KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI (KNUST)

SCHOOL OF MEDICAL SCIENCE

DEPARTMENT OF MOLECULAR MEDICINE

CHARACTERIZATION OF BIOCHEMICAL RISK FACTORS OF SENILE CATARACT AMONG ADULTS VISITNG KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA.

BY

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CHARACTERIZATION OF BIOCHEMICAL RISK FACTORS OF SENILE CATARACT AMONG ADULTS VISITNG KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This work has not been submitted for any other degree.

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ABBREVIATION/ACRONYMS

ABBREVIATION	MEANING
• AGE	Advanced Glycated End-Product
• AR	Aldose Reductase
• ATP	Adenosine Triphosphate
• BMI	Body Mass Index
• CI	Confidence Interval
• CL^-	Serum Chloride Anion
• FBG	Fasting Blood Glucose
• G6P	Glucose -6-Phosphate
• GSH	Glutathione
• GSSG	Glutathione Disulfide
• HDL-C	High Density Lipoprotein Cholesterol,
• HMP	Hexose Monophosphate
• IOL	Implantation Of An Intraocular Lens
• K^+	Serum Potassium Cation,
• KM	Affinity Constant
• LDL-C	Low Density Lipoprotein Cholesterol
• LRTEST	Log Likelihood-Ratio Test
• MIP	Major Intrinsic Protein
• NA^+	Serum Sodium Cation,
• NAD	Nicotinamide Adeninedinucleotide
• NADP	Nicotinamide-Adenine Dinucleotide Phosphate
• NADH	Reduced Nicotinamide-Adenine Dinucleotide
• ORs	Odd Ratios
• PKC	Protein Kinase C Isoforms
• PSC	Posterior Subcapsular Cataract
• ROS	Reactive Oxygen Species
• SDH	Sorbitol Dehydrogenase
• TC	Total Cholesterol
• TG	Triglycerides
• VA	Visual Acuity
• VLDL	Very Low Density Lipoprotein
• WHO	World Health Organization

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. 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 and characterized the biochemical risk factors for senile cataract among Ghanaian adults and to elucidate the association between serum biochemical indices and senile cataract for future cataract prognosis, screening program and to develop appropriate preventive strategies.

This was a case-control study of outpatients attending eye clinic department of the Komfo Anokye Teaching Hospital (KATH) between February 2009 and July 2010. A total of 200 outpatients above 40 years comprising 100 cases (clinically newly diagnosed adult cataract patients) and 100 controls (patients who are clinically without the condition) were sampled for the study. Laboratory test values in cases and controls were compared and expressed as odds ratio and 95% confidence interval.

The study found mean concentration of fasting blood glucose (4.92±2.09 mmol/L) of the cases was lower than the control grouping but within the normal reference ranges and was associated with senile cataract (ORs (95% CI) 1.3; 1.O6-1.49; p=0.008).

There was elevated serum Na^+ level in those suffering from senile cataract (143.2±6.76 mmol/L) than the controls (139.3±1.96; p=0.0000). It was significant factor associated with the senile cataract (ORs (95% CI) 0 6 95% CI (0.47-0.72; p=0.000)).

The mean serum K^+ level (4.21±0.50 mmol/L) of senile cataract patients was lower than the controls (4.38±0.45) and also associated with senile cataract with ORs (95% CI) 2.48 (1.47-0.72; p=0.010).

Persons with low HDL C abnormalities have ORs (95% CI) 3.17 (1.79-5.61; p=0.000) and were significantly associated with senile cataract among the study population.

The mean uric acid concentrations level was (210.0±113.8μmol L-1) lower in the cases compared with (311.1±117μmol L-1) controls and was significantly associated with senile cataract among the study population (ORs (95% CI) 1.01 (1.01-1.02) P=0.000).

After adjusting for potential confounders, fasting blood glucose (ORs (95% CI) 1.O6-1.49 ;p=0.008), sodium (Na^+) ORs (95% CI) 0 6 (0.47-0.72; p=0.000), potassium (K^+) ORs (95% CI) 2.48 (1.47-0.72; p=0.010), triglyceride (TG) ORs (95% CI) 0.58 (0.35-0.98) p=0.040), uric acid (UA) ORs (95% CI) 1.01 (1.01-1.02) P=0.000) and high density lipoprotein-Cholesterol (HDL-c) ORs (95% CI) 3.17 (1.79-5.61; p=0.000) were identify and characterized as a significantly human biochemical indices associated with development of senile cataract among the study population.

