ABSTRACT

Senile Cataract, the opacity of the lens due to age is a major public health problem which if not detected and treated early could lead to blindness, and other morbidities. It is the leading cause of avoidable blindness in Ghana. Various risk factors have been identified with aetiology and pathogenesis of senile cataract including: reduced functional status, social interaction and quality of life, depression and falls. However, there are few reports on the biochemical risk factors elsewhere and no such study report could be found in Ghana.

The study seeks to identify the biochemical risk factors and derrangement associated with senile cataract, elucidates the association between serum biochemical markers (its intrinsic processes that leads to such derangement) and senile cataract for future prognosis and management outcome of such condition compared to those who are clinically without the condition. Case-control study of outpatients attending eye clinic department of the Komfo Anokye Teaching Hospital (KATH) between July 2009 and September 2010 were recruited for the study. A total of 200 out patients above 40years comprising 100 clinically newly diagnosed adults cataract patients and 100 patients who are clinically without the condition. Laboratory test values in cases and controls were compared and expressed as odds ratio at 95% confidence interval.

Biochemical analysis of the Patients with cataract (cases) the matched control pairs reveals that, four out of ten (4/10) biochemical variables measured were significant risk factors associated with the senile cataract development. (Table 4.4) Persons with HDL C abnormalities were twice as likely to develop senile cataract (odds ratio, 2.52 p=0.095). Within the normal reference ranges of FBG, and low uric acids (UA) levels increased risk for senile cataract (odds ratio, 1.20; p=0.106 and odds ratio1.01 p=0.011 respectively). Exposure to sodium in the

absence of other biochemical risk factors remained the most significant factor associated with senile cataract (odds ratio, 0.60; p=0.001). there was a statistically significant difference between the mean serum Na+ level (Rf=135-145) in senile cataract patients (143.2±6.76) and normal individuals (139.3±1.96; p=0.0000).The low level of mean concentrations of High Density Lipoprotein (1.114±0.42 mmol L-1, p =1.000) in cataract patients were different compared to that in control patients (1.04±0.10 mmol L-1 and 2.94±1.06 respectively Also, the mean uric acid concentrations level was 210.0±113.8 µmol L^{-1} lower in the cases compared to 311.1±117 µmol L^{-1} of the controls (p=1.000). The mean concentration of fasting blood glucose (R_f=3.6-6.4). of the cataract patients 4.92±2.09 mmol L^{-1} were also lower than the control group of 6.01±2.96 mmol L^{-1} p= 0.9986 but the differences were not significant.

The mean concentration of High Density Lipoprotein -Cholesterol [HDL-C] mmol L^{-1} in cataract patients 1.14±0.42 vs. 1.04±0.10 mmol L^{-1}; p=0.000 (reference range, 1.15-1.68 mmol L^{-1}) was different compared to that in control patients). Below HDL-C level of 1.5 mmol/l subjects had a three-fold higher calculated probability of developing senile cataract. (ORs 3.17(1.79-5.61) and HDL–C were significantly associated with senile cataract among the study population. Similarly, mean serum uric acid [UA]level (reference ranges was 143-417 µmol L-1) were lower in the cataract group than in the control group (210.0±113.8 vs. 311.1±117; p=0.000) respectively and ORs (95%CI) 0f 1.01(1.01-1.02) whilst the mean concentration of fasting blood sugar (FBS) of the cataract patients 4.92±2.09 mmol L^{-1} were also lower than the control group of 6.01±2.96 mmol L^{-1})) respectively and ORs (95%CI) 0f 1.25(1.O6-1.49)p=0.008 (reference range (3.6-5.8) mmol/l).(table 4.2).The trend in the likelihood ratio test indicate a very strong association between increasing order of level of exposure to sodium, uric acid, HDL-C, FBS and the probability of developing the senile cataract

The study found that Human serum components such [Sodium], [Uric acid], [HDL-CHOLESTEROL], Fasting Blood Glucose are biochemical risk factors association with the

developing of senile cataract and diets with high Na+ content may leads to high level of serum Na^+ associated with senile cataract. Whilst in aging processes biochemical derangement such as reduced efficiency of metabolic processes and a decrease in antioxidant defense mechanism may result in low serum level of HDL-C and reduction in serum uric acid levels. These intrinsic processes may mire uric acid strong endogenous antioxidant role and both HDL-C antioxidative and anti-inflammatory activities leading to such derangement associated with senile cataract. Human blood component play significant role as both potential osmotic and oxidative stress associated with age related or senile cataract causing avoidable blindness.

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PROBLEM STATEMENT

In recent times, "cataract reflect a systemic processes" has become a hypothetical statement associated with aging, implicated in the common usage of terms such as "age related" or "senile" cataract". Globally, senile cataract accounts for 50% of blindness and remains the leading cause of visual impairment all over the world, despite improvements in surgical outcomes (WHO 2005).The main causes of blindness as a proportion of total blindness are cataract (47.8%), Glaucoma (12.3%), and age related macular degeneration (8.7%). Other causes include cornea opacity (5.1%), diabetic retinopathy (4.8%), and childhood blindness (3.9%), and trachoma (3.6%), onchocerciasis (0.8%) (WHO 2004)A meta-analysis of population based surveys on blindness prevalence in Asia, Africa, and the industrialized countries indicates that women bear approximately two-thirds of the burden of blindness in the world (WHO "Gender and Blindness"(2002). Approximately 75 percent of population over 75 years suffers from lens opacity and blindness has profound human and socioeconomic consequences in all societies in Ghana. The costs of lost productivity and of rehabilitation and education of the blind constitute a significant economic burden for the individual, the family and society. As a global and national burden the question arises as to whether various clinical background factors such as age, sex, smoking, alcohol, diabetes, and hypertension are adequate information available for early identification of biochemical risk factors for senile cataract.

STATEMENT OF HYPOTHESIS

With the exception of confounders, the biochemical elements of senile cataract formation between the established cases of cataract and those who are clinically without the condition will not be significant enough to affect clinical dysfunction associated with cataract. Thus, biochemical determinants of clinically established cataract will not affect the clinical outcome of such condition. However, the determinants of biochemical risk factors and their

derrangement associated with degradation of senile cataract formation may be necessary to assess future prognosis and management outcome of such condition. This hypothesis is tested by investigating the association between serum electrolyte levels, fasting blood sugar, uric acids, and serum lipid profile that might indicate dysfunctions associated with cataract

PROBLEM ANALYSIS DIAGRAM

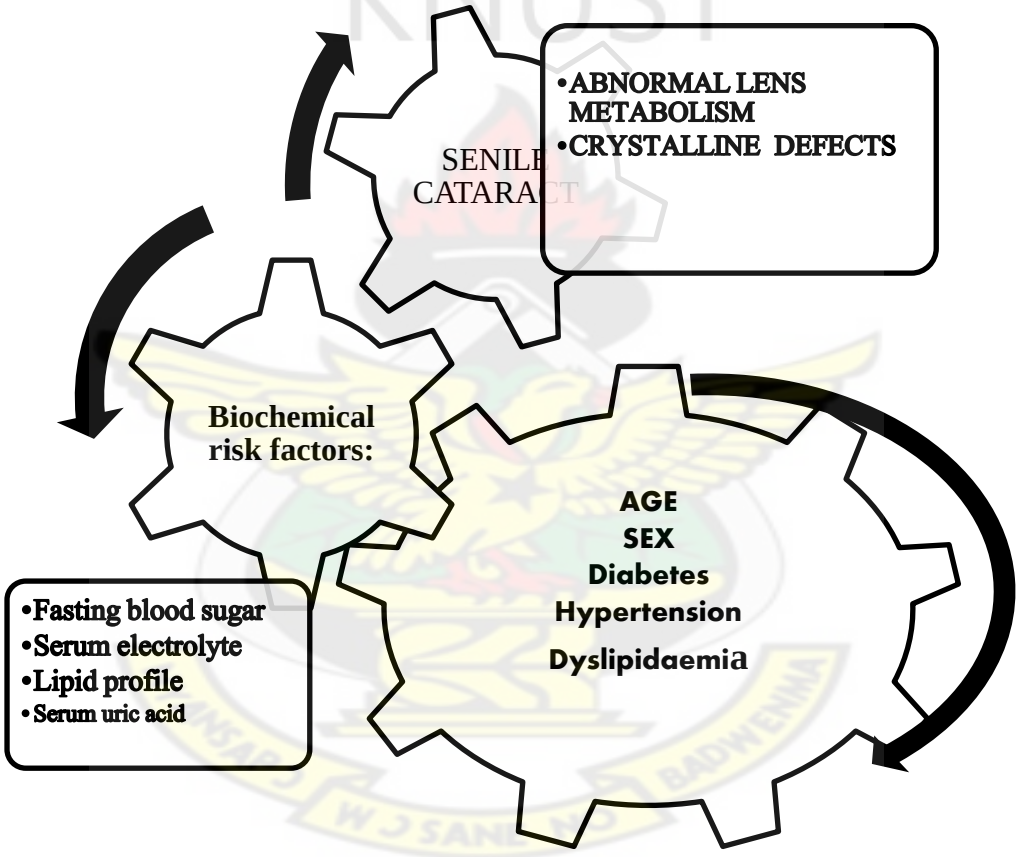


Figure.1.Biochemical and structural changes that take place within the human crystalline linking the degenerative processes that occur in other parts of the body as a consequence of aging and other confounders that leads to the clinical derrangement associated with senile cataract.

AIM OF THE STDUY

The general aim of the research was to identify the biochemical risk factor of senile cataract liking the degenerative processes that occur in other parts of the body as a consequence of aging among Ghanaian Adults (≥40years) Visiting Eye Clinic in Komfo Anokye Teaching Hospital in Kumasi. This may help target higher risk populations for screening programmes, and develop appropriate preventive strategies.

THE SPECIFIC OBJECTIVES WERE:

1. to identify and characterized the biochemical risk factors for senile cataract among adults above 40 years in relation to various clinical background factors such as age, sex, smoking, alcohol consumption, diabetes, dyslipidaemia, hypertension and medication.

2. To elucidate the association between serum biochemical indices and senile cataract.

3. To estimate and compare the annual reportage of senile cataract burden among outpatient visiting KATH eye clinic over the study period.

4. To provide a database on cataract prognosis and biochemical indices on senile cataract for future clinical research and development

JUSTIFICATION OF THE STUDY

In Ghana, cataract formation is more commonly observed among adults subjects. It is the leading cause of avoidable blindness. The elderly suffer disproportionately with a loss of vision from eye diseases. The elderly are more likely to have ocular disease and to suffer greater severity of that disease. Unreported data from KATH indicated that in 2009, the yearly eye clinic report at the KATH saw a total of 23783 attendances with 3534 newly diagnosed cases of cataract and 699 cataract surgeries were done.

Cataractogenesis is one of the earliest secondary complications of diabetes mellitus. Even though senile cataract has been found to be more common among older people, populations of older adults have a higher prevalence of senile cataract and ocular diseases associated with systemic diseases than among any other population groups. No data exist on its prognosis and biochemical indices for future clinical research and development in Ghanaian patients.

Development of nonsurgical procedures requires a much deeper understanding of which the proposal for this study seek to identify and characterize the biochemical risk factors for senile cataract among adult above 40 years in relation to various clinical background factors such as age, sex, oxidative stress, smoking, alcohol, diabetes, hypertension obesity by investigating the association between serum electrolyte levels, fasting blood sugar, uric acids, and serum lipid profile that might indicate dysfunction associated with senile cataract.

CHAPTER ONE

Introduction and background

Globally, cataract accounts for 50% of blindness and remains the leading cause of visual impairment all over the world, despite improvements in surgical outcomes (WHO 2005). This number is expected to rise due to an aging population and increase in life expectancy. Cataract, an opacification of the crystalline lens in the eye, can be caused by many factors including the natural aging process, metabolic abnormalities, nutritional disorders, chronic ocular inflammation and trauma. Diagnosis is made with ocular examination using slit-lamp biomicroscopy. Although cataracts are not preventable, their surgical treatment is one of the most cost-effective interventions in healthcare.

1.1 Definition of Blindness

In the 10th revision of the WHO International Statistical Classification of Diseases, Injuries and Causes of Death, 'low vision' is defined as visual acuity of less than 6/18 but equal to or better than 3/60, or a corresponding visual field loss to less than 20° in the better eye with the best possible correction. 'Blindness' is defined as visual acuity of less than 3/60, or a corresponding visual field loss to less than 10° in the better eye with the best possible correction. Visual impairment' includes both low vision and blindness (WHO, 1993.). Normal or near normal vision ≥ 6/18 in both eyes; Visual impairment < 6/18 to ≥ 6/60 in better eye, ≥ 6/60 in worse eye; Unilateral blindness, < 6/60 in worse eye, ≥ 6/60 in better eye; Moderate bilateral blindness, < 6/60 in worse eye, ≤ 6/60 to ≥ 6/120 in better eye; and Severe bilateral blindness = counting fingers < 3 meters in both eyes (< 6/120)(Ghana Med J. 2005 June).

1.2 Causes of Blindness

The main causes of blindness as a proportion of total blindness are cataract (47.8%), Glaucoma (12.3%), and age related macular degeneration (8.7%). Other causes include cornea opacity (5.1%), diabetic retinopathy (4.8%), childhood blindness (3.9%), trachoma (3.6%) and onchocerciasis. (0.8%) (WHO, 2004).

The contribution of cataracts to blindness globally is likely to grow due to an aging population and unsuccessful attempts to control this blinding condition in low and middle-income countries (WHO 2005).

