phlebotomy was performed to obtain 4ml of venous blood from each subject. The blood samples were transferred into 10ml plain bottles, allowed to clot, dislodged and spun at 3000rpm for 5 minutes to obtain serum. The serum was separated and dispensed into a dry and chemically clean 5ml plain serum container after which the sample was stored at -20^{0}C until assay.

2.3 Laboratory Methods

Estimation of glutathione was carried out using the modified standard Ellman's method [12]. Total antioxidant capacity was determined by the method of

Koracevic et al. [13]. Total plasma peroxides were determined using the FOX-2 method [14] with minor modifications [15]. The ratio of total plasma peroxides to total antioxidant potential (TPP) was the Oxidative Stress index, an indication of the degree of oxidative stress.

$$\text{OSI (\%)} = \frac{\text{TPP micromole } (H_2O_2) \times 100}{\text{TAP micromole per liter}}$$

2.4 Statistical Analysis

Data analysis was done using the statistical package for social sciences (SPSS version 22.0). Student's t-test was used to test mean differences between groups, analysis of variance (ANOVA) was used to test the significance of variations within the group and among group means and Fisher's least significant difference (LSD) post hoc test was used for comparison of multiple groups means. A probability value of $P < .05$ was considered statistically significant. Microsoft Excel was used to plot the correlation graphs.'

3. RESULTS

Comparison of the age, body mass index (BMI), waist-hip ratio (WHR), systolic and diastolic pressure, glutathione (GSH), total antioxidant capacity (TAC), total plasma peroxides (TPP) and oxidative stress index (OSI) between the cataract subjects and controls are shown in Table 1. The cataract patients had significantly higher ($P<.05$) WHR, systolic and diastolic pressures, TPP and OSI compared to the controls while GSH and TAC levels were significantly lower ($P<.05$) in the cataract subjects compared to the controls. Table 2 shows a comparison of age, BMI, WHR, Systolic and Diastolic pressure, GSH, TAC, TPP and OSI among cataract subjects with no visual impairment, visual impairment and blindness respectively. The GSH and TAC showed significant variation ($P<.05$) among the groups. Other parameters showed no significant variation ($P>.05$). Table 3 is a post hoc analysis of the mean GSH and TAC levels between the various cataract groups. The GSH and TAC were significantly lower ($P<.05$) in cataract subjects with visual impairment than in those with no impairment while GSH and TAC were significantly lower ($P<.05$) in cataract subjects with blindness than in cataract subjects without visual impairment. Table 4 shows the comparison between cataract subjects with no visual impairment and control subjects. The WHR, systolic and diastolic pressure, TPP and OSI were significantly higher ($P <.05$) in cataract subjects with no visual impairment when compared to control subjects. Fig. 1 shows the correlation plot of OSI against TAC in the cataract subjects studied using Pearson's correlation. A significant negative correlation ($r = -0.607$, $P<.05$) was observed between OSI and TAC in the test group. Fig. 2 shows the correlation plot of OSI against TPP in the test subjects studied using Pearson's correlation. A positive correlation ($r = 0.212$, $P = .07$) was observed between OSI and TPP in cataract patients. Fig. 3 shows the correlation plot of OSI against Diastolic pressure in the test subjects studied using Pearson's correlation. A significant positive correlation ($r = 0.296$, $P = .01$) was observed between OSI and diastolic pressure in cataract patients.

Table 1. Shows the comparison between cataract patients and controls

Parameters/Groups	Cataract Patients (n = 75)	Controls (n = 42)	t-cal	P value
Age (years)	59.4±13.5	58.7±15.21	0.251	0.802
BMI (kg/m^2)	24.5±4.50	24.5±5.98	0.011	0.992
WHR	0.92±0.05	0.86±0.06	6.386	0.000*
Systole (mmHg)	140.2±20.58	121.9±14.61	5.073	0.000*
Diastole (mmHg)	82.7±12.72	76.1±11.24	2.830	0.005*
GSH (mmol/L)	4.1±1.39	5.1±1.31	-3.907	0.000*
TAC (µmol/L)	1121.9±681.98	1724.9±944.74	-3.982	0.000*
TPP (µmol/L)	153.7±35.04	124.4±22.35	4.872	0.000*
OSI (%)	21.5±21.53	9.6±5.64	3.505	0.001*