In log Likelihood-ratio test (LRTEST) controlling for age and sex, elevated serum Na+ level in those suffering from senile cataract was significant factor associated with senile cataract ORs (95% CI) 0.60 (0.45 - 0.80), P=0.001). Persons with lower serum potassium (K^+) ORs (95% CI) 2.48 (1.47-0.72; p=0.010) are twice likely to develop senile cataract. Persons with low HDL CHOL abnormalities were twice likely to develop senile cataract (ORs (95% CI), 2.52 p=0.095). The trend LRTEST indicated a very strong association between increasing order of level of exposure to (Na^+), HDL-c, UA, FBG and the probability of developing the senile cataract.

The studies clearly established a half (50%) of any study population with the age of ≥70 years especially women are most likely to develop senile cataract. This study strongly suggests an association between serum biochemical indices and senile cataract. The association exist between low levels of High Density Lipoprotein-cholesterol (HDL) , low levels uric acid, Fasting blood Glucose(FBG), Serum sodium (Na+), serum potassium (K+), Triglyceride (TG) and Senile cataract.

Our findings add to an evolving biochemical and laboratory animal based hypothesis that changes observed in the blood level constituent raised or lowered can be used for diagnostic and prognostic purposes and their detection makes up a large part of routine clinical chemistry especially in an elective cataract surgery procedure. It is essential to have access to the biochemical picture of such a patient prior to, during and immediately after surgery in order to effectively treat adverse reactions and that further studies may be necessary to ascertain whether or not certain dietary habit has a part to play in the aetiogenesis of Senile Cataract in various regions of Ghana.

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PROBLEM STATEMENT

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).

It is the leading cause of avoidable blindness in Ghana. Senile cataract affects mainly the elderly reducing functional capacity and quality of life and increasing number of adults will be at risk of blindness as population grow and age.

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. Estimates are that if cataract onset could be delayed by 10 years, the number of cataract surgeries could be reduced annually by 45%.

As a global and national burden, identifying biochemical modifiable systemic markers associated with senile cataract may help target higher risk populations for screening program and develop appropriate preventive strategies.

STATEMENT OF HYPOTHESIS

With the exception of confounders of age, sex and any other systemic disease, the biochemical elements of established senile cataract will not significantly affect clinical dysfunction associated with cataract. This hypothesis is tested by investigating the association between serum electrolyte levels, fasting blood glucose, uric acids, and serum lipid profile that might indicate dysfunctions associated with cataract