The patterns of the causes of blindness in adults and children vary considerably across the world. The causes of blindness also vary by race, with whites being more commonly affected with macular degeneration and blacks having a higher prevalence of untreated cataract and open –angle glaucoma (Sommer *et al.*, 1991).

1.3 Cataract

Any opacity in lens or capsule is known as "Cataract" and may be broadly divided into early-onset (congenital or juvenile) and age-related cataract (senile cataract)(Vijaya, Gupta *et al.*,1997).

1.3.1 Definition of cataract

Cataract refers to opacification of the 'crystalline' lens in the human eye (Chitkara *et al.,* 2004). It is opacity of the natural, crystalline lens of the eye and remains the most common cause of visual loss in humans.

1.3.2 Classification of cataract

Different groups have used various parameters for the classification which are summarized in Table 1.1 below. Barber (1973) discussed and reviewed some parameters on which lenses can be classified whilst Kanski (1984) categorized cataract lenses on the basis of their morphology, development and etiology.

Table 1.1 Classification of Cataract lenses by parameter and the various types of cataract

PARAMETER	Developmental classification	Morphological classification	Etiological classification		Secondary classification
			Type	Cause	
TYPE OF CATARACT	Immature	Capsular	Senile	Aging	Anterior verities
		a) congenital			
		b) acquired			
	Mature	Subcapsular	Traumatic	Infrared irradiation	Hereditary disorders
		a) Anterior		ionization irradiation	
		b) Posterior			
	Hypermature	Nuclear	Metabolic	Diabetes	
		a) Congenital		Galactosemia	
		b) Senile		Fabry's disease	
	Morganian	Cortical	Toxic	Conticosteroid	
		a) Congenital		Chlorpromazine	
		b) Senile			
		Lamellar			

1.3.3 Presentation and diagnosis of cataract

People with cataract can present with one or more of the following symptoms: gradual diminution of visual acuity, glare, frequent change in eyeglasses prescription and change in colour appreciation.

General symptoms include cloudy vision, glare at night time, Halo around lights, double or multiple vision and Changes in colors and contrast (Cataract symptom, 2004).

The senile cataract condition may be discovered by a general practitioner or optometrist, followed by referral to an ophthalmic surgeon for confirmation of the diagnosis and management. The diagnosis is made with ocular examination using slit-lamp biomicroscopy after dilatation with 1.0% tropicamide (Mydriacy) 2.5% Phenylephrine hydrochloride (Neosynephrine**)** reagents**.**

1.3.4 Treatment options of cataract

The cure for cataract is surgery. However, this surgery is not equally available to all, and where it is available it does not produce equal outcomes. Surgical removal of the cataract is currently the only treatment option once the lens has opacified. This is usually accompanied by implantation of an intraocular lens (IOL) to replace the focusing power of the natural lens (Fedorowicz, *et al.*, 2005). Each participant underwent best –corrected distance visual acuity measurement with Snellen chart. The Snellen eye chart is used, with rows of letters decreasing in size to determine how clearly a person can actually see. The Preferred Practice Pattern of the American Academy of Ophthalmology (2004) recommends Snellen chat visual acuity tests as the best guide for appropriateness of surgery with respect to the patient's functional and visual needs, environment, and risk factors. Visual acuity screening is a widely used approach to identify reduced vision. Visual acuity of 6/6 (20/20) as measured on the Snellen chart is usually considered normal. Visual acuity, the sharpness of near and distance vision, is tested separately for each eye. One eye is covered with a piece of paper or the palm of the hand placed lightly over the eye to

allow testing of the distance and near vision in the opposite eye via reading the letters or telling the direction of the letter on the chart.

1.3.5 Risk factors of cataract

West *et al.,* (1995) indicated that identification and awareness of risk factors for cataract could have an important benefit by reducing patient's dependence on the family and society. Delaying the incidence of cataract depends upon the identification of risk factors for senile cataract. In public health perspective; risk factors for cataract are readily classified as unmodifiable and potentially modified.

1.3.5.1 Unmodifiable risk factors for cataract

Age is by far the strongest known risk factor for cataract formation. Age represents the cumulative effect of the complex interaction of exposure to many factors over time that contributed to the development of senile cataract. As widely documented, (Leske *et al.*, 1983; West *et al.,* 1995) age was the principal risk factor related to the development of lens opacities. Riaz (2006); reported that age-related cataract accounts for more than 40% of cases of blindness throughout the world with the majority of people blind from cataract found in the developing world.

In spite of evidence that unoperated cataract is more common among blacks than among whites, information is limited on racial differences in cataract prevalence. The Barbados Eye Study (1997) provides prevalence data on lens opacity in a predominantly black population. Cortical opacity was the most

frequent type of cataract, and women had a higher frequency of opacification (Leske *et al.,* 1997). Women have a higher risk for most types of cataracts (Delcourt et al., 2000), though evidence suggests estrogen may protect against cataract formation. (Klein et al., 1994) The anti-estrogen drug tamoxifen (used to block estrogen receptors) increases risk of cataract when taken long-term (Cumming RG, 1997).

Kahn *et al*., (1977) and Weale (1981) reported that in gender, the relative susceptibility to cataract change with age as well as individual response to physical trauma.

The most exciting recent developments in cataract epidemiology have been the identification of a strong genetic component. Twin studies in the United Kingdom suggest that approximately half of nuclear and two thirds of cortical cataract can be accounted for by hereditary factors (Hammond *et al*., 2000). Dominant genes have been implicated for cortical cataract and additive genetic effects for nuclear. These findings are generally consistent with those from population-based studies (Heiba 1995; McCarty 1999). However, at this stage, nothing can be done to alter an individual's genetic makeup in relation to senile cataract.

In a two case-control studies to examined correlation between family history as a risk factor and cataract, the Age Related Eye Disease Study (AREDS 2001) reported that family history were risk factor for cortical cataract, posterior subcapsular cataract, and mixed cataract cases, whereas Leske et al (1997) found a significant association between family history as a risk factor for mixed cataract only.

1.3.5.2 Modifiable risk factors for cataract

Important risk factors for age-related cataract include exposure to sunlight (Heck *et al.,* 2004) and ultraviolet-B (UV-B) radiation (Worgul *et al*., 1976), the presence of diabetes and the use of therapeutic drugs such as corticosteroids (Hodge et *al.,* 1995;Garbe *et al.,* 1998), and recreational drugs such as nicotine and alcohol (Munoz *et al.,* 1993; Delcourt *et al*.,2000). The occurrences of severe diarrhoea and dehydration have been suggested by some studies (Minassian *et al.,* 1989). The role of dietary antioxidant vitamins is unclear and often contradictory (Leske *et al.,* 1997; Waagbo et al., 2003). An increased risk of lens opacities in smokers has been demonstrated in cross-sectional, case-control, and prospective studies (Klein *et al.,* 2003; Nirmalan *et al.,* 2004).

Risk for cataract is greater among individuals with lower socioeconomic status or educational level. This is attributed to nutritional deficiencies from poor diet, increased exposure to disease, poor general health, and greater exposure to conditions inducing cataract development. (West *et al.,* 1995)In humans, occurrence of cataract has been associated with protein deficiency (Chatterjee *et al.,* 1982), tryptophan metabolism (Cotlier *et al.,* 1981), vitamins and minerals (Jacquest *et al.,* 1988).

A strong preposition of the cause of nuclear cataract in humans is the effect of sunlight. In vitro experiment have revealed that an exposure to lens protein turn yellow setting out a stage of cataract formation (Heck *et al.,* 2004). Geographical areas with more hours of sunshine have a greater prevalence of cataract, showing an association between ultraviolet B irradiation and cataract formation (Heck *et al.,* 2004). Exposure to X-rays or gamma radiation is a risk

factor for cortical and posterior subcapsular cataracts in humans. Radiologists routinely minimize exposing the lens to ionizing radiation. When this is not possible, cataracts frequently develop and require surgical treatment (Worgul *et al.*, 1976).

Diabetics are five times more susceptible to the development of cataracts than non diabetics (Ederer *et al.,* 1981). The accumulation of sorbitol (due to elevated sugar levels in eye by the action of Aldose reductase) has been shown to cause lens damage (Kinoshita *et al.*, 1974), results in osmotic imbalance and changes in membrane permeability leading to lens opacity.

In some population, especially in Pakistan and India, repeated bouts of diarrhea has been shown to contribute to high prevalence of cataract formation (Harding 1980).Diarrhea results in elevated blood urea levels leading to carbamylation of lens proteins (Harding *et al.*, 1991) causing unfolding of proteins leading to cataractogenesis.

Risks for all cataract types also increase with heavy alcohol consumption (Delcourt *et al.*, 2000).

1.3.6 Epidemiology of cataract

According to World Health Organization (WHO), cataract is the leading cause reversible blindness and visual impairments in more than 17million (47.8%) of the 37 million blind individuals worldwide, and this number is projected to reach 40 million by 2020. (WHO 2005) and in the Beaver Dam Eye Study (BDES), the prevalence of cataract increases with age It reported that 38. 8 % of men and 45.9% of women older than 75 years had visually significant cataract. (Klein *et al*. 1998).

In Ghana, cataract formation is more commonly observed among adults subjects. It is the leading cause of avoidable blindness. The elderly suffer disproportionately with a loss of vision from eye diseases. The elderly are more likely to have ocular disease and suffer greater severity of the disease.

Blindness has profound human and socioeconomic consequences in all societies. The costs of lost productivity and of rehabilitation and education of the blind constitute a significant economic burden for the individual, the family and society. Populations of older adults have a higher prevalence of primary ocular disease and systemic diseases associated with ocular disease compared with younger adults. In addition, older adults have normal age-related changes in vision and difficulty recognizing or reporting visual impairment because of the presence of comorbid conditions, such as cognitive impairment. Vision impairment is consistently associated with decreased functional capacity and quality of life in older persons, including the ability to live independently, with more severe vision impairment associated with greater negative effects. Improving sensory function is one of the cardinal approaches involving health promotion among the elderly particularly the provision of facilities which will

improve community access to early detection of visual impairment and treatment (Klein 1991; Wormald 1992).

Although, the aim of improving visual impairment is clearly to produce improvements in other clinical outcomes such as improved quality of life or a reduction in falls. Any benefits arising from vision assessment will necessarily be dependent on improved vision.

A meta-analysis of population based surveys on blindness prevalence in Asia, Africa, and the industrialized countries indicates that women bear approximately two-thirds of the burden of blindness in the world. (WHO “Gender and Blindness" 2002)

The challenges of cataract research, influencing government policy and modifying the behaviour of the public are therefore worth taking up.

CHAPTER TWO

Literature review

2.1 Diseases of the eye lens

Disease of the lens occurs when there are changes of aqueous humor component, osmotic character of lens capsule, metabolic disturbance caused by various factors example (diabetes mellitus, trauma) resulting in lens protein degeneration, and the transparent lens becoming opaque. Cataract refers to opacification of the 'crystalline' lens in the human eye (Chitkara *et al;* 2004).Typically, cataracts are defined as lens opacities associated with some degree of visual impairment (West S. K et al 1995). Cataract is the most common cause of visual loss in humans (Vijaya, Gupta et al. 1997).Lens opacity, reflects systemic processes that are associated with aging, implicated in the common usage of terms such as "age related" and "senile cataract". the biochemical and structural changes that take place within the human crystalline lens have been likened to degenerative processes that occur in other parts of the body as a consequence of aging (Asbell *et al;* 2005).

2.2 Senile cataract

The commonest form of cataract mainly the in age group between 45 years or more is referred to as age-related or senile cataract (Donnelly CA. 1997). Among various types of cataracts "Senile cataract", accounts for the vast

majority of the cases. Since senile cataract develops over a period of years or decades, it may result from very subtle changes in the intraocular composition. Senile cataract is a marker of generalized tissue aging. Studies by Hirsch and Schwartz (1983) shared the view that senile cataracts reflect systemic phenomena rather than only a localized ocular disease. An aging lens undergoes metabolic changes that predispose it to cataracts.

2.2. Types of senile cataract

There are three types of senile cataract: nuclear cataract, cortical cataract and posterior subcapsular cataracts. Disease progression in all types of cataract is indicated by increased lens opacity, though opacity manifests differently in each type. Each type of age-related cataract has a specific mechanism that leads to their development. These include: oxidative damage, protein aggregation, breakdown of the glutathione, damage to fiber cell membranes, protein breakdown, abnormal lens epithelial cell migration, or aberrant changes in lens fiber cells. Opacity follows a gradient but progressive colouration of the lens from shades of yellow to brown as the cataract condition advances (Annon 2004).

2.2.1 Nuclear cataract

Nuclear cataracts show increased oxidative damage to lens proteins and lipids (Spector *et al*., 1995), causing protein-to-protein interactions that cause aggregation and increased light scattering.

Evidence suggests a strong connection between aging and increased amounts of oxidized glutathione in the lens nucleus indicative of an imbalance between

protein and lipid oxidation, and glutathione-dependent reduction (Bova *et al.*, (2001) and Sweeney *et al.,* (1998).

Nuclear cataract formation may be caused by separation of lens cell cytoplasm (a jelly-like substance) into protein-rich and protein-poor liquid phases (Clark, *et al.,* (2000)) accounting for the opacity.