*Values are expressed as Mean ± SD, BMI= Body mass Index; WHR= Waist-to-Hip-Ratio; GSH= Glutathione; TAC= Total antioxidant capacity; OSI= Oxidative Stress Index; * = Significant at P<0.05*

Table 2. Shows the comparison between cataract patients with visual impairment, no visual impairment and blindness respectively

Parameters/Groups	Cataract with no visual Impairment (n = 25)	Cataract with visual impairment (n = 25)	Cataract with blindness (n = 25)	F-ratio	P value
Age (years)	59.4±14.20	59.7±14.50	58.96±12.10	0.020	0.980
BMI (kg/m^2)	23.6±3.72	25.7±5.23	24.2±4.34	1.434	0.245
WHR	0.93±0.04	0.92±0.06	0.92±0.05	0.202	0.818
Systole (mmHg)	137±22.30	140±20.90	143±18.90	0.484	0.618
Diastole (mmHg)	84.8±16.40	79.5±8.73	83.8±11.70	1.243	0.295
GSH (mmol/L)	4.69±1.37	3.8±1.17	3.8±1.48	3.555	0.034*
TAC (µmol/L)	1429.6±964.03	1038.8±394.07	897.5±437.22	4.466	0.015*
TPP (µmol/L)	145.1±27.99	158.9±42.43	156.9±32.97	1.134	0.327
OSI (%)	17.9±19.48	20.3±20.65	26.4±24.17	1.011	0.369

*Values are expressed as Mean ± SD,BMI= Body mass Index; WHR= Waist-to-Hip-Ratio; GSH= Glutathione; TAC= Total antioxidant capacity; OSI= Oxidative Stress Index; *= Significant at P<0.05*

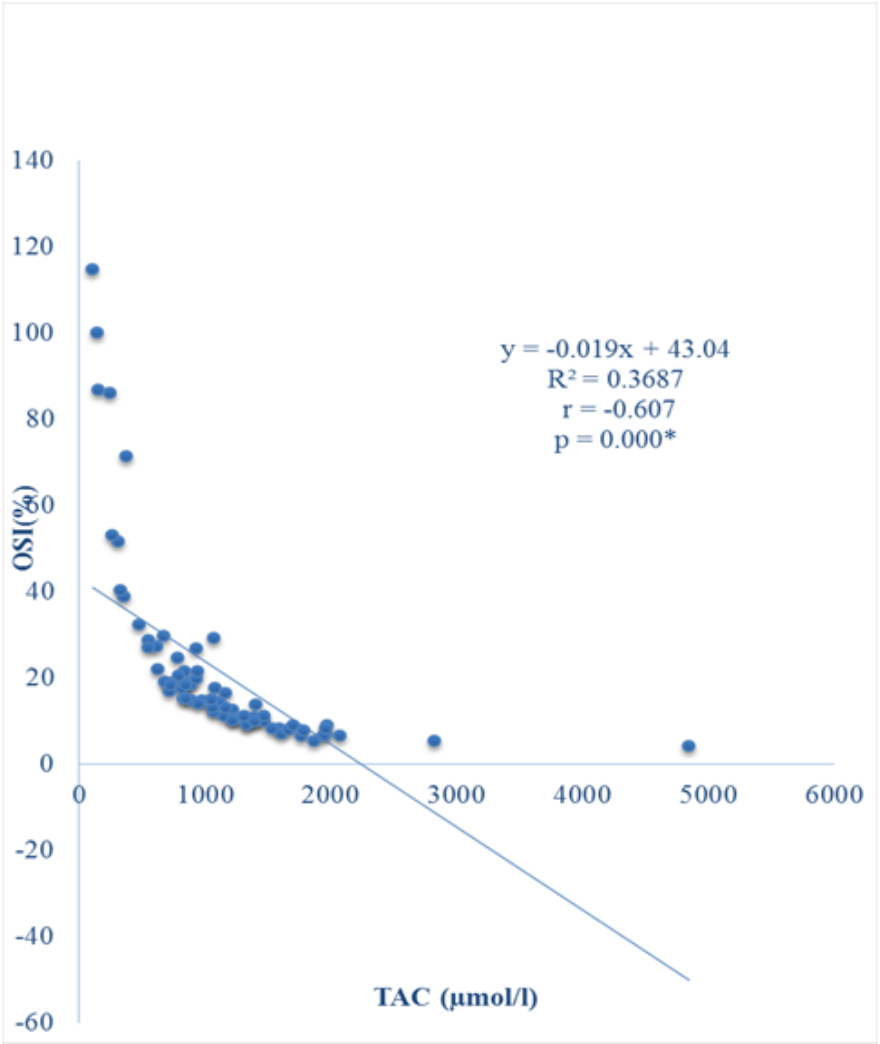

Fig. 1. Correlation plot of Oxidative Stress Index (OSI) against Total Antioxidant Capacity (TAC) in cataract patients