PROBLEM ANALYSIS DIAGRAM

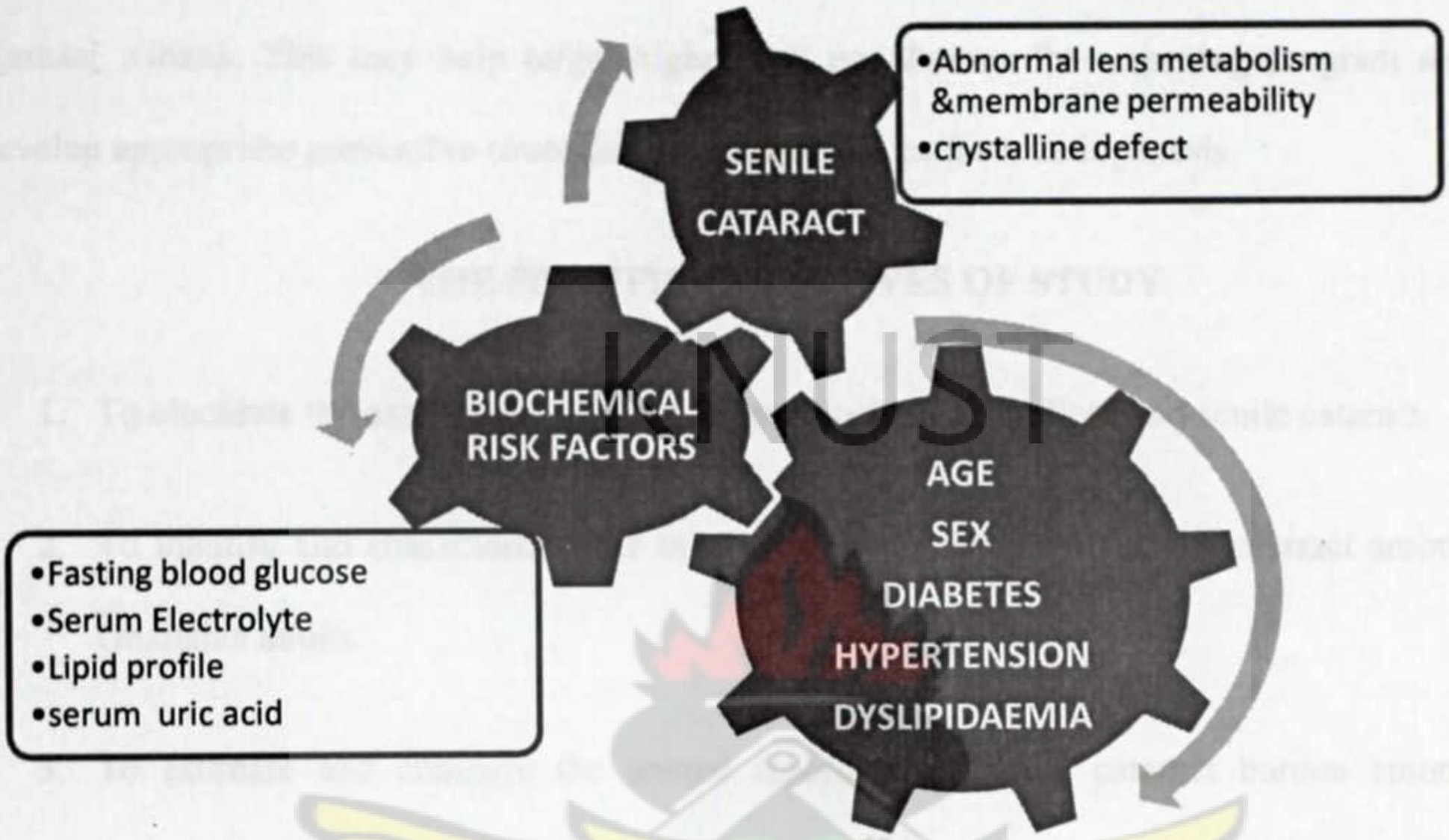

Figure 1 Different pathophysiological features of senile cataract

AIM OF THE STUDY

The general aim of the research was to identify the biochemical risk factor of senile cataract linking 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, Kumasi ,Ghana. This may help target higher risk populations for screening program and develop appropriate preventive strategies associated with cataract aetiogenesis.

THE SPECIFIC OBJECTIVES OF STUDY

1. To elucidate the association between serum biochemical indices and senile cataract.
2. To identify and characterized the biochemical risk factors for senile cataract among Ghanaian adults.
3. To estimate and compare the annual reportage of senile cataract burden among outpatient visiting KATH eye clinic.
4. To provide a database on cataract prognosis and biochemical indices on senile cataract for future clinical research and development reference.

JUSTIFICATION OF THE STUDY

The opacity of the lens due to age is a major public health problem which if not detected and treated early could lead to avoidable blindness and other effects including reduced functional status, social interaction and quality of life, depression and falls.

In Ghana, cataract formation is more commonly observed among adult subjects. The elderly suffer disproportionately with a loss of vision from eye diseases. Unreported data from Komfo Anokye Teaching Hospital (KATH) in the year 2009, the annual eye clinical report indicated a total of 23783 attendances with 3,534 newly diagnosed cases of cataract and 699 cataract surgeries were done.

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. Development of nonsurgical procedures requires a much deeper understanding of 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 and obesity.

However, there are few reports on the biochemical risk factors of senile cataract elsewhere and to date no study report exist on its prognosis and the biochemical indices for future clinical research and development among Ghanaian patients. This was pursued by investigating the association between serum electrolyte levels, fasting blood glucose, uric acids, and serum lipid profile that might indicate dysfunction associated with senile cataract.

CHAPTER 1

1. 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

A simple definition of blindness refers to loss of vision resulting in a person being unable to walk unaided. '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. '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. Visual impairment' includes both low vision and blindness. (Vision 2020) The indicators of the normal eyes and blind eye in Ghana are as follows:

- ✓ 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;

- ✓ Severe bilateral blindness = counting fingers < 3 meters in both eyes (< 6/120) WHO, (1997).

1.1.1 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).

1.2 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).

1.2.1 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.

1.2.2 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 and Shun -shin (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	Age	Anterior verities
		Congenital			
		Acquired			
	Mature	Subcapsular	Traumatic	Infrared irradiation	Hereditary disorders
		Anterior			
				Ionization irradiation	
		Posterior			
	Hypermature	Nuclear	Metabolic	Diabetes	
		Congenital		Galactosemia	
		Senile		Fabry's disease	
	Morganian	Cortical	Toxic	Corticosteroid chlorpromazine	
		congenital			
		Senile			
		Lamellar			

1.2.3 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). Senile cataract 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 dilation with 1.0% tropicamide and 2.5% Phenylephrine hydrochloride reagents.