2.2.2 Cortical cataract

Cortical opacities start in small regions of the lens periphery and gradually spreading around the circumference of the lens. Several mechanisms may initiate the cortical cataract: damage to the fiber cell plasma membrane, loss of protective molecules (such as glutathione), excessive breakdown of proteins (proteolysis), and damage to systems responsible for calcium homeostasis. These factors are interrelated and the derangement of any of the process can directly affect the other (Beebe *et al.*, 2003). For example, Loss of calcium homeostasis may cause opacification around the lens periphery and towards the nucleus resulting in elevated calcium levels which can damage to cells in cortical cataracts (Duindam *et al.*, 1998). Elevated calcium leads to proteolysis, protein aggregation, and light scattering.

2.2.3 Posterior subcapsular cataracts

Posterior subcapsular cataracts are caused by environmental stresses such as ultraviolet light, diabetes, and drug ingestion (West et *al*., 1995; Jobling *et al.*, 2002).

Light scattering occurs in a cluster of swollen cells at the back of the lens, beneath the lens capsule.

2.3 Structure and composition of crystallin lens

The crystalline lens is a transparent, biconvex structure whose functions are to maintain its own clarity, to refract light and to provide accommodation. It is composed of the capsule, lens epithelium, cortex, and nucleus. The lens consists of multiple layers of cells without the usual cellular organelles for energy production and other regenerative mechanisms for cellular biostability (Berman 1991). A lens is formed from specialized epithelial cells during embryonic development. In an adult lens, only a few epithelial cells replicate, proliferating slowly, and producing new fiber cells that elongate and accumulate crystalline (lens proteins). Crystallins are the major structural proteins of the eye lens (most of them are present in the fiber cells) and account for approximately 90% of the total proteins. All vertebrate lenses contain α- β- and γ-crystallins. These major proteins in the lens (α, β, and γ-crystallins) are constantly subjected to age-related changes such as oxidation, deamidation, truncation, glycation, and methylation. In the human lens, α-crystallins (molecular mass 800-1000 kDa) make up 40% and comprise two related proteins, αA- and αB-crystallins. The β-crystallins (molecular mass 40-200 kDa) are of two types - acidic βA-species and basic βB-species, based on overall charge (Kannabiran *et al*., 2000).

2.3.1 Crystallin Lens Proteins

Transparency and proper light refraction of the lens depend on a unique arrangement of tightly packed fibers, which in turn rely on a defined protein structure. The human lens has a protein concentration of 33% of its wet weight, which is twice that of most other tissues such as brain = 10% and muscle = 18% (Cotlier, 1981).

A lens protein is divided into 2 groups: water soluble and water insoluble proteins (Morner, 1894).

The water-soluble fraction accounts for about 80% of lens proteins and consist mainly of crystalline. The crystallins are intracellular proteins contained within the epithelium and plasma membrane of the lens fiber cells. Crystallins are subdivided into 3 major groups: alpha, beta and gamma. However, accumulated evidence, including DNA sequencing, indicates that the beta and gamma crystallins are part of the same family, and generally referred to as the betagamma crystallins (BCSC Section 11 2007-2008).

Alpha crystallins constitute 32% of the lens proteins. They are the largest, with a molecular weight ranging from 600 to 4000 kiloDaltons (kD), depending on the tendency of subunits to aggregates. The alpha crystallins are composed of a mixture of different-sized macromolecular aggregates of 4 major subunits and up to 9 minor subunits. Each subunit polypeptide has a molecular weight of about 20 kD. The subunits are held together by hydrogen bonds and hydrophobic interactions. Alpha crystallins appear to be specifically involved in the transformation of epithelial cells into lens fibers. The rate of synthesis of alpha crystallins is 7 times higher in epithelial cells than in the cortical fibers, indicating a significant decrease in rate of synthesis after the transformation.

The *beta crystallins* account for 55% (by weight) of the water-soluble proteins in the lens. The *gamma crystallins* are the smallest of the crystallins, with a molecular weight in the range of 20 kD. They make up approximately 1.5% of adult mammal lens protein but constitute as much as 60% of soluble lens protein in weanling animals

Structure and solubility characteristics of lens protein

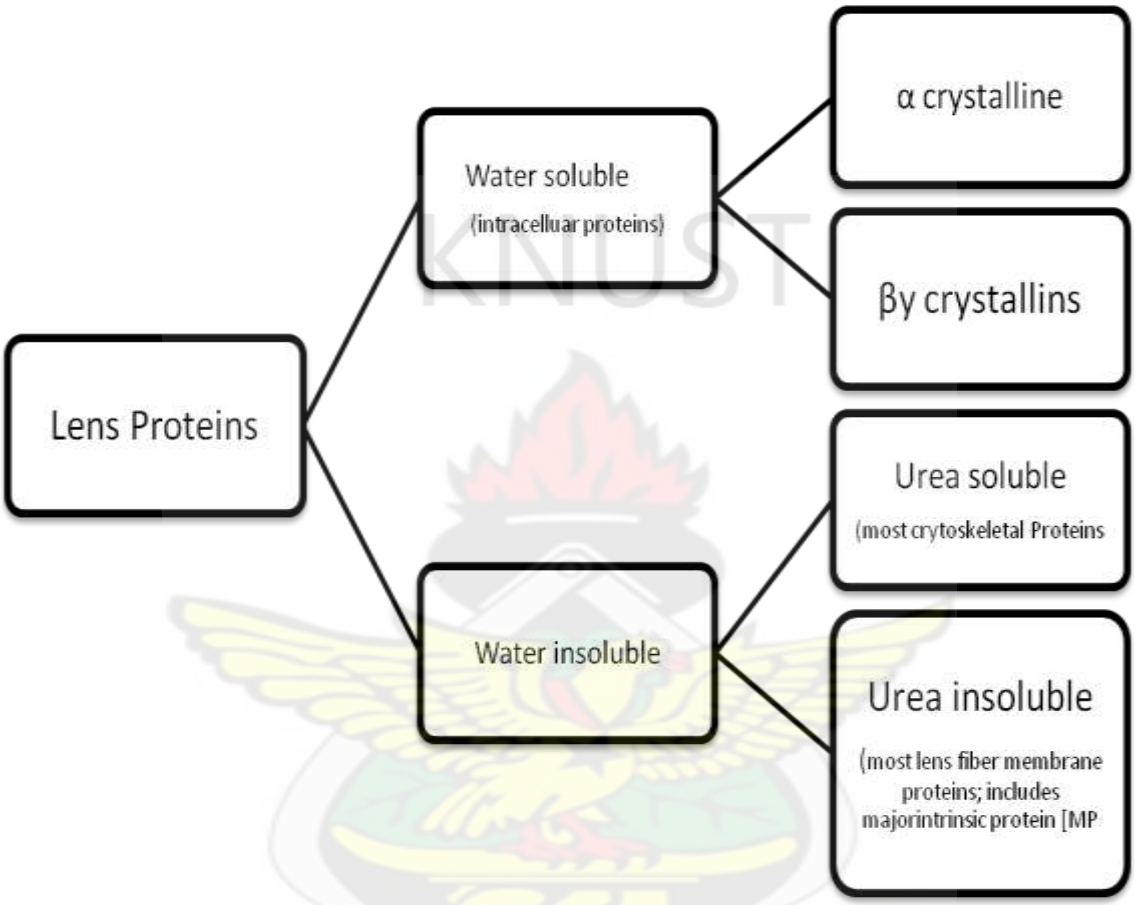


Figure 3.0 Structure and solubility characteristics of lens protein

The lens protein can be further separated into two fractions; soluble and insoluble in 8 molar urea. The *urea-soluble* fraction contains cytoskeleton proteins that provide the structural framework of the lens cells. The urea-insoluble fraction contains the lens fiber plasma membranes that resemble erythrocyte plasma membranes in many respects. Several proteins are associated with these fiber plasma membranes. It makes up nearly 50% of the membrane proteins and it is known as the major intrinsic protein (MIP). This protein, with a molecular weight of 28 kD, breaks down with age to a 22-kD protein. The relative proportions of these two proteins (28kD/22kD) become about equal at 20-30 years of age. The 22-kD protein predominates in the nucleus. The MIP first appear in the lens just as the fibers begin to elongate and can be detected in membranes throughout the mass of the lens. It is not found in the epithelial cell and seems to be associated with the differentiation of epithelial cells into fibers cells. The MIP, concentrated in the gap junctions, is the predominant protein of the junction-enriched membrane proteins. It is an inherent part of the membrane, where it has been localized by immunofluorescence (Hejtmancik *et al.*, 2000).

2.4 Decrease in lens protein concentration with age

Although aging brings about a natural decrease in the absolute amount of protein in the lens, this reduction is even more remarkable in cataractous lenses. As mentioned earlier, the percentage of soluble protein also decreases, from approximately 81% in adult transparent lenses to only 51.4% in cataractous lenses. Loss of proteins from the lens probably represents an escape of intact

crystallins through the lens capsule. Researchers have found that, in cortical cataracts, the levels of both alpha and gamma crystallines in the aqueous humor increase; in nuclear cataracts, the level of alpha crystallins increases, whereas that of gamma crystallins decreases (Hejtmancik *et al.,* 2000).

In the lens, energy production largely depends on glucose metabolism. Glucose enters the lens from the aqueous component both by *simple diffusion* and by a mediated transfer process called *facilitated diffusion*. Most of the glucose transported into the lens is phosphorylated to glucose -6-phospahte (G6P) by the enzyme hexokinase. This reaction is 70-1000 times slower than that of other enzymes involved in lens glycolysis and is, therefore, rate limited in the lens. Once formed, G6P enters one of two metabolic pathways: anaerobic glycolysis or the hexose monophosphate (HMP) shunt. The more active of these two pathways is anaerobic glycolysis, which provides most of the high-energy phosphate bonds required for lens metabolism. Substrate-linked phosphorylation of ADP to ATP occurs at two steps along the way to lactate. The rate-limiting step in the glycolytic pathway itself is at the level of the enzyme phosphofrunctokinase, which is regulated through feedback control by metabolic products of the glycolytic pathway. This pathway is much less efficient than aerobic glycolysis because only 2 net molecules of ATP are produced for each glucose molecule utilized whereas aerobic glycolysis produces an additional 36 molecules of ATP from each glucose molecule metabolized in the citric acid cycle (oxidative metabolism). Because of the low oxygen tension in the lens, only about 3% of the lens glucose passes through the Krebs citric acid cycle to produce ATP; however, even this low level of aerobic metabolism produces about 25% of the lens ATP (Andley *et al.*, 2000)

The less active pathway of utilization of G6P in the lens is the hexose monophosphate (HMP) shunt, also known as the pentose phosphate pathway. About 5% of lens glucose is metabolized by this route, although the pathway is stimulated in the presence of elevated levels of glucose. The activity of the HMP shunt is higher in the lens than in most tissues, but its role is far from established. As in other tissues, it may provide NADPH (the reduced form of nicotinamide-adenine dinucleotide phosphate (NADP) for fatty acid biosynthesis and ribose for nucleotide biosynthesis. It also provides the NADPH necessary for glutathione reductase and aldose reductase activities in the lens. The carbohydrate products of the HMP shunt enter the glycolytic pathway and are metabolized to lactate (BCSC Section 112007-2008).

Aldose reductase is the key enzyme for lens sugar metabolism in the polyol pathway that converts glucose to fructose via sorbitol dehydrogenase (SDH). This enzyme has been found to play pivotal role in the development of "sugar" cataracts. Aldose Reductase (AR) is a monomeric reduced nicotinamide adenine dinucleotide (NAD) phosphate (NADPH)-dependent enzyme and a member of aldo-keto reductase superfamily. AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH. Sorbitol dehydrogenase, the second enzyme in the polyol pathway that converts sorbitol to fructose, also contributes to oxidative stress, most likely because depletion of its cofactor NAD/ leads to more glucose being channeled through the polyol pathway. The accumulation of sorbitol produced (due to elevated sugar levels in eye by the action of aldose reductase) lens damage (Kinoshita *et al.,* 1979)

The affinity constant K_m for aldose reductase is about 700 times that for hexokinase. Because the affinity is actually the inverse of K_m, aldose reeducate has a very low affinity for glucose compared to hexokinase. Less than 4% of lens glucose is normally converted to sorbitol.

As previously stated, the hexokinase reaction is rate-limited in phosphorylating glucose in the lens and is inhibited by the feedback mechanisms of the products of glycolysis. Therefore, when glucose increases in the lens, as occurs in hyperglycemic states, the sorbitol pathway is activated relatively more than glycolysis and sorbitol accumulates. Sorbitol is metabolized to fructose by the enzyme polyol dehydrogenase. Unfortunately, this enzyme has a relatively low affinity (high K_m), meaning that considerable sorbitol will accumulate before being further metabolized. This characteristic results in retention of sorbitol in the lens.

A high NADPH/NADH ratio drives the reaction toward the making of sorbitol in the forward direction. The accumulation of NADP that occurs as a consequence of activation of the sorbitol pathway may cause the stimulation of the HMP shunt that is observed in the presence of elevated lens glucose. Along with sorbitol, fructose also builds up in a lens incubated in a high-glucose environment. Together, the two sugars increase the osmotic pressure within the lens, drawing in water. At first, the energy-dependent pumps of the lens are able to compensate, but ultimately they are overwhelmed. The result is swelling of the fibers, disruption of the normal cytoskeleton architecture and lens opacification.

The pivotal role of aldose reductase in cataractogenesis in animals is apparent from studies of the development of sugar-induced cataract in various animal species. Those species that have high aldose reductase activities develop lens opacities, whereas those lacking aldose reductase do not. In addition, specific inhibitors of this enzymatic activity, applied droop systematically to one eye, decrease the rate of onset and severity of sugar cataracts in experimental studies (BCSC Section 2 2007- 2008).