Table 3. Comparison of mean GSH and TAC in the cataract group using LSD post hoc analysis

Parameters	Groups		Mean difference	P-value
	Cataract with no visual impairment	Cataract with visual impairment		
GSH (mmol/L)	4.69±1.37	3.8±1.17	0.87	0.025*
TAC (µmol/L)	1429.6±964.03	1038.8±394.07	390.76	0.038*
	Cataract with no visual impairment	**Cataract with blindness**		
GSH (mmol/L)	4.69±1.37	3.8±1.48	0.88	0.023*
TAC (µmol/L)	1429.6±964.03	897.5±437.22	532.08	0.005*

*GSH = Glutathione, TAC = Total antioxidant capacity, * = significant at p < 0.05*

Table 4. Shows the comparison between cataract patients with no impairment and control subjects

Parameters/Groups	Cataract with no visual Impairment (n = 25)	Controls (n = 42)	t-cal	P-value
Age (years)	59.4±14.20	58.7±15.21	0.200	0.842
BMI (kg/m^2)	23.6±3.72	24.5±5.98	-0.676	0.501
WHR	0.93±0.04	0.86±0.06	5.058	0.000*
Systole (mmHg)	137±22.30	121.9±14.61	3.433	0.001*
Diastole (mmHg)	84.8±16.40	76.1±11.24	2.594	0.012*
GSH (mmol/L)	4.69±1.37	5.1±1.31	-1.308	0.196
TAC (µmol/L)	1429.6±964.03	1724.9±944.74	-1.229	0.224
TPP (µmol/L)	145.1±27.99	124.4±22.35	3.331	0.001*
OSI (%)	17.9±19.48	9.6±5.64	2.608	0.047*

*Values are expressed as Mean ± SD, BMI= Body mass Index; WHR= Waist-to-Hip-Ratio; GSH= Glutathione; TAC= Total antioxidant capacity; OSI= Oxidative Stress Index; * = Significant at P<0.05*

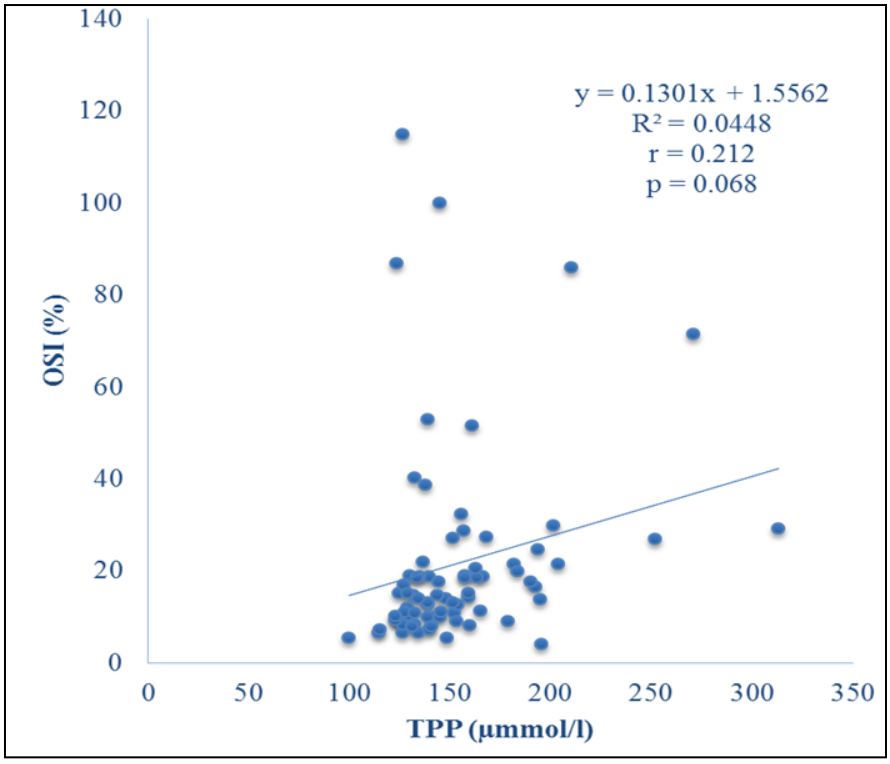

Fig. 2. Correlation plot of Oxidative Stress (OSI) against Total Plasma Peroxides (TPP) in cataract patients