1.2.4 TREATMENT OPTIONS OF CATARACT

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. The cure for cataract is surgery. However, surgery is not equally available to all, and where it is available it does not produce equal outcomes. Surgery 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.

1.2.5 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.

1.2.5.1 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 accounted 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) provided 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 for long-term (Cumming, 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 suggested 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 consistent with those from population-based studies (Heiba 1995; McCarty 1999). However, 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.

1.2.5.2 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 (Jacques' *et al.,* 1988).

A strong preposition of the cause of nuclear cataract in humans is the effect of sunlight. *In vitro* experiments 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 resulted in elevated blood urea levels leading to carbamylation of lens proteins (Harding *et al.*, 1991) causing unfolding of proteins leading to cataract

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

1.2.5.3 EPIDEMIOLOGY OF CATARACT

According to World Health Organization (WHO), cataract is the leading cause of reversible blindness and visual impairments in more than 17million of the 37 million blind individuals worldwide, and this number is projected to reach 40 million by 2020 (WHO, 2005).

In Africa, the prevalence of blindness (1.7%) and prevalence of cataract is 0.6%. Cataract is seen primarily in adults, and the incidence grows rapidly after age 50, affecting 50% of individuals between 65 to 74 and 70% of individuals age 75 and older (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, 2002). In the Beaver Dam Eye Study (BDES), the prevalence of cataract increases with age. BDES reported that 38. 8 % of men and 45.9% of women older than 75 years had visually significant cataract (Klein 1998)

In Ghana, cataract formation is more commonly observed among adult subjects. Unreported data from Komfo Anokye Teaching Hospital (KATH) in the year 2009, the annual eye clinical report indicated a total of 23783 attendances with 3,534 newly diagnosed cases of cataract and 699 cataract surgeries were done fewer than expected to achieve annually in Kumasi, Ghana.

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

CHAPTER 2

LITERATURE REVIEW

2. DISEASES OF THE EYE LENS

Disease of the lens occurs when there are changes of aqueous humour component; osmotic character of lens capsule, metabolic disturbance caused by various factors for example diabetes mellitus and trauma resulting in lens protein degeneration, and the transparent lens becoming opaque. Decreased lens transparency results in increased light scattering as light passes through the lens and then to the retina where the diminished focus of light impairs vision.

The opacification of the 'crystallin' lens in the human eye is called cataract (Chitkara et al; 2004). Cataract is the most common cause of visual loss in humans (Vijaya, Gupta *et al.* 1997) and is considered clinically significant when opacification interferes with visual function and associated with some degree of visual impairment (West S. K *et al.,* 1995). Cataract adversely impacts vision by light absorption in a less transparent lens. The mechanism by which the lens becomes opaque is still unknown, but is undoubtedly due to the age-dependent changes that affect several tissues including the lens of the eye. The proposed underlying mechanism for cataracts involves: disruption of the structure of the lens fiber cells, increases in protein aggregation, or cytoplasm dysfunction in the lens cell (Harding J *et al;* 1991). 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).

2.1 SENILE CATARACT

The commonest form of cataract occurs mainly in the 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 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.

2.1.1 TYPES OF SENILE CATARACT

There are three types of senile cataract: nuclear cataract, cortical cataract and posterior subcapsular cataracts. Disease progression in all these 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).

2.1.1.1 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, 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.

2.1.1.2 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.

2.1.1.3 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.

2.2 STRUCTURE AND COMPOSITION OF CRYSTALLIN LENS

The crystallin 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). The 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 crystallin (lens proteins). Crystallin 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, deamination, 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- crystallin (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 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 two (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.

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

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 Beta gamma crystallins (BCSC Section 11 2007-2008).