2.6 Pump-Leak theory underlying the transport of ions in the lens

The combination of active transport and membrane permeability is often refer to as the pump-leak system of the lens. According to the pump-leak theory, potassium and various other molecules such as amino acids are actively transported in the anterior lens via the epithelium. They then diffuse out with the concentration gradient through the back of the lens, where there are no active transport mechanisms. Conversely, sodium flows in through the back of the lens along a concentration gradient and then is actively exchanged for potassium by the epithelium (BCSC Section 11 2007-2008)

In support of this theory, an anteroposterior gradient was found for both ions: potassium was concentrated in the anterior lens and sodium was concentrated in the posterior lens. Most of the NA^{+}, K^{+}- ATPase activity is found in the lens epithelium. The active transport mechanisms are lost if the capsule and attached epithelium are removed from the lens but not if the capsule alone is removed by enzymatic degradation with collagenase. These findings support the hypothesis that the epithelium is the primary site for active transport in the lens. Thus,

sodium is pumped across the anterior face of the lens into the aqueous humor, and potassium moves from the aqueous humor into the lens. At the posterior surface of the lens (the lens-vitreous interface), the movement of solute occurs largely by passive diffusion. Much of the diffusion throughout the lens occurs from cell to cell through the low-resistance gap junctions. The unequal distribution of electrolytes across the lens cell membranes results in an electrical potential difference between the inside and outside of the lens. The inside of the lens is electronegative, measuring about -70mV. Calcium homeostasis is also critical to the lens. The normal intracellular level of calcium in the lens is about 30mV, whereas the exterior calcium level is close to 2μM. This large transmembrane calcium gradient is maintained primarily by the calcium pump (Ca^{2}+-ATPase). The lens cell membranes are also relatively impermeable to calcium. Loss of calcium homeostasis can disrupt lens metabolism. Increased levels of calcium can result in many changes, including depressed glucose metabolism, formation of high-molecular-weight protein aggregates, and activation of disruptive proteases.

Membrane transport and permeability are also important considerations in lens nutrition. Active amino acid transport takes place at the lens epithelium by a mechanism dependent on the sodium gradient, which is brought about by the sodium pump. A variety of substances, including ascorbic acid, myo-inositol, and choline, have specialized transport mechanisms in the lens.

2.7 Aqueous humour of the eye

Aqueous humour is a clear liquid that resembles cerebrospinal fluid in physical properties but not in chemical composition. It is secreted in the posterior chamber by ciliary process then proceeds to anterior chamber through pupil (Cole, 1977).

The aqueous humour is produced from blood secretions. Serum electrolytes concentration directly affects electrolytes of aqueous humuor and the lens metabolism (Van Heyningaen *et al.,* 1961). The secretion is not an ultrafiltrate of plasma because it is produced by energy dependent processes in the epethelia layer of the ciliary body (Macknight et al. (2000).

Any physiological or experimental condition that affects the eye should have significant influence on chemical composition of intraocular fluids (Bito, 1977), therefore, the aqueous humour of human eye is of crucial interest for the pathogenesis of ocular diseases (Hannappel *et al.,* 1985; Brown *et al.,* 1986).

Although a good correlation between aqueous humour composition and senile cataract has not been shown, attempts have been made to study aqueous humour in cataract and other ocular disease.

In a case control study comparing the level of uric acid in aqueous humor of patients with cataract and control group, the aqueous humuor was taken from 32 patients with senile and presenile cataract during surgical operation by means of anterior chamber puncture. The uric acid was determined by indirect method with uricase. The mean content of uric acid in aqueous humor of patients with cataract was 187.13 **µ** mol /l and in the control group 309.34µmol/l. The

difference between the groups was statistically significant (Kałuzny *et al.*, 1996).

The antioxidant properties of uric acid have long been recognized and as a result of its comparatively high serum concentrations, it is the most abundant scavenger of free radicals in humans. Also, uric acid as a strong endogenous antioxidant may play an important role in pathogenesis of cataract Waring *et al* (2001).

2.8 Pathophysiology of senile cataract and associated disorders

Again, it has been stated that the antecedent of the adoption of western – lifestyle coupled with reduced physical have contributed to the increased in the development of type II DM. Multiple mechanisms have been implicated in the development of cataract in diabetics . Patterson (1956) reported that there was lenticular opacity when the blood sugar was more than 225 mg%. This major finding could therefore , correlate with other studies elsewhere that a causal as well as an initiating factor is thought to be the changes observed when the lens-forming glucose and protein molecules react to produce a large intraocular mass disrupting the lenticular fibers thereby damaging the inorganic ion balance. Two possible routes were explored by Stevens (1998); modification of the lens proteins leading to Advanced Glycation End product (AGE) formation and modification of the ATPase pumps leading to increased osmotic and oxidative stress in the lens and to eventual opacification (Zhao *et al.*, 1998 and Obrosova *et al.*, 1999).

Increased risk of cataract among adults with DM (hyperglycemia) and hypertension has been reported in literature elsewhere. Example, Bikibele et *al.*, (2003) Ibadan hospital based case control study found a correlation between uncontrolled hypertension and the risk for visually disabling age –related cataract (ORs1.3 , p>0.05). There is a positive correlation between sodium levels and elevated blood pressure (Suvillan (1986).An association between raised sodium levels and cataract may also be mediated through the effect of salt intake on hypertension.

2.8.1 Cataractogenesis

Cataractogenesis is a highly complex and multifactorial process. Theories of cataractogenesis generally recognize different pathophysiologic features for the senile cataract. Cataractogenesis is essentially a disturbance in the state of lens proteins. The biochemical and structural changes that take place within the human crystalline lens have been likened to degenerative processes that occur in other parts of the body as a consequence of aging (Asbell *et al.;* 2005)

Cataract develops when the main structural protein in the lens, alpha-crystallin aggregates into clumps that distort and cloud the lens and impair vision. The lens provides an environment where these processes proliferate at a rate faster than that in other parts of the body. The mechanism of the initial insult to the crystallin molecule could take a number of forms. The causal as well as an initiating factor is thought to be the changes observed when the lens-forming glucose and protein molecules react to produce a large intraocular mass disrupting the lenticular fibers thereby damaging the inorganic ion balance.

Studies have shown that glucose is osmotic active, intracellular dehydration occurs due to the increased osmotic effect of the glucose in the extracellular fluid space. This has led to the theory that sugars play a key role in aging; in the same way that hyperglycemia could be the responsible factor for the long-term pathologies associated with diabetes such as cataract (Monnier 1988).

2.8.2 Contribution of diabetes mellitus in lens opacity

Diabetes mellitus and hyperglycemia are major modifiable risk factors for the development of lens opacities in the African-descent population.

Multiple mechanisms have been implicated in the development of cataract in diabetics (Anselm et al., 2004).

Four main hypotheses about how hyperglycaemia causes diabetic complications have been proposed. These are:

- increased polyol pathway flux;
- increased advanced glycation end-product (AGE) formation;
- activation of protein kinase C (PKC) isoforms and
- increased hexosamine pathway flux

2.8.2.1 Involvement of polyol pathway in cataractogenesis

A number of mechanisms have been proposed to explain the potential detrimental effects of hyperglycaemia-induced increases in polyol pathway flux. Hyperglycaemia-induced increases in polyol pathway flux include sorbitol-

induced osmotic stress, decreased (sodium ion and potassium ion) ATPase activity, an increase in cytosolic NADH/NAD^+ and a decrease in cytosolic NADPH. Sorbitol does not diffuse easily across cell membranes, and it was originally suggested that this resulted in osmotic damage to microvascular cells (Cheng *et, al.*, 1986).

In polyol pathway, both NADPH and NAD^+ are consumed as cofactors for the enzymes Aldose reductase (AR) and sorbitol dehydrogenase (SDH). Osmotic stress due to accumulation of sorbitol and oxidative stress due to changes in the ratio of NADPH/$NADP^+$ and reduced NAD (NADH)/NAD^+ are the major cause of various complications of secondary diabetes. Reduction of glucose to sorbitol by Aldose reductase (AR) constitutes the first and the rate-limiting step of the polyol pathway that converts glucose to fructose via sorbitol dehydrogenase (SDH).

Sorbitol dehydrogenase, the second enzyme in the polyol pathway that converts sorbitol to fructose, also contributes to oxidative stress, most likely because depletion of its cofactor NAD/ leads to more glucose being channeled through the polyol pathway.

In the presence of normal glucose (5.5 mM), Aldose Reductase (AR)-catalyzed reduction represents less than 3% of total glucose utilization, whereas in the presence of high glucose (20 mM), more than 30% of the glucose is used by AR (Gonzalez *et al.*,1984). For every 100 mg/100 ml increase of blood glucose there is a fall of 1.6 mEq Na^+ per liter of water. Lens potassium level is 125 mmol/kg of lens water and lens sodium is 14-26 mmol/kg of lens water. These two cations are in balance with each other, which is mainly due to Na^+-k^+- ATP-

ase pump and lens membrane permeability. Alteration in either of these ions leads to cation imbalance in lens which in turn results in cataract formation. Alteration in cation concentration of aqueous humour which is attributed to alterations in serum cation concentration can be known as a risk factor for cataract formation (Delamere 2001). Multiple studies have been done to clarify the relationship between human biochemical elements and cataract formation. Interestingly, in some of these studies relationship between some serum biochemical elements (such as Na^+) and cataract have been verified (Clayton *et al* 1982)

Philips *et al* (1980) studies notify significant and meaningful difference between serum Na^+ of those suffering from age-related cataract versus those not. Diets with high Na^+ content are a risk factor for age-related cataract formation. High Na^+ content of the diet leads to high level of serum Na^+, which in turn contributes to formation of age-related cataract

2.8.2.2 Intracellular dehydration of senile cataract formation

The occurrences of severe diarrhoea and dehydration have been suggested by some studies as a risk factor for senile cataract (Minassian *et al.,* 1989). In the Australia study, the relation between senile cataract and hydration of the lens has long been documented. Lower total hydration of the lens may be associated with cataract as the human lens ages. Thus, in an elderly individual whose peripheral tissues may be suffering an already marginal perfusion due to atherosclerosis, this may lead to tissue dehydration and metabolic imbalance. As the glucose level increases, production of the polyol sugars (fructose and sorbitol) occurs in the lens, producing an elevation in the osmotic pressure and

thus a movement of water into the lens fibers. Intracellular dehydration occurs due to the increased osmotic effect of the glucose in the extracellular fluid space. The swelling of the lens causes myopia, a symptom commonly found in poorly controlled diabetic patients. If this process continues, alterations in electrolytes, amino acids, ATP, and other substrates occur, which result in the precipitation of proteinaceous material, causing lenticular opacity or cataract. (Karl *et al.,* 2008).

2.8.3 Dyslipidaemia and cataract formation

In the Beaver Dam Eye Study, HDL-cholesterol concentrations were inversely related to cortical cataract in women, and higher ratios of total to HDL-cholesterol were alongside related to posterior subcapsular cataract (PSC) in men (Klein *et al*.,1997). Dyslipidaemia was defined as follows: serum total cholesterol >5.2 mmol/L; serum LDL >2.58 mmol/L; serum triglycerides (TG) >1.7mmol/L; and serum HDL <1.03 mmol/ L by (Friedewald *et al.,* 1997).

A more practical system categorizes dyslipidaemia as primary or secondary and characterizes them by increases in cholesterol only (pure or isolated hypercholesterolemia), increases in TGs only (pure or isolated hypertriglyceridemia), or increases in both cholesterol and TGs (mixed or combined hyperlipidemia). This system does not take into account specific lipoprotein abnormalities (eg, low HDL or high LDL) that may contribute to disease despite normal cholesterol and TG levels.

Secondary causes contribute to most cases of dyslipidaemia in adults. The most important secondary cause in developed countries is a sedentary lifestyle with excessive dietary intake of saturated fat, cholesterol, and trans fats.

Trans fats are polyunsaturated or monounsaturated fatty acids to which hydrogen atoms have been added; they are commonly used in many processed foods and are as atherogenic as saturated fat.

Several observations suggest an association between cataract and low HDL or high triglycerides. Results in other studies have failed to show an association between cataracts and plasma HDL-cholesterol concentrations (Mark *et al.*, 1988; Miglior *et al.,* 1989). The odds of having cataract were 86% higher in Lithuanian women with elevated triglycerides (Paunksnis *et al.,* 2007).The association between cataract and low HDL or high triglycerides has long been documented with Meyer *et al.*, (2003) in the South Africa study. An extremely strong association ($p<0.0001$) was found to exist between HDL cholesterol levels and the development of lens opacities. Abnormal serum lipoprotein levels was a risk factor among the dyslipidaemia subjects for the development of human lenticular opacities

3.8.4 Oxidative damage and protective mechanisms in cataractogenesis

Free radicals are generated in the course of normal cellular metabolic activities and may also be produced by external agents such as radiant energy. These highly reactive free radicals can lead to the damage of lens fibers. Human studies, as well as in vitro and in vivo animal experiments strongly suggest that there is an association between increased oxidative stress and the development of cataract (Altomare *et al.* 1996, Boscia *et al*. 2000).