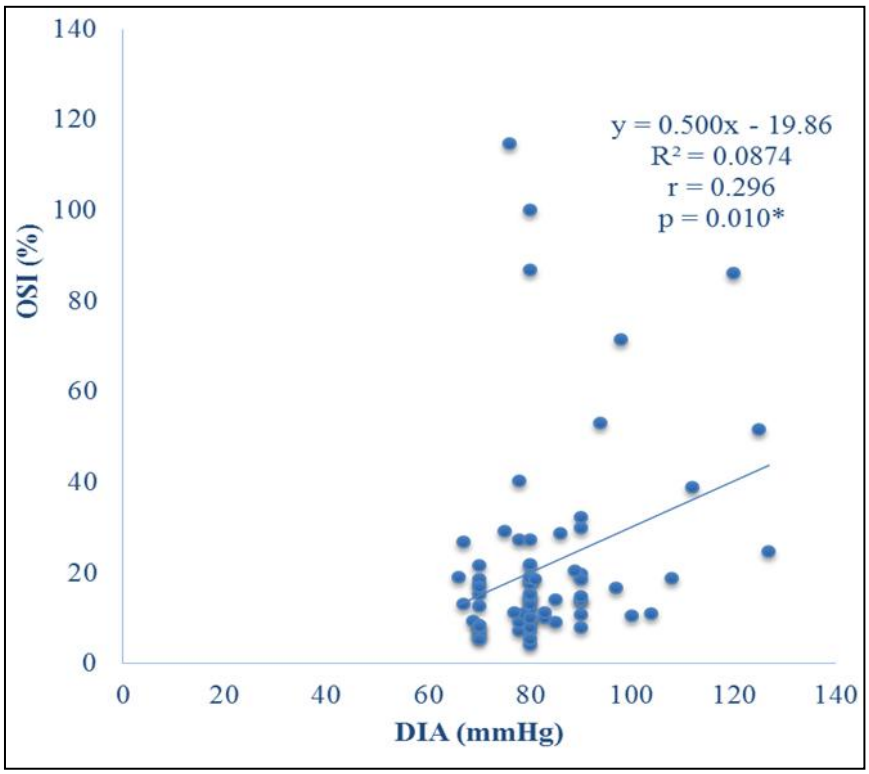

Fig. 3. Correlation plot of Oxidative Stress Index (OSI) against Diastolic pressure in cataract patients

4. DISCUSSION

Free radical formations occur endogenously in the cell as a normal physiological process and as a consequence of both enzymatic and non-enzymatic reactions [16]. "Exogenous sources include drugs, UV light, ionizing radiation exposure and other activities that we come in contact with in our daily life. Free radicals play a dual role as both toxic and beneficial compounds. Their activities are checked by the antioxidant system and the delicate balance between their two antagonistic effects is an important aspect of life. At high concentrations, the free radicals generate oxidative stress; a state of imbalance between the production of reactive oxygen species and the cellular antioxidant defense mechanisms. In the cells of the eyes, the reactive oxygen species may initiate a surge of toxic biochemical reactions such as peroxidation of membrane lipids and extensive damage of the proteins which cause intracellular protein aggregation and precipitation" [17]. The biomarkers of Oxidative Stress of 75 diagnosed cataract patients and 42 healthy individuals (control subjects) were assessed in this project work. Parameters assessed include glutathione (GSH), Total antioxidant capacity (TAC), Total plasma peroxides (TPP) and Oxidative stress index (OSI). The total plasma peroxides and Oxidative Stress index were significantly higher in cataract patients than in the control subjects. This is supported by earlier studies [18,19] their works showed increased levels of the lipid peroxidation product, malondialdehyde as a consequence of increased plasma peroxides and Oxidative Stress in cataract patients. Kaur et al. [20] reported higher levels of plasma peroxides in cataract patients when compared to the control group which is similar to our findings. The higher levels of total plasma peroxides seen in cataract patients may be a result of increased altered redox state caused by cataract predisposing or risk factors such as familial, aging, diabetes, diarrhea, malnutrition, ultraviolet radiation from sunlight, smoking, excessive alcohol consumption, hypertension and renal failure [21] and increased metabolic risk [22]. The higher levels of free radicals attack important macromolecules such as DNA, proteins, lipids, etc, leading to cell damage and homeostatic disruption [23]. Glutathione and total antioxidant capacity were seen to be lower in cataracts than in the control subjects and their levels were also observed to further decrease with the severity of the cataract. It was observed that those with blindness and visual impairment have significantly lower GSH and TAC compared to those without impairment, this suggests that lower TAC and GSH are associated with a higher risk of developing complications of cataracts including visual impairment and blindness. This is to the studies of Kisic et al. [24] and Bhatia et al. [25]. The higher levels of the Peroxides increase oxidative