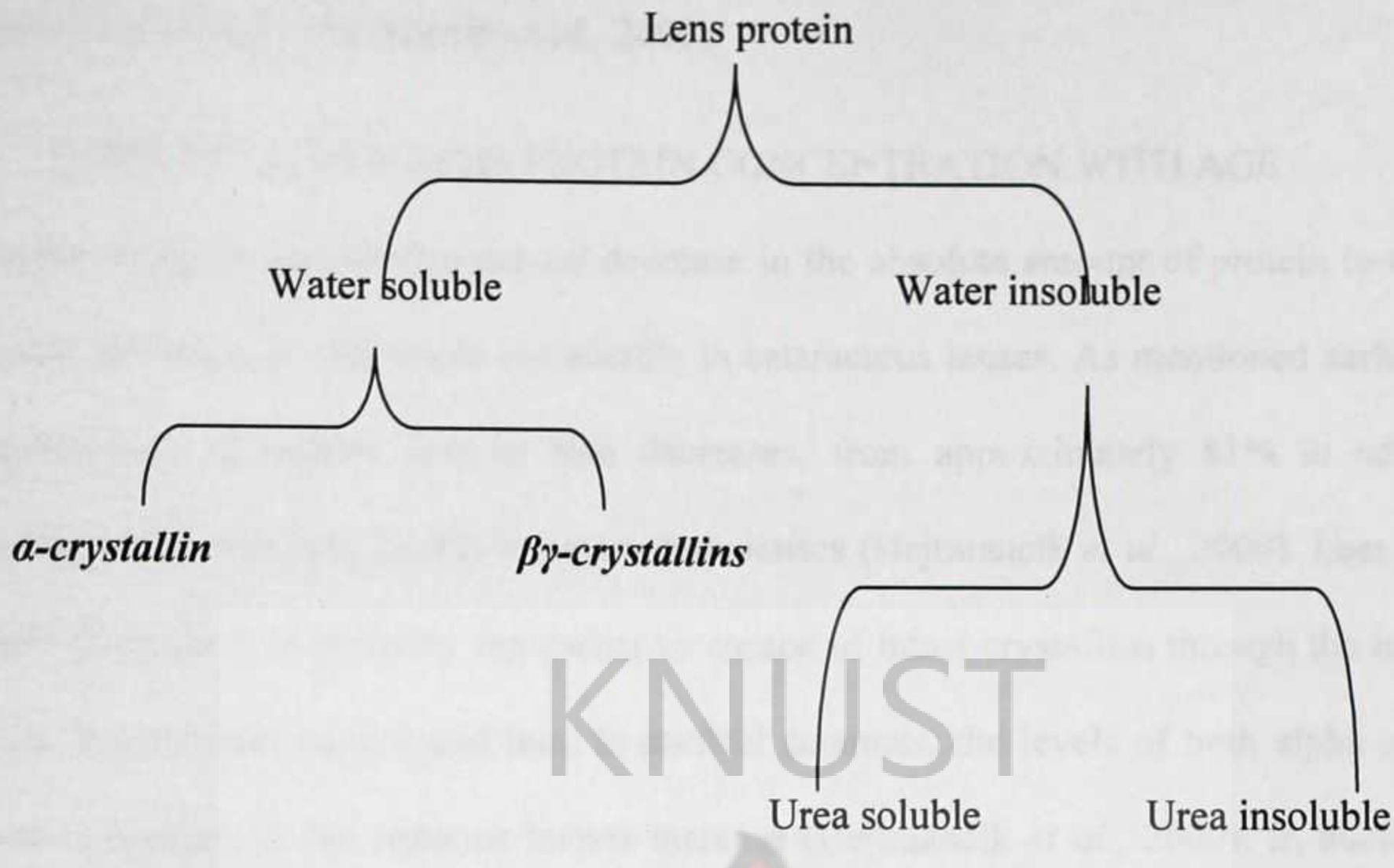

Figure 2, Structure and solubility characteristics of lens protein (BCSC, lens and cataract 2007-2008).

The lens protein can be separated into two fractions: soluble and insoluble. 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. The protein 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. The

MIP is the inherent part of the membrane, where it has been localized by immunofluorescence (Hejtmancik *et al.,* 2000).

2.4 ASSOCIATION OF 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 (Hejtmancik *et al.,* 2000). 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 crystallins in the aqueous humor increase (Hejtmancik *et al.,* 2000). In nuclear cataracts, the level of alpha crystallins increases, whereas that of gamma crystallins decreases (Hejtmancik *et al.,* 2000).

2.5 ENDOCRINOLOGY AND BIOCHEMISTRY OF EYE LENS

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). Glycolysis is the process by which sugars (like glucose) are metabolized to produce the energy currency of the body, adenosine triphosphate (ATP). When glycolysis occurs in differentiated lens fiber cells deep within the lens, the absence of oxygen (anaerobic glycolysis) only allows 10% of the energy available to be conserved. The glucose comes from the aqueous humor, the fluid sac between the lens and cornea. Energy from glucose is derived from (aerobic) oxidative pathways in superficial lens fiber cells and epithelial cells containing mitochondria. In animal studies, 50% of the ATP produced by epithelial cells came

from oxidative metabolism and glycolysis accounted for almost all ATP produced in most lens fiber cells (Winkler *et al*., 1991)

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-phosphate (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 the 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 phosphofructokinase, 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). As a result 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, 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. HMP 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. lens and cataract, 2007-2008).