The antioxidant properties of uric acid have long been recognized and as a result of its comparatively high serum concentrations, it is the most abundant scavenger of free radicals in humans. Also, uric acid as a strong endogenous antioxidant may play an important role in pathogenesis of cataract Waring *et al* (2001). Reductions in serum uric acid levels are clinically relevant in view of its antioxidant properties; uric acid may have potentially important and beneficial effects on the general body oxidative stress due to defect in Xanthine oxidase acting as a dehydrogenase, it removes hydrogen from Xanthine or Hypoxanthine and attaches it to NAD, thereby generating NADH. Because NADPH is used for several critical reductive metabolic steps, such as the detoxification of reactive oxygen species and hydroperoxides (e.g., by the glutathione reductase/glutathione peroxidase system), a large drain on the NADPH pool could compromise the ability of the cell to protect itself from oxidative stress

Under normal physiological conditions, Xanthine oxidase acts as a dehydrogenase, it removes hydrogen from Xanthine or hypoxanthine and attaches it to NAD, thereby generating NADH. However, in certain conditions, such as the disruption of blood flow to a tissue, Xanthine dehydrogenase is converted to a ROS producing oxidase form. AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH. Sorbitol dehydrogenase, the second enzyme in the polyol pathway that converts sorbitol to fructose, also contributes to oxidative stress, most likely because depletion of its cofactor NAD^+ leads to more glucose being channeled through the polyol pathway**.** Evidence linking oxidative stress, cataract formation and serum levels

of antioxidants has led investigators such as Tao *et al* (2004) to assess the role of antioxidant intake in the development of age related cataract. High serum concentrations of antioxidants such as alpha-tocopherol, beta-carotene, retinol, ascorbic acid and selenium have been associated with decreased prevalence of cataract in various observational studies (Knekt 1992; Rouhiainen 1996; Lyle 1999).

The lens is equipped with several enzymes that protect against free radical or oxygen damage. These include glutathione peroxidase, catalase, and superoxide disumutase.

Superoxide dismutase catalyzes the destruction of O_2, and produces hydrogen peroxide:

$$\mathbf{2O^-_2 + 2H^+ \longrightarrow H_2O_2. + O_2.}$$

Catalase may break down the peroxide by the reaction:

$$\mathbf{2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2}$$

Gluthathione peroxidase catalyzes the reaction:

$$\mathbf{2GSH + LOOH \xrightarrow{Gluthathione\ peroxidase} GSSG + LOH + H_2O}$$

The glutathione disulfide (GSSG) is then reconverted to glutathione (GSH) by glutathione reductase, using the pyridine nucleotide NADPH provided by HMP shunt as the reducing agent:

$$\mathbf{GSSG + NADPH + H^+ \xrightarrow{Glutathione\ reductase} 2GSH + NADP^+}$$

Thus, glutathione acts indirectly as a major free radical scavenger in the lens. Both vitamin E and ascorbic acid are present in the lens. Each of these substances can act as a free radical scavenger and thus protect against oxidative damage.

The biochemical mechanism of cataractogenesis

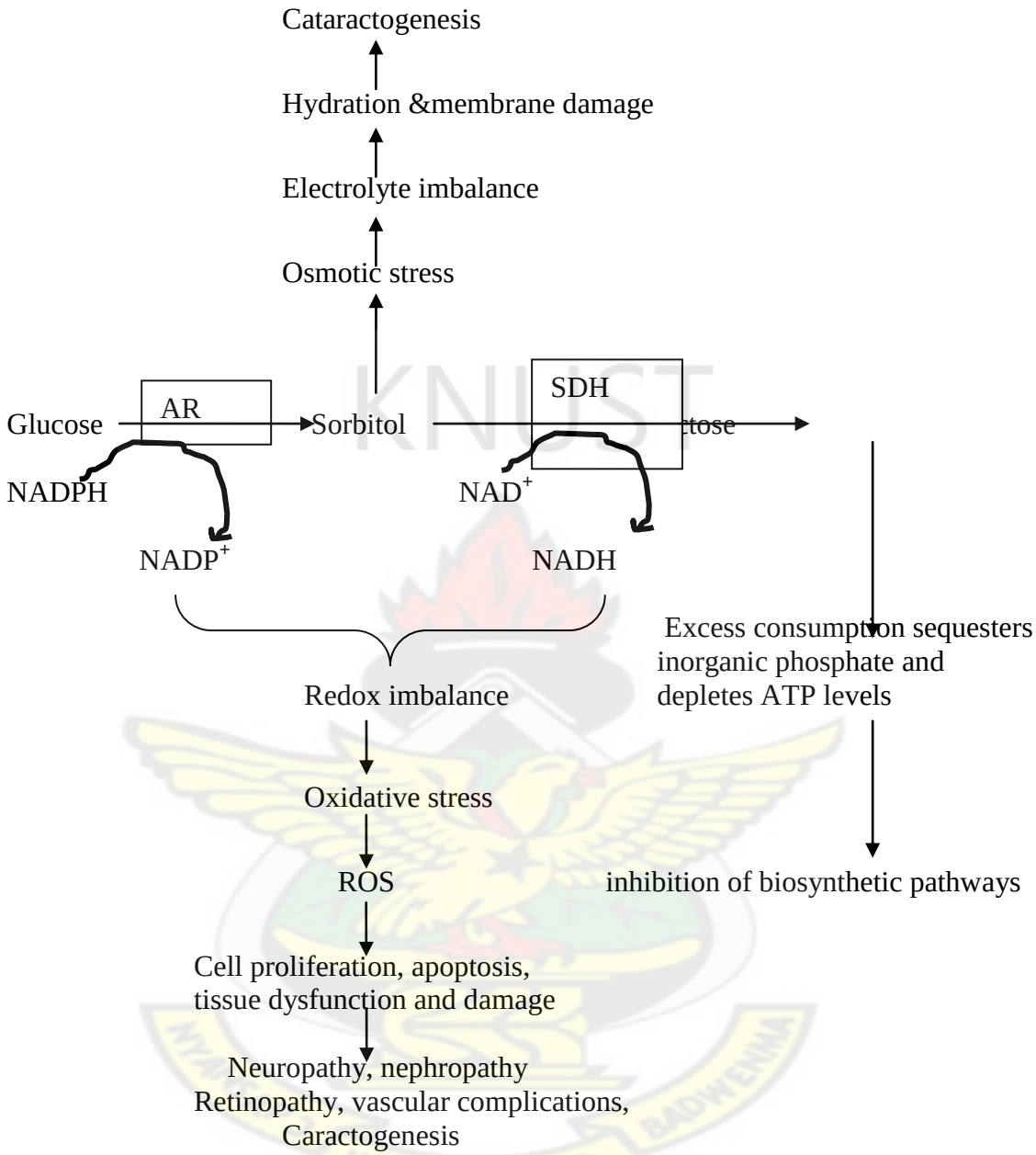


Figure 3 Involvement of polyol pathway in diabetic complications. During hyperglycemia, reduction of glucose to sorbitol by Aldose reductase (AR) constitutes the first and the rate-limiting step of the polyol pathway that converts glucose to fructose via sorbitol dehydrogenase (SDH). In this pathway, both (nicotinamide adenine dinucleotide phosphate) (reduced nicotinamide adenine dinucleotide) NADPH and NAD^+ are consumed as cofactors for the enzymes AR and SDH. Osmotic stress due to accumulation of sorbitol and oxidative stress due to changes in the ratio of NADPH/$NADP^+$ and reduced NAD (NADH)/NAD^+ are the major cause of various complications of secondary diabetes. Reactive oxygen species (ROS) (Verma *et al.*, 1977; Kinoshita *et al.*, 1981)

CHAPTER THREE

Equipment, materials and methods

3.1 Equipments

Cobas Integra 400 plus Auto-analyzer

Electrolyte Analyzer 9180X

Universal 320 Centrifuges

Slit Lamp biomicroscope

Ophthalmoscope

White –Westinghouse refrigerator

3.1.2 Materials

Visual acuity Snellen chart for KATH eye clinic

Sphygmomanometer & stethoscope

Bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China)

Fluoride oxalate tube

Vacutainers tube

Syringes

Nunc CryoTubes

3.1.3 Reagents

1.0% tropicamide (Mydriacy)

2.5% Phenylephrine hydrochloride (Neosynephrine)

3.2 Study design

Case control study design was used in this study. The study population consisted of three hundred (200) participants between the ages of 40 years and above, of whom 100 were cases and 100 were controls. Cases and controls were general ophthalmology outpatients seen at the Komfo Anokye Teaching Hospital Eye Clinic in Kumasi from January 2010 to June 2010.All procedures used in these studies were approved by the Committee for Human Research and development; Komfo Anokye Teaching Hospital.

3.3 Participant (subject) selection

Out - patients visiting the eye clinic were selected on the basis of presence of cataract in one or both eyes as (cases).

The diagnosis is made with ocular examination using slit-lamp biomicroscopy after dilatation with 1.0% tropicamide (Mydriacy) 2.5% Phenylephrine hydrochloride (Neosynephrine**)** reagents by optometrist, followed by referral to an ophthalmic surgeon for confirmation of the diagnosis and management. For each patient (case), a matched healthy eye (normal eye) without cataract was considered for the study as controls.

The cases and controls were of the same age, sex, related or unrelated and resided in the same area of the city. Cases were adult of 40 years and above with low vision defined as visual acuity of less than 6/18 but equal to or better than 3/60 (Blindness' is defined as visual acuity of less than 3/60).

The Controls were matched age, sex, and adult of 40 years and above with visual acuity not worse than 6/9 with no correction in both eyes, neither operated with no significant evidence of cataract in one or both eyes.

3.4 Data collection and subject consent

Participants were contacted at the eye clinic. The objectives and the importance of the study were explained to the people both in the local dialect and the English language. The out patients were given the opportunity to ask questions for clarification. If they consent, qualified participant were given the consent form, and explain as to how to fill it. Approved questionnaire were administered to the qualified participant's .Anthropometric measurements as well as blood pressure were taken by qualified nurses. Visual acuity and other diagnosis of senile cataract status were performed by a specialist ophthalmologist. In addition to a clinical examination, data collection during the study included a personal data, anthropometric measurements, history of some known risk factors of cataract. Participants were asked to come for laboratory investigation the next day after they have fasted 12hrs over night.

3.5 Anthropometric measurement

Anthropometric measurements included height to the nearest centimetre without shoes against a wall-mounted ruler .weight.

Qualified participants were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, and China) to nearest 0.1 kg in clothing.

The body mass index (BMI) was calculated as weight over the height squared (kg/m^2). BMI was classified into four categories according to WHO recommendations: individuals with a healthy weight (BMI 20 – 24.9), overweight (BMI 25 – 29.9), underweight (BMI < 20 kg/m^2) and obese (BMI > 30 kg/m^2).

Blood pressure was taken by trained personnel using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 min in accordance with the recommendation of the American Heart Association (Kirkendall et al., 1967). Duplicate measurements were taken with a 5 min rest interval between measurements and the mean value was recorded to the nearest 2.0 mm Hg.

3.6 Visual acuity determination

Visual acuity screening is a widely used approach to identify reduced vision. Visual acuity of 6/6 (20/20) as measured on the Snellen chart is usually considered normal. Visual acuity, the sharpness of near and distance vision, is tested separately for each eye. One eye is covered with a piece of paper or the palm of the hand placed lightly over the eye to allow testing of the distance and near vision in the opposite eye.The Preferred Practice Pattern of the American Academy of Ophthalmology (2004) recommends Snellen visual acuity tests as the best guide for appropriateness of surgery with respect to the patient's functional and visual needs, environment, and risk factors.

Each participant underwent best –corrected distance visual acuity measurement with Snellen chart. Visual acuity was defined as average value of the corrected acuity of both eyes before examination. Visual acuity and other diagnosis of senile cataract status were done by specialist ophthalmologist.The Snellen eye chart is used, with rows of letters decreasing in size to determine how clearly a person can actually see.

3.7 Sample collection and preparation

3.7.1 Human biochemical analysis

Venous blood samples were collected after an overnight fast (10 – 16 hours). About five millilitres (5mls) of venous blood were drawn and three (3) ml was dispensed into vacutainers plain tubes and 2 ml into fluoride oxalate tubes. After centrifugation at 500 g for 15 min, the serum and plasma were stored at - 80°C until required for analysis.

Serum biochemistry was performed with Cobas Integra 400 plus Auto-analyzer (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined included: fasting blood sugar (FBS),serum lipid profile (total cholesterol, HDL (high density lipoprotein), LDL (low density lipoprotein), VLDL (very low density lipoprotein) and triglycerides), serum uric acid and serum electrolyte was performed with Electrolyte Analyzer 9180x.

3.7.1.1 Fasting blood glucose determination

Glucose concentration in the samples was estimated with the hexokinase method. Hexokinase (HK) phosphorylates glucose with ATP to produce glucose-6-phosphate, which is then oxidized by glucose-6-phosphate dehydrogenase to 6-phosphogluconate with the simultaneous reduction of NAD^+ to NADH. The resulting increase in absorbance at 340nm is directly related to the concentration of glucose in the sample.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + NAD^+ \xrightarrow{\text{G6PDH}} \text{Phosphogluconate} + \text{NADH}$$

3.7.1.2 Serum electrolyte determination

Serum electrolyte determination was performed with Electrolyte Analyzer 9180x (mmol/l) which uses ion-selective electrodes (ISEs) measurement principle for determination of the concentration or the activity of ions in aqueous media. The use of ion-sensitive electrodes (ISEs) for determination of the concentration or the activity of ions in aqueous media has been known for a long time.