stress and decrease antioxidant defense hence causing a reduced TAC in cataract patients, this is also in line with the negative correlation observed between oxidative stress index and total antioxidant capacity in this work. Since TAC is a combination of the activities of both the enzymatic and non-enzymatic antioxidant systems, the result observed suggests a decrease in both antioxidant systems in cataract patients. One of the several possibilities for the occurrence of lower glutathione concentration in the blood is an increased consumption for the removal of peroxides and free radicals [26]. "Glutathione is the obvious compound that defends the lens against oxidative insults, being directly involved in reducing the disulfides, being a pivotal cofactor in the detoxication of hydrogen peroxides and acting as a free radical quencher" [26]. "The waist-to-hip ratio (WHR) of the test group was also significantly higher than the control group. An increased WHR has been demonstrated as an aggravating factor that further increases oxidative stress and disrupts the oxidant-antioxidant balance" [27]. "The mechanism is through the presence of excessive adipose tissues around the abdominal region which have been identified as a source of pro-inflammatory cytokines, including tumor necrotic factor-alpha, interleukin, interleukin-1beta (IL-1B) and interleukin-6 (IL-6)" [28]. "Tissue necrosis factor-alpha (TNF-alpha) is a critical cytokine that influences the inflammatory response, the immune system, adipose cell apoptosis, as well as lipid metabolism, it also influences increased hepatic lipogenesis and insulin signaling and induces oxidative stress. Free radical production can also be induced by TNF-alpha through the binding of specific receptors and promoting nuclear factor-kappa (NF-KB) signaling" [29]. "It also increases the interaction of electrons with oxygen to generate superoxide anions" [30]. "The accumulation of abdominal fat which can be indirectly measured through WHR is an important cardiovascular disease risk factor. This is due to its association with a series of metabolic disorders such as diabetes mellitus, hypertension and dyslipidemia" [31]. "The cataract patients had significantly higher systolic and diastolic blood pressures. Recently, it has been hypothesized that oxidative stress is a key player in the pathogenesis of hypertension" [32], "this tally with the significant positive correlation observed between oxidative stress index and diastolic pressure in this present study. Reactive oxygen species have an important role in the homeostasis of the vascular wall, hence they could contribute to the mechanism of hypertension" [33]. However hypertension has also been known to further aggravate oxidative stress and deplete the antioxidant system; important sources of ROS are the vascular wall. The major stimuli are the mechanical stretch on the vascular wall and the activation of the rennin-angiotensin system. In particular, angiotensin II activates NADPH/NADH oxidase of the vascular smooth muscle cells, resulting in the release of reactive oxygen species [34], this alters the antioxidant-oxidant system which has been implicated in cataractogenesis.

5. CONCLUSION

The findings of this study suggest that increased waist-hip-ratio, increased blood pressure, lipid peroxidation, Depletion of Glutathione (GSH) and Total Antioxidant Capacity (TAC) and increased oxidative stress may be associated with the formation of cataracts and further depletion of GSH and TAC may cause the progression of cataract to blindness.

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ETHICAL CONSIDERATION

All experiments were performed by the ethical standards laid down in the Helsinki Declaration of 1975, as revised in 2000. Ethical approval was obtained from the Health and research ethics committee of the Cross River State Ministry of Health, Calabar.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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