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 adeninedinucleotide (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 channel through the polyol pathway. The accumulation of sorbitol causes lens damage (Kinoshita *et al.,* 1979).

The affinity constant Km for aldose reductase is about 700 times that for hexokinase. Because the affinity is actually the inverse of Km, 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 Km), meaning that considerable sorbitol will accumulate before being further metabolized. This characteristic result 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 drop systematically to one eye, decrease the rate of onset and severity of sugar cataracts in experimental studies (BCSC. fundamentals &principles of ophthalmology, 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 referring 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 into the anterior lens via the epithelium. They then diffuse out along 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, lens & cataract; 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 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 (BCSC, lens & cataract; 2007-2008).

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. Ascorbic acid is in the lens and surrounding ocular tissues in substantial quantities. Dehydroascorbate (the oxidized form of ascorbic acid) can enter the lens through a glucose transporter (Tsukaguchi *et al.*, 1999).

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 the pupil (Cole, 1977).

The aqueous humour is produced from blood secretions. Serum electrolytes concentration directly affects electrolytes of aqueous humour 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 epithelia 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 (Kałuzny *et al.,* 1996).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 serves as a strong endogenous antioxidant play an important role in pathogenesis of cataract (Waring *et al.,* 2001).

2.8 PATHOPHYSIOLOGY OF SENILE CATARACT AND ITS ASSOCIATED DISORDERS

Cataractogenesis is a highly complex and multifactorial process. Theories of cataractogenesis generally recognize different pathophysiological features for the senile cataract. 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. 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).

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).

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).

Lower total hydration of the lens may be associated with cataract as the human lens ages. The occurrences of severe diarrhoea and dehydration have been suggested by some studies as a risk factor for senile cataract (Minassian *et al.,* 1989).

2.9 CONTRIBUTION OF DIABETES MELLITUS TO 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 mechanisms 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.

2.9.1 INVOLVEMENT OF POLYOL PATHWAY IN CATARACTOGENESIS

A number of causes 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, aldose Reductase (AR)-catalyzed reduction represents less than 3% of total glucose utilization, whereas in the presence of high glucose, more than 30% of the glucose is used (Gonzalez *et al.,* 1984).

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^+ ATPase pump and lens membrane permeability. Alterations in either of

these ions lead to cation imbalance in lens which in turn results in 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 noticed significant and meaningful difference between serums Na^+ of those suffering from age-related cataract versus those suffering not from age-related. 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.

2.10 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).

2.11 DYSLIPIDAEMIA AND CATARACT FORMATION

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 <1.04 mmol/ L by (Friedewald *et al.*, 1997).

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 concordantly related to posterior subcapsular cataract (PSC) in men (Klein *et al* 1994). The National Cholesterol Education Program Adult Treatment Panel III Approach to Dyslipidemias identifies HDL-cholesterol (mmol/L) < 40 (< 1.04) Low ≥ 60 (≥ 1.55) High. A more practical system categorizes dyslipidemias 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 hyperlipidemias). This system does not take into account specific lipoprotein abnormalities (e.g. low HDL or high LDL) that may contribute to disease despite normal cholesterol and TG levels. Secondary causes contribute to most cases of dyslipidemia 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)

Although hypertriglyceridemia was found in the Framingham study to predict incident PSC prospectively during follow-up in men only and patients with PSC are reported to have significantly higher plasma triglyceride concentrations than patients with nuclear or cortical cataract, PSC is by far the less frequent type of opacity, representing only 5% of cases.(Hiller *et al* 2003).

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 by Meyer *et al.,* (2003) in their study in South Africa study. An extremely strong association ($p<0.0001$) was found to exist between HDL cholesterol levels and the development of lens opacities.

2.12 OXIDATIVE DAMAGE AND PROTECTIVE MECHANISMS IN CATARACTOGENESIS

Free-radical action is directly linked to cataracts and is a major cause of damage to eyes and cataract formation (Christen *et al.,* 1992).

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 lens is equipped with several enzymes that protect it 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.