An ion-selective electrode (ISE) is an electrode which exhibits an electrical response which is a function of concentration of a specific ion contained in a solution which is in contact with the ISE and a reference electrode. Ion selective electrodes operate on the basis of the Nernst principle which defines a logarithmic relationship between the potential of a solution and its ionic concentration. The operation of ion selective electrodes is based on the fact that there is a linear relationship between the electrical potential developed between an ISE and a reference electrode immersed in the same solution, and the Logarithm of the

activity (or "effective concentration") of the ions in the solution. This relationship is described by the Nernst equation:

$E = E^0 + (2.303RT/ nF) \times Log(a)$

Where E = the total potential (in mV) developed between the sensing and reference electrodes.

E0 = is a constant which is characteristic of the particular ISE/reference pair. (It is the sum of all the liquid junction potentials in the electrochemical cell) 2.303 = the conversion factor from natural to base10 logarithm.

R = the Gas Constant (8.314 joules/degree/mole).

T = the Absolute Temperature.

n = the charge on the ion (with sign).

F = the Faraday Constant (96,500 coulombs per mole).

Log(a) = the logarithm of the activity of the measured ion.

An ion selective electrode responds selectively to an ion for which the electrode is designed to analyze and is used to measure ion concentrations, ion activities, etc. The determination of a given ion's concentration in a solution is based on the fact that within certain limits the potential of the electrode is directly proportional to the logarithm of the ion's activity. Ion selective electrode sensors that can measure the activity or concentration of analyte ions and metabolites are useful in the analysis of biological fluids including blood, urine, plasma, saliva, spinal fluid, and serum. An ion selective electrode apparatus usually comprises an ion selective electrode and a reference electrode. The ion selective electrode includes an ion selective membrane on the surface of it.

Circulating sodium concentration is commonly measured by both direct and indirect ion-sensitive electrode (ISE)

Sodium electrode is a glass capillary electrode used for in vitro diagnostic measurement of sodium ions present in fluid samples. It is designated with a Na+ making on the surface of the housing.

Potassium electrode is a membrane electrode used for in vitro diagnostic measurement of Potassium ions present in fluid samples. It is designated with a K+ making on the surface of the housing.

Chloride electrode is a membrane electrode used for in vitro diagnostic measurement of Chloride ions present in fluid samples. It is designated with a Cl^- making on the surface of the housing.

3.7.1.3 Serum uric acid determination

Uric acid is converted by oxidation by uricase to allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzene-sulphonic acid (chlorophenol sulphonic acid) and 4-aminophenazone (4AP) to form a red-violet quinonimine compound, which is proportional to the amount of uric acid present.

$$\text{Uric} + 0_2 + 2H_20_2 \xrightarrow{\text{uricase}} \text{Allantoin} + C0_2 + H_20_2$$

$$2\ H_20_2 + \text{chlorophenol sulphonic acid} + 4\text{AP} \xrightarrow{\text{peroxidase}} \text{N-(4-antipyryl}$$

-3- chloro-5-sulphonate –p-benzo- quinoneimine

3.7.1.4 Fasting lipid profile determination

3.7.1.4.1 Total cholesterol

The method for this assay is based on that described by Trinder, (1969). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free

cholesterol produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour is directly proportional to the total cholesterol concentration.

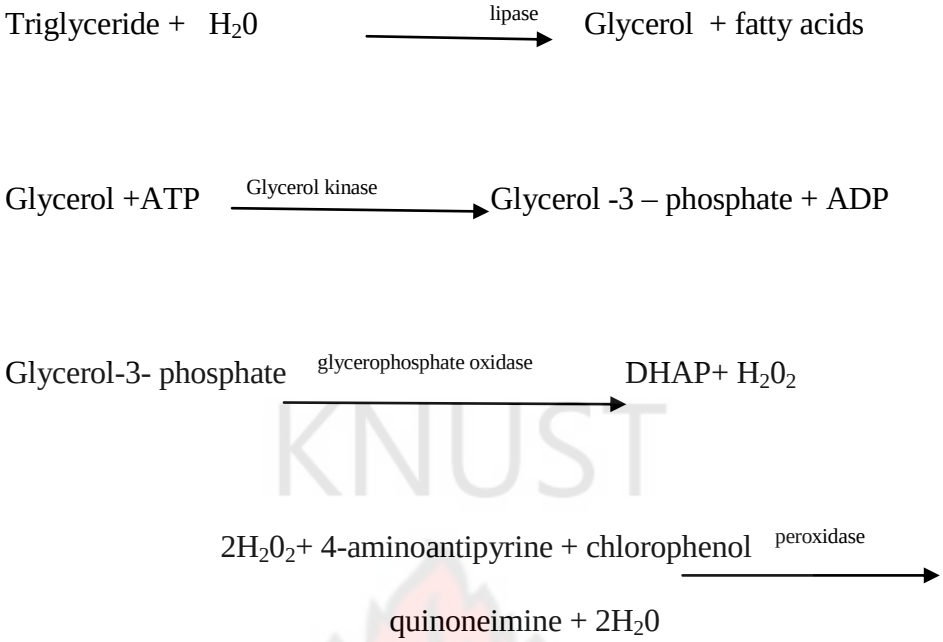
Cholesterol $+H_20$ —(cholesterol esteras)→ Cholesterol + Fatty acids

Cholesterol $+0_2$ —(cholesterol oxidase)→ Cholest-4-en-3-one$+H_20_2$

H_20_2 + 4 – aminophenazone + Phenol —(peroxidase)→ H_20+ Quinoneimine

3.7.1.4.2 Triglyceride

The method for this assay is based on a modified Trinder colour reaction to produce a fast linear endpoint reaction riglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H2O2) by glycerophosphate oxidase. The hydrogen peroxide the reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.



3.7.1.4.3 HDL –cholesterol

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg2+ ions. The cholesterol concentration in the HDL is then determined by the method described by Trinder for the assay of cholesterol.

3.7.1.4.4 LDL cholesterol

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald *et al.*, 1972). LDL-cholesterol = TC − [HDL-cholesterol + (TGs ÷ 5)] (Friedewald formula). This calculation is valid only when TGs are < 400 mg/dL and patients are fasting. LDL = TC – TG/2.2 -HDL (mmol/l)

3.7.1.4.5 Very low density lipoprotein (VLDL)

VLDL is estimated by TG ÷ 5 because the cholesterol concentration in VLDL particles is usually $^{1}/_{5}$ of the total lipid in the particle**.**

3.8 Statistical analysis

Results are presented as Means ± SD. Unpaired *t*-test was used to compare the means of all continuous variables. The Chi-square test statistic was used to assess the statistical significance of categorical variables. Conditional logistic analysis was used to estimate the association between the significant identified biochemical variables and the senile cataract. Odds analysis and confidence intervals for biochemical risk factors of senile cataracts was done using the log likelihood ratio test statistic. Log likelihood ratio test was used to observe the strength in the association between level of exposure, the trend in the likelihood and the probability of developing the senile cataract. The odds ratio (ORs) of biochemical for risk factors of senile cataract among cases and control was considered to be statistically significant when a p-value < 0.05. All statistical analyses were performed using Stata /IC version 10.0 (http://www.stata.com/ stata@stata.com) for windows.

CHAPTER FOUR

RESULTS

4.1 General characteristics of the study population

The general characteristics of the study population stratified by cases and controls are shown in Table 4.1.Patients with cataract (cases) were significantly older (67.58 ± 1.31years) than the matched control pairs (62.17 ± 0.79 years respectively) ($p < 0.0003$). About 50% of all 70-year-olds suffer from a cataract requiring surgery and there were 62% female and 38%male with senile cataract out of the 100 cases studied. The average body mass index of control group 24.40 ± 3.39 kg m-2) was significantly higher than the cases with 22.40 ±3.37 kg m-2 ; $p < 0.0001$ and 19% out of the 100 cases were known hypertensive. The mean systolic Bp (126.3 ± 28.37 mmHg) and diastolic Bp (80.5±16.96mmHg) in patients with cataract were lower in comparison to the control patients (128.3 ± 22.38 mmHg, 81.41±15.97 mmHg respectively). The distributions of known diseases were gout (5%), diabetes (9%), and hypertension (19%) for cases and gout (0%) diabetes (1.01%), hypertension (4.04%) for control respectively. Among 100 cases studied, 74% had visual impairment (acuity, ≥ 6/60) in left eyes and 68% visual impairment (acuity, ≥ 6/60) in right eyes. The distribution for normal visual acuity for left and right eyes was 26% and 32 respectively for normal or near normal vision (acuity < 6/18 ≥ 6/18) in both eyes.

Table 4.1 General Characteristics of the study population stratified by cases and controls visiting Komfo Anokye Teaching Hospital in Kumasi Ghana, between 2008, and 2010

Variables	Patients with cataract	Controls	P value
	Mean ± SD	**Means ± SD**	
***Age (years)**	67.58±1.31	62±0.79	0.0003
Gender: F/M (%)	62% /38%	61%/39%	0.884
***BMI (Kg/m2**	22.40±3.37	24.19±3.39	0.0001
***SBP (mmHg)**	126.3±28.49	128.3±22.38	0.821
***DBP (mmHg)**	80.5±16.96	81.41±15.97	0.8669

Known diseases(%)	**CASES**	**CONTROLS**	**P-value**
Diabetes	(9%)	(1.01%)	0.0000
Hypertension	(19%)	(4.04%)	0.0000
Gout	(5%)	(0%)	0.0000
Unknown	(67%)	(94.95%)	0.0000

	CASES		**CONTROLS**		**P-value**
Visual Acuity	VR	VL	VR	VL	
Normal eyes (%)	32%	26%	91%	95%	0.0000
Impaired eyes (%)	68(68%	74%	9%	5%	0.0000

Age/sex	**CASES (N=100)**			**CONTROLS (N=100)**		
	Female (%)	Male (%)	Total	Female (%)	Male (%)	Total
40-50	5 (8.06%)	3 (7.89%)	8 (8%)	8 (13.11%)	4(10.26%)	12(12%)
51-60	13(20.97%)	7(18.42)	20(2%)	16 (26.23%)	8(20.5%)	24(24%)
61-70	11(17.74)	11(28.9%)	22(2%)	8 (13.11%)	15(38.46%)	23(23%)
>71	33(53.23%)	17(44.7%)	50(5%)	29(47.54%)	12(30.77%)	41(41%)
Total	62(100%)	38(100%))	100	61(100%)	39(100)	100

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure

4.2 Characteristics of biochemical variables among the study population

Table 4.2 present characteristics of biochemical variables among the study population.Ten (10) biochemical variables were measured using KATH reference range (R_f). Comparing the serum electrolyte levels, there was a statistically significant difference between the mean serum Na^+ level (R_f=135-145) in senile cataract patients (143.2±6.76) and normal individuals (139.3±1.96; p=0.0000). The mean serum K^+ level R_f=3.5-5.5).of senile cataract patients and normal individual's was4.21±0.50 and 4.38±0.45; p=0.9965 respectively. The mean serum Cl^-level R_f =97-110) between the cases and control were and there was statistically insignificant.

Conversely, The mean concentrations of total cholesterol (4.80 ± 1.08 mmol L^{-1}, p = 0.9182), triglyceride 0.14±0.34 (p=0.0130) and Very Low Density Lipoprotein (0.34 ± 1.08 mmol L^{-1}, p = 0.6703) in patients with cataract was lower than in the control with mean concentrations of (5.03±1.23, 0.51±0.73 and 0.45±2.03 mmol L^{-1} respectively). The low level of mean concentrations of High Density Lipoprotein (1.114±0.42 mmol L^{-1}, p =1.000) and Low Density Lipoprotein Cholesterol was (3.06 ± 1.10 mmol L^{-1}, p= 0.2207) in cataract patients were different compared to that in control patients (1.04±0.10 mmol L^{-1} and 2.94±1.06 respectively). Also, the mean uric acid concentrations level was 210.0±113.8 µmol L^{-1} lower in the cases compared to 311.1±117 µmol L^{-1} of the controls (p=1.000). The mean concentration of fasting blood glucose (R_f=3.6-6.4). of the cataract patients 4.92±2.09 mmol L^{-1} were also lower than the control group of 6.01±2.96 mmol L^{-1} p= 0.9986 but the differences were not significant.