2.13 HYDROGEN PEROXIDE AND CATARACT

Cataract formation is initiated by the free radical hydrogen peroxide found in the aqueous humor (Garner & Davies *et al.*, 2000). Hydrogen peroxide oxidizes glutathione, or conversely, glutathione chemically reduces hydrogen peroxide, ultimately damaging the energy-producing system of the eye and allowing sodium to leak into the lens. Excess sodium attracts water to maintain osmolality, which initiates the edema phase of a cataract. Normal body heat in the lens catalyzes oxidation of the lens' proteins, which become opaque and insoluble (similar to the process by which egg protein changes from clear to opaque upon cooking). Free radicals break down fatty acids in membranes and lens protein fibers, generating more free radicals. This cross-links (or denatures or breaks down) the laminate-like structural proteins inside the lens capsule. The lens capsule can swell or shrink (dehydrate) and these changes in pressure breaks lens fiber membranes, forming microscopic spaces that trap water and debris (Bantseev & Bhardwaj R *et al* 1997).

$$20^{2-} + 2H^{+} \longrightarrow H_2O_2. + O_2$$

Catalase may break down the peroxide by the reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Gluthathione peroxidase catalyzes the reaction:

$$2GSH + LOOH \longrightarrow GSSG + LOH + H_2O$$

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:

$$GSSG + NADPH + H^{+} \longrightarrow 2GSH + NADP^{+}$$

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.

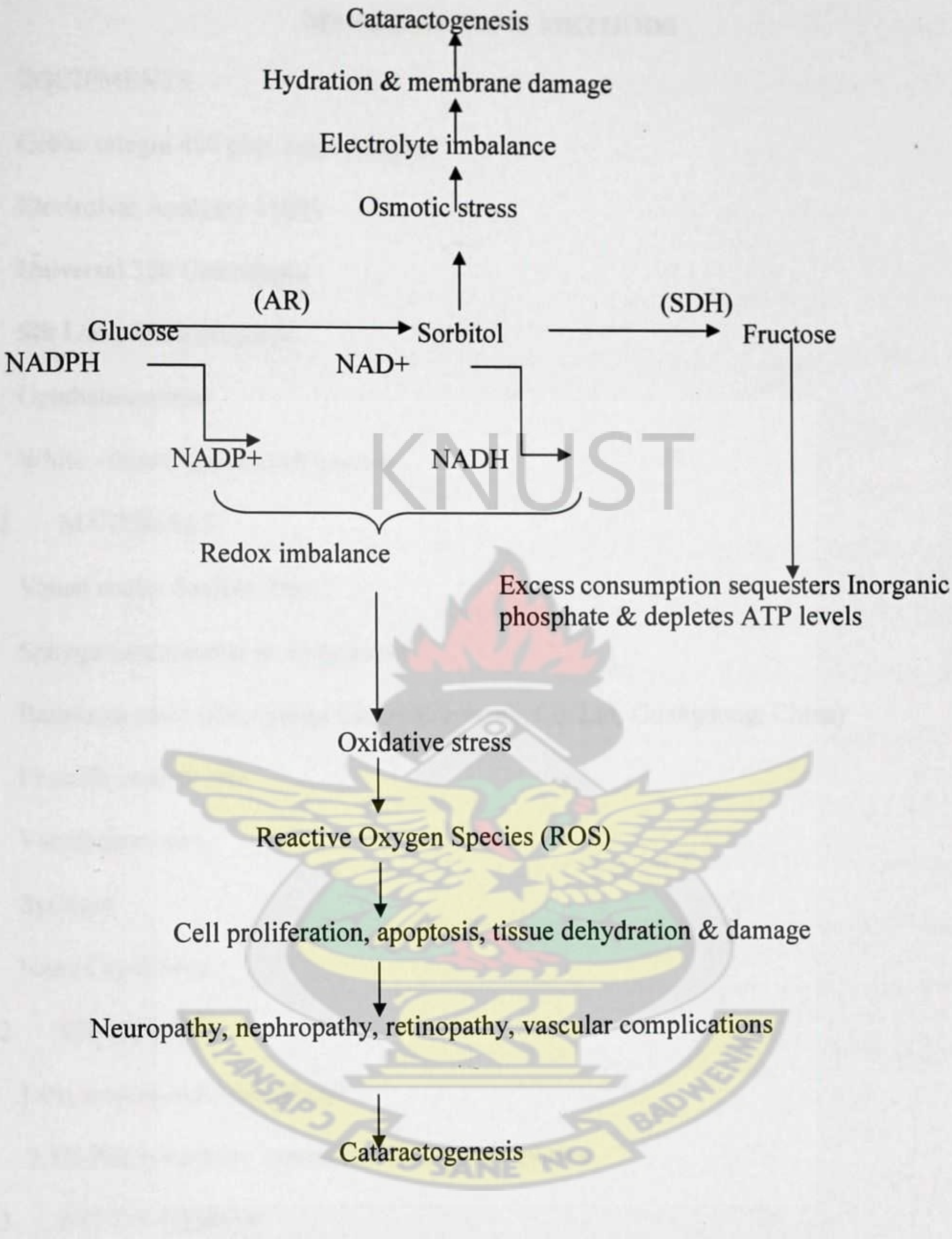

Figure 3 Involvement of polyol pathway in diabetic complications: Conversion glucose to fructose, NADPH and NAD^+ are consumed as cofactors for the enzymes aldose reductase (AR), Sorbitol dehydrogenase (SDH) (Verma *et al.*1977 & Kinoshita *et al.* 1981)