Table 4.2 Characteristics of biochemical variables (Mean ± SD and 95% CI) among the study population stratified by cases and control

Variables	Patients with cataract		Control		P value	R_f KATH laboratory
	Mean ± SD	**95% CI**	**Means ± SD**	**95%CI**		
FBS (mmol L^{-1})	4.92±2.09	4.51-5.34	6.01±2.96	5.40-6.61	0.9986	**3.6-6.4**
Na^+ (mmol L^{-1})	143.2±6.76	141.9-144.6	139.3±1.96	138.9-139.7	0.0000	**135-145**
K^+ (mmol L^{-1})	4.21±0.50	4.12-4.29	4.38±0.45	4.29-4.47	0.9965	**3.5-5.5**
Cl^- (mmol $L^{-1)}$	105.5±3.65	104.7-106.2	105.2±2.35	104.5-105.5	0.1616	**97-110**
TC (mmol L^{-1})	4.80±1.08	4.58-5.01	5.03±1.23	4.78-5.29	0.9182	**3.90-5.20**
TG (mmol $L^{-1)}$	0.14±0.34*	0.07-0.21	0.51±0.73	-0.18-0.11	0.013	**0.5-2.26**
HDL -C (mmol $L^{-1)}$	1.14±0.42	1.05-1.21	1.04±0.10	1.47-1.86	1.0000	**1.15-1.68**
LDL-C (mmol L^{-1})	3.06±1.10	2.84-3.28	2.94±1.06	2.73-3.15	0.2207	**0.0-2.6**
VLDL (mmol L^{-1})	0.34±1.08	0.132-0.56	0.45±2.03	0.46-0.85	0.6703	
URIC ACID	210.0±113.8	187.4-232.6	311.1±117.9	286.4-335.8	1.0000	**143-417**

Results are presented as mean ± SD at 95% confidence interval. P value defines the level of significance when study population with cataract was compared to the controls. FBG = fasting blood glucose, TC = total cholesterol, TG = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL = very low density lipoprotein, Na^+=serum sodium cation, K^+=serum potassium cation, Cl^-serum chloride anion

4.3 Biochemical variables associated with senile cataract

Table 4.3 Present conditional logistic regression analysis of the biochemical variables associated with senile cataract among the study population. Out of the ten (10)biochemical variables measured, blood levels of six (6): fasting blood glucose (FBG) 1.3 (1.O6-1.49 ;p=0.008), sodium (Na^+)mmol L^{-1} 0 6 (0.47-0.72; p=0.000), potassium (K^+ mmol L^{-1}) 2.48 (1.47-0.72; p=0.010), triglyceride TG (mmol L^{-1}) 0.58 (0.35-0.98) p=0.040), uric acid(UA) 1.01 (1.01-1.02) P=0.000) and high density lipoprotein-Cholesterol (HDL-C mmol L^{-1}) 3.17 (1.79-5.61; p=0.000) were significant and associated with senile cataract among the study population.

The other four variables however, were associated with senile cataract but were not significant. Cl^- (mmol L-^{1}) 0.95 (0.87-1.05; p=0.313, total cholesterol TC mmol L^{-1}) 1.24 (0.95-1.62; p= 0.115), low density lipoprotein –cholesterol LDL-C mmol L^{-1}) 0.88 (0.67-1.15; p= 0.363 and very low density lipoprotein VLDL mmol L^{-1}) 1.04 (0.87-1.25; p= 0.664)

Table 4.3 ORs and 95% CIs conditional logistic regression analysis of the biochemical variables associated with senile cataract among the study population

VARIABLES	ORs (95%CI)	P value
Fasting blood glucose(FBG)	1.25(1.O6-1.49)	0.008
Sodium (Na^+)	0.58(0.47-0.72)	0.000*
Potassium (K^+)	2.48(1.47-0.72)	0.01
Chlorine (Cl^-)	0.95(0.87-1.05)	0.313
Total cholesterol (TC)	1.24(0.95-1.62)	0.115
Triglyceride (TG) (	0.58(0.35-0.98)	0.04
HDL –C -1)	3.17(1.79-5.61)	0.000*
LDL-C	0.88(0.67-1.15)	0.363
VLDL	1.04(0.87-1.25)	0.664
URIC ACID	1.01(1.01-1.02)	0.000*

Factors with P<0.05 were considered as statistically significant. The results are presented as odd ratio (ORs) and 95% confidence intervals (CIs) P value defines the level of significance when study population with cataract was compared to the controls. FBG = fasting blood glucose, TC = total cholesterol, TG = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL = very low density lipoprotein.

4.4 Log likelihood-ratio test of significant biochemical risk factors of senile cataract

The significant and essential biochemical variables related to individual risk of senile cataract were presented in table 4.4. The trend in the likelihood ratio test indicate a very strong association between increasing order of level of exposure to sodium, uric acid, HDL-C, FBS and the probability of developing the senile cataract. Persons with HDL C abnormalities were twice as likely to develop senile cataract (odds ratio, 2.52 p=0.095). Within the normal reference ranges, FBS, and low uric acids (UA) levels increased risk for senile cataract (odds ratio, 1.20; p=0.106 and odds ratio1.01 p=0.011 respectively). Exposure to sodium in the absence of other biochemical risk factors remained the most significant factor associated with senile cataract. It is the only protective risk factor for senile cataract (odds ratio, 0.60; p=0.001).

Table 4.4 ORs and 95% CIs for significant and essential biochemical risk factors of senile cataract among the study population

Biochemical risk factors	ORs(95% CI)	P value
FBS*	1.20(0.96-1.50)	0.106
Na**	0.60(.45-0.80)	0.001
HDL CHOL**	2.52(0.85-7.50)	0.095
URIC ACID***	1.01(0.00-1.01)	0.011

P<.05 were considered as statistically significant (**** highest level of strength).

CHAPTER FIVE

DISCUSSION

In the present study, it was observed that older people aged between 40 -70 years especially women are most likely to develop senile cataract due to exposure to significant and essential biochemical factors such as sodium, uric acid, high density lipoprotein cholesterol and fasting blood glucose. The trend in the likelihood ratio test indicates a very strong association between increasing order of level of exposure to sodium, uric acid, high density lipoprotein cholesterol and fasting blood glucose and the probability of developing the senile cataract.

5.1 Effects of sex and age of the senile cataract formation

The burden of senile cataract was higher among the 70 years age group. Patients with cataract (cases) were significantly older (67.58 ± 1.31years) than the matched control pairs (62.17 ± 0.79; p 0.003) years respectively. There were 62% female and 38%male with senile cataract out of the 100 cases studied. The biochemical and structural changes that take place within the human crystalline lens have been likened to degenerative processes that occur in other parts of the body as a consequence of aging. Reasons for poor nutritional status in older people are multi-faceted and include the physiological, psychological and social changes associated with aging which affect food intake and body weight, exacerbated by the presence of illness. The exact mechanism tying age and the risk of senile cataract cannot be determined from present

data. It has been suggested that the incidence of senile cataract rises exponentially with the age after 50 years (Kahn et al, 1977). Weale (1981) reported that, in gender, the relative susceptibility to cataract change with age, but so does their response to physical trauma. This suggest a hormonal influence and recent epidemiological studies show that women undergoing hormone replacement therapy have a reduced incidence of cataract compared with control cohort of the same age (Cumming *et al.,* (1997). In order to curtail the combining effect of age and sex in the outcome of this study, both variables were treated as confounders.

This study support the population based surveys study on blindness prevalence in Asia, Africa, and the industrialized countries indicating that women bear approximately two-thirds of the burden of blindness in the world. (WHO "Gender and Blindness"(2002)

It is important to note a significant and essential of some biochemical factors in the blood which might indicate the dysfunctions and derrangement associate with human senile cataract. Four biochemical factors: sodium, uric acid, high density lipoprotein cholesterol and fasting blood glucose. (Table 4.4) were found to be risk factors associated with senile cataract. It has been reported earlier that, one of the secondary complication of diabetes mellitus is diabetic retinopathy and cataract formation but the biochemical mechanism supporting this has been inconsistent due to the challenges of lens glucose and sorbitol metabolism and the production of aqueous humour which the environment for nutrient utilization. The distributions of known diseases were gout (5%), diabetes (9%), and hypertension (19%) for cases and gout (0%) diabetes (1.01%), hypertension (4.04%) for control respectively in this study Studies by Leske *et al*., 1983 and West *et al.,* 1995) have strongly established age and Diabetes mellitus

(DM) as the principal risk factors in the aetiogenesis of cataract among people of African-descent. However, in multiple regression tests, the level of blood sugar was not the significant factor associated with cataract for diabetes mellitus patients.

5.2 Diabetes, Fasting Blood Glucose (FBG) and senile cataract

In Ghana, a diabetic patient was defined as one who had a fasting blood glucose concentration (FBG) ≥6.4 mmol L^{-1} while fasting 12 hours overnight for the purpose of this study. The desired limit in reference range of FBS is 6.4mmol/l. The WHO criterion mandates the presence of diabetes mellitus, (WHO, 1999) define as Fasting blood glucose (FBG) ≥5.6 mmol L^{-1} or previously diagnosed type 2 diabetes (Alberti et al., 2006).Only 9%, were diabetic among cases and the mean concentration of fasting blood glucose of the cataract patients was 4.92±2.09 mmol L^{-1} were lower than the control group of 6.01±2.96 mmol L-^{1} showed no statistical significance (Table 4.2). Within the normal reference ranges, FBG, levels increased risk for senile cataract (odds ratio, 1.20; p=0.106) (Table 4.4). This clearly indicate that glucose play an important in the biochemical mechanism of cataract formation (figure 2.1). This is explained by the involvement of polyol pathway in diabetic complications. Reduction of glucose to sorbitol by Aldose reductase (AR) constitutes the first and the rate-limiting step of the polyol pathway that converts glucose to fructose via sorbitol dehydrogenase (SDH). In this pathway, both (nicotinamide adenine dinucleotide phosphate) (reduced nicotinamide adenine dinucleotide) NADPH and NAD^{+} are consumed as cofactors for the enzymes AR and SDH. Osmotic stress due to accumulation of sorbitol and oxidative stress due to changes in the ratio of $NADPH/NADP^{+}$ and reduced NAD (NADH)/NAD^{+} are the major cause of various

complications of secondary diabetes (Verma *et al.,* 1977; Kinoshita *et al.,* 1981) The accumulation of sorbitol has been shown to cause lens damage osmotic imbalance and changes in membrane permeability leading to lens opacity. A causal as well as an initiating factor is thought to be the changes observed when the lens-forming glucose and protein molecules react to produce a large intraocular mass disrupting the lenticular fibers thereby damaging the inorganic ion balance. The epithelium of the lens helps to maintain the ion equilibrium and permit transportation of nutrients, minerals, and water into the lens. This type of transportation, referred to as a "pump-leak system," permits active transfer of sodium, potassium, calcium, and amino acids from the aqueous humor into the lens as well as passive diffusion through the posterior lens capsule. Maintaining this equilibrium is essential for the transparency of the lens and is closely related to the water balance. The water content of the lens is normally stable and in equilibrium with the surrounding aqueous humor.

5.3 Serum sodium as a risk factor for senile cataract

In the current study, the mean serum Na^+ level (R_f=135-145) in senile cataract patients (143.2±6.76) and normal individuals (139.3±1.96; p=0.0000) were statistically significantly different (Table 4.2). Though the mean of serum Na^+ of the senile cataract patients were in the sodium reference range of (135-145) but it was in the upper limit and in comparison with control group, the serum sodium of those with clinical senile cataract condition was elevated. Donnelly et al., (2004) also reported that the fasting blood sugar (fasting) level was higher in the cataract group than in the control group. This major finding could therefore, correlate with other studies elsewhere that a causal

as well as an initiating factor is thought to be the changes observed when the lens-forming glucose and protein molecules react to produce a large intraocular mass disrupting the lenticular fibers thereby damaging the inorganic ion balance.

In log likelihood regression analysis of all the biochemical indicators associated with senile cataract, serum Na^+ was the most significant in this study which verifies other studies. The trend in the likelihood ratio test indicate a very strong association between increasing order of level of exposure to sodium, uric acid, HDL-C, FBS and the probability of developing the senile cataract. Exposure to high sodium level in the absence of other biochemical risk factors remained the most significant factor associated with senile cataract. It is the only protective risk factor for senile cataract (odds ratio, 0.60; p=0.001) (Table4.4)). Interestingly, an association between raised sodium levels and cataract may also be mediated through the effect of salt intake on hypertension. There is a positive correlation between sodium levels and elevated blood pressure (Suvillan (1986).

One of the proposed risk factors for cataract formation is serum sodium ion level. Interestingly in some of these studies relationship between some serum biochemical elements (such as Na^+) and cataract have been verified. Clayton *et al*., (1982) & Philips *et al* (1980) studies notify significant and meaningful difference between serum Na^+ of those suffering from age-related cataract versus those not. Lens has high content of potassium and low content of sodium.The lens metabolism is associated with aqueous humour, (Daliles *et al* 1995 and Luntz *et al* 2000;) and the aqueous humour is produced from blood secretions. Serum electrolytes concentration directly affects electrolytes of aqueous humor and in turn lens metabolism (Van Heyningaen *et al* 1961). Lens potassium level is 125 mmol/kg of lens water and lens sodium is 14-26

mmol/kg of lens water. These two cations are in balance with each other, which is mainly due to Na^{+}-K^{+} ATP-ase pump and lens membrane permeability. Alteration in either of these ions leads to cation imbalance in lens which in turn results in cataract formation. Hence alteration in cation concentration of aqueous humour which is attributed to alterations in serum cation concentration can be known as a risk factor for cataract formation. Delamere (2001)

The mechanism of the initial insult to the crystallin molecule could take a number of forms. While numerous studies have shown that glucose is osmotic active, intracellular dehydration occurs due to the increased osmotic effect of the glucose in the extracellular fluid space.

Elevated levels of blood (and aqueous humor) glucose overwhelm this pathway, leading to production of sugar alcohols, which in turn increase osmotic pressure and cause lens swelling and cataract. Osmotic swelling of diabetic lens may render the cells leaky, enhancing the loss of GSH accumulated in the lens. Disrupted cell membrane by osmotic stress may also interfere with amino acid transport into the lens, and hence the biosynthesis of GSH. The swelling of the lens causes myopia, a symptom commonly found in poorly controlled diabetic patients. If this process continues, alterations in electrolytes, amino acids, ATP, and other substrates occur, which then finally result in a precipitation of proteinaceous material, causing lenticular opacity or cataract. As it seems, high Na+ content of the diet may leads to high level of serum Na+, which in turn contributes to formation of age-related cataract

5.4 Lipoprotein disorders (Dyslipidaemia) and the risk of senile cataract

Different serum lipids (TG, TC, HDL, LDL, and VLDL) vary significantly in various population groups due to difference in geographical, cultural, economical, social conditions, dietary habits and genetic makeup. Dyslipidaemia was defined as follows: serum total cholesterol >5.2 mmol/L; serum LDL >2.58 mmol/L; serum triglycerides (TG) >1.7 mmol/L; and serum HDL <1.03 mmol/ L by (Friedewald *et al.*, 1997). Serum lipoprotein abnormalities are characterized by high triglycerides concentrations [TG], low high density lipoprotein- cholesterol concentrations [HDL-C], normal total and low cholesterol density lipoprotein – cholesterol concentrations [LDL-C]. By National Cholesterol Education Program Adult Treatment Panel III, approach to dyslipidaemia identify HDL-cholesterol (mg/dl (mmol/l)) < 40 (< 1.03) =Low and HDL-cholesterol ≥ 60 (≥ 1.55) =High as lipid disorders

In this study, the significant type of lipid abnormality observed among the clinically diagnosed senile cataract patients was low high density lipoprotein-cholesterol concentrations [HDL-C] also called hypoalphalipoproteinemia possibly due to diet or an abnormality in liver function. The lower cholesterol might imply a defect in metabolism of cell membrane important in cataractogenesis.

The low level of mean concentrations of High Density Lipoprotein (1.114±0.42 mmol L^{-1}, p =1.000) in cataract patients were different compared to that in control patients (1.04±0.10 mmol L^{-1} and 2.94±1.06 respectively see Table 4.2). This low level high density lipoprotein-Cholesterol (HDL-C

mmol L^{-1}) 3.17 (1.79-5.61; p=0.000) was significantly associated with senile cataract among the study population see Table4.3). Persons with HDL C abnormalities were twice as likely to develop senile cataract (odds ratio, 2.52 p=0.095).

A more practical system categorizes dyslipidaemia as primary or secondary. Secondary causes contribute to most cases of dyslipidaemia in adults. This specific lipoprotein abnormality e.g., low levels HDL may contribute to disease despite normal cholesterol and TG levels. It has been proposed that most of the lipid in the mammalian lens is associated with the intercellular membrane structure and extracellular glucose diffuses into the lens uncontrolled by the hormone insulin. Insulin resistances or reduced action of insulin at the tissue level explain low level HDL-C concentration observed in this study. Other factors such mutation in ATP-binding cassette transporters' A1 (ABCA1) gene expression in HepG2 cell, familiar HDL deficiency and decreased lipoproteins lipase activity may also lead to inadequate transport of phospholipids and cholesterol to the extracellular resulting in the hypercatabolism of lipid – poor nascent HDL particles. This may result in low circulation HDL

Apolipoprotein A1 is produced directly from liver (and to much lesser extent, from the gastrointestinal tract) and associated with phospholipids to form nascent HDL

There is evidence so to suggest that the rate of catabolism of apolipoprteinA1 is a more important determinant of circulating HDL-C concentration the rate of production (Brinton *el al.*, 1991)

The association between cataract and low HDL or high triglycerides has long been documented with Meyer *et al.,* (2003) in the South Africa study. An extremely strong association ($p<0.0001$) was found to exist between HDL cholesterol levels and the development of lens opacities. Several observations suggest an association between cataract and low HDL or high triglycerides. Another study reported High-density lipoproteins (HDL) having both antioxidative and anti-inflammatory activities, in addition to their better-known cardioprotective role in reverse cholesterol transport. (Klinmov *et al*.1993; (von Eckardstein *et al,* 2005). With above observation, low serum level of HDL –C is a biochemical risk factor association senile cataract and should be given more attention.

5.5 Low uric acid as oxidative stress biomarker associated with higher risk of age related cataract or senile cataract

Reductions in serum uric acid levels are clinically relevant because high levels are often associated with gout. Again, Elevation of serum uric acid concentration occurs as a physiologic response to increased oxidative stress-for example, during acute exercise-thus providing a counter-regulatory increase in antioxidant defenses The reference ranges (143-417µmol L-1, Hyperuriceamia =uric acid >416.4 µmol L^{-1} for men and 356.9 µmol L^{-1} for women) were used in this study..Uric acid level was significantly lower in the senile cataract patients than in the controls and only 5% reported for known gout condition in the present study. The mean uric acid concentrations

level was 210.0±113.8 µmol L^{-1} significant lower in the cases compared to 311.1±117 µmol L^{-1} of the controls Uric acid (UA) were significantly associated with senile cataract among the study population.1.01 (1.01-1.02) P=0.000) low uric acids (UA) levels increased risk for senile cataract odds ratio1.01 p=0.011 respectively

Aging and oxidative stress, in particular, affects the entire body. AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH. Sorbitol dehydrogenase, the second enzyme in the polyol pathway (converts sorbitol to fructose), also contributes to oxidative stress, most likely because depletion of its cofactor NAD/ leads to more glucose being channeled through the polyol pathway **(**See figure 2.1)

Depletion of cofactor by action AR and Sorbitol dehydrogenase enzymes in the polyol pathway in aetiogenesis of cataract at the tissue level explain low level uric acid concentration observed in this study may have potentially important and beneficial effects within on the general body oxidative stress. Evidence linking oxidative stress, cataract formation and serum levels of antioxidants has led investigators to assess the role of antioxidant intake in the development of age related cataract (Tao *et al* (2004).

High serum concentrations of antioxidants such as alpha-tocopherol, beta-carotene, retinol, ascorbic acid and selenium have been associated with decreased prevalence of cataract in various observational studies (Knekt 1992; Rouhiainen 1996; Lyle 1999).

In view of its antioxidant properties; uric acid may have potentially important and beneficial effects within on the general body oxidative stress possibly due diet low in purine intake by adults, defective gene that regulate the formation of xanthine oxidase in the liver and intestinal mucosa (defect in xanthine oxidase acting as a dehydrogenase), increased oxidative stress associated with aging.

Uric acid is derived from nucleic acid and free purine nucleotides in a complex degradation through the nucleoside intermediates of hypoxanthine and xanthine using xanthine oxidase

Xanthine oxidase acts as a dehydrogenase (it removes hydrogen from xanthine or hypoxanthine and attaches it to NAD) thereby generating NADPH.

NADPH is used for several critical reductive metabolic steps, such as the detoxification of reactive oxygen species and hydroperoxides (e.g., by the glutathione reductase/glutathione peroxidase system), AR-catalyzed reductive pathway may impose a significant strain on NADPH supply. A large drain on the NADPH pool could compromise the ability of the cell to protect itself from oxidative stress

This current study finding was consistent with Leske *et al* (2004), Lens Opacities Case-Control study; Leske found that, lens opacities were associated with lower levels of riboflavin, vitamin E, iron, and protein nutritional status. Higher uric acid levels increased risk (odds ratio, 1.74 for mixed opacities

Kałuzny J et al 1996 investigated the level of uric acid in aqueous humor of patients with cataract.The mean content of uric acid in aqueous humor of patients with cataract was 187.13umol/l and in the control group 309.34 umol/l. The difference between the groups is statistically significant. The results suggest that uric acid as strong endogenous antioxidant may play an important role in pathogenesis of cataract. as a result of its comparatively high serum concentrations,. A randomized, placebo-controlled double-blind study of the effects of systemic administration of uric acid, 1,000 mg, in healthy volunteers, compared with vitamin C, 1,000 mg observed a significant increase in serum free-radical scavenging capacity from baseline during uric acid and vitamin C infusion, using two methodologically distinct antioxidant assays. The effect of uric acid was substantially greater than that of vitamin C.

Vitamin C (ascorbic acid) is essential for normal ocular metabolism and occurs in the lens at a concentration 30-50 times higher than blood. This concentration is second only to the central nervous system and adrenal cortex. Prior to cataract formation, vitamin C concentrations significantly drop. Vitamin C provides protective benefits for the lens by: kern *et al* (1987).

Reddan, *et al.*, (1999) also reported that cataract formation is associated with a breakdown in the mechanism that regulates utilization of glutathione and vitamin C and/or decreases their concentration in the lens and surrounding structures.

An aging lens undergoes metabolic changes that predispose it to cataracts. Some metabolic changes occur from reduced oxygen and nutrient supply which increases eye vulnerability to free-radical damage.

The eye is protected by cellular antioxidants: glutathione and vitamin C.

Healthy eyes are protected from free radical damage by a mechanism that produces and recycles antioxidants in the eye that neutralize free radicals.

Glutathione (GSH) is a very small specialized protein (a tripeptide) consisting of three amino acids: glutamic acid, cysteine, and glycine. Glutathione is concentrated within the lens and is readily oxidized by damaging oxidants. Those oxidants are chemically reduced (neutralized) as glutathione is chemically oxidized in cytoplasm of cells within the lens. When glutathione levels decline in the epithelial cells (or the entire lens), cell damage and cataract formation can occur unabated.

Decreased glutathione and vitamin C are associated with cataracts. Tessier *et al* 1998 and Brubajer *et al.,* (2000) Evidence suggests a strong connection between aging and increased amounts of oxidized glutathione in the lens nucleus indicative of an imbalance between protein and lipid oxidation, and glutathione-dependent reduction.(Bova *et al* 2001 Sweezny *et al* 1998) Glutathione can benefit lens function (Reddy 1990) by: Preserving the physicochemical integrity of proteins in the lens, Sweeny *et al* (1998), maintaining action of the sodium-potassium transport pump and molecular integrity of lens fibers (protein) Sweeny *et al* (1998), maintaining molecular

integrity of lens fiber membranes and acting as a free radical scavenger to protect membranes and enzymes from oxidation, Kaluzny *et al.,* (1996), preventing free-radical-induced photochemical generation of harmful by-products , Winkler *et al.,(*1994), Reactivating oxidized vitamin C, which improves antioxidant capability in the lens, (Head ,2001).A suggested glutathione dose is 500 mg daily.

Oxidized glutathione can be regenerated (i.e., reduced) by the enzyme glutathione reductase that uses the coenzyme called reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is the cofactor derived from the dietary or supplemental B vitamin: niacin or niacinamide, also known as vitamin B3.Reddy (1990). In addition to GSH and NADPH, numerous other nonenzymatic antioxidants are present in the cells, most prominently vitamin E (α–tocopherol) and vitamin C (ascorbate). Vitamin E is a major antioxidant found in the lipid phase of membranes and, like other chemically related molecules, acts as a powerful terminator of lipid peroxidation. Under certain conditions, such as the disruption of blood flow to a tissue, xanthine dehydrogenase is converted to a ROS–producing oxidase form. The lens' oxygen concentration is lower than most parts of the body because it has no direct blood supply (Helbig et *al* 1993). The lens depends on glycolytic metabolism to produce much of the adenosine triphosphate (ATP) and reducing agents for metabolism (Winkler et al 1991

CHAPTERSIX

CONCLUSION AND RECOMMENDATION

The present study on identification and the characterization of biochemical risk factors associated with clinically established senile cataract leads to the following conclusions.

- ✓ This study provides the first report on the serum biochemical risk factors of senile cataract among Ghanaians in a case-control study design.
- ✓ About 50% of all 70-years-old suffer from a cataract requiring surgery
- ✓ More women about 62% have a medical condition of senile cataract than men 38% male with out of the 100 cases studied.
- ✓ The trend in the likelihood ratio test indicate a very strong association between increasing order of level of exposure to serum sodium, uric acid, high density lipoprotein –cholesterol , fasting blood glucose and the probability of developing the senile cataract. These biochemical risk factors should be re-examine with the knowledge that they can cause significant molecular oxidative damage resulting osmotic and oxidative stress associated with aging.
- ✓ Reductions in serum uric acid levels, low serum level of HDL–C (Dyslipidaemia), raised sodium levels and normal FBS are biochemical markers of oxidative and osmotic stress and should also be given prioritized attention in Ghanaian with senile cataract.
- ✓ This result was consistent with other study elsewhere and support the hypothesis that with the exception of confounders, cataractogenesis is connected to the aging process, associated with increased osmotic and oxidative stress, (a consequence of free radical attacks), reduced efficiency of metabolic processes and a decline in antioxidant defenses.

Future prognosis and management of senile cataract

Though, an increasing number of visually impaired and blind people gaining access to cataract surgical services due to the development of prevention of blindness

programmes in many countries, there are numerous hurdles in developing preventive measures and drug therapy that needs to be overcome. Identifying risk factors through large-scale epidemiological research and the subsequent primary prevention and elucidation of the mechanism of formation for the development of drug therapy is anticipated; however, their immediate realization is problematic. Therefore, until these goals are realized, the practical challenge would be to improve the existing surgical methods such as developing newer instruments and to ensure that there are enough ophthalmologists to respond to the increasing number of cataract patients requiring surgeries. Senile cataract is by far the most frequent form of cataract.

To assess future prognosis and management outcome of such condition, biochemical investigation need to consider especially FBS, Na+, HDL-C AND URIC ACID levels. Changes in these biochemical elements characterize the electrolyte status for elective surgery, oxidative stress and decline in antioxidant defenses system.It must be recognized that

- Diets with high Na+ content are a risk factor for age-related cataract formation.
- HDL –C, both antioxidative and anti-inflammatory activities, in addition to their better-known cardio protective role in reverse cholesterol transport play an important role in pathogenesis of cataract.
- Uric acid serve as strong endogenous antioxidant and reductions in serum uric acid levels are clinically relevant in terms of a decline in antioxidant defenses.

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APPENDIX