CHAPTER 3

MATERIALS AND METHODS

1. EQUIPMENTS

Cobas Integra 400 plus Auto-analyzer

Electrolyte Analyzer 9180x

Universal 320 Centrifuges

Slit Lamp biomicroscope

Ophthalmoscope

White –Westinghouse refrigerator

1.1 MATERIALS

Visual acuity Snellen chart

Sphygmomanometer & stethoscope

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

Fluoride oxalate tube

Vacutainers tube

Syringes

Nunc CryoTubes

1.2 REAGENTS

1.0% tropicamide (Mydriacy)

2.5% Phenylephrine hydrochloride (Neosynephrine

1.3 STUDY DESIGN

Case control study design was used in this study. The study population consisted of two hundred (200) participants between the ages of 40 years and 80 years, 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 ethical committee of the Komfo Anokye Teaching Hospital;and Kwame Nkrumah University of Science and Technology ; School of Medical Sciences, Kumasi, Ghana.

1.4 PARTICIPANT (SUBJECT) SELECTION

Outpatients visiting the eye clinic were selected on the basis of presence of cataract in one or both eyes as (cases). For each patient (case), a matched healthy eye (normal eye) without cataract was considered for the study as controls. The diagnosis was made with ocular examination using slit-lamp biomicroscopy after dilatation with 1.0% tropicamide 2.5% phenylephrine hydrochloride 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 between 40 – 80 years 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 between 40-80 years with visual acuity not worse than 6/9 with no correction in both eyes, neither operated and no significant evidence of cataract in one or both eyes.

1.5 DATA COLLECTION AND SUBJECT CONSENT

Participants were contacted at the eye clinic. The objectives and the importance of the project were explained to the people both in the local dialect and the English language. The outpatients were given the opportunity to ask questions for clarification. If they give consent qualified participant were given the consent form, and explain as to how to fill it. Approved questionnaire were administered to the

qualified participants. Anthropometric measurement as well as blood pressure was determined by qualified nurses. Visual acuity and other diagnosis of senile cataract status were done by 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 formation through questionnaire. Participants were asked to come for laboratory investigation the next day after they had fasted 12hrs over night.

1.6 ANTHROPOMETRIC MEASUREMENT

Anthropometric measurements included height to the nearest centimeter without shoes were measured against a wall-mounted ruler. Qualified participants were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler.

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 sitted for >5 minute in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mm Hg.

1.7 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 and other diagnosis of senile cataract status were done by specialist ophthalmologist.The Snellen eye chart was used, with rows of letters decreasing in size to determine how clearly a person can actually see.

1.8 SAMPLE COLLECTION AND PREPARATION

1.8.1 BIOCHEMICAL ANALYSIS

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 an over-night fast (10-16 hours). After centrifugation at 5000rpm for 15 minutes, the serum and plasma were stored at - 80°C until assayed.

Serum biochemistry was performed with Cobas Integra 400 plus Auto-analyzer (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined include: fasting blood glucose (FBG), 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.

1.8.2 FASTING BLOOD GLUCOSE

Glucose concentration in the samples was estimated using 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 by the reaction shown below. The resulting increase in absorbance at 340nm is directly proportional to the concentration of glucose in the sample.

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

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

1.8.3 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. 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.This relationship is described by the Nernst equation:

$$\mathbf{E = E^0 + (2.303RT/\ nF)\ x\ Log\ (a)}$$

Where

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

- E^0 = 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.

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.

1.8.4 FASTING LIPID PROFILE DETERMINATION

1.8.4.1 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 hydrogen peroxide in the presence of phenol and peroxidase Oxidizes 4-aminophenazone to quinoneimine chromogen and measured at 500 nm. The intensity of the final red

colour is directly proportional to the total cholesterol concentration. The reaction to the determination of cholesterol is shown below.

$$\boldsymbol{Cholesterol + H_2O \xrightarrow{cholesterol\ esterase} Cholesterol + Fatty\ acids}$$

$$\boldsymbol{Cholesterol + O_2 \xrightarrow{cholesterol\ oxidase} Cholest-4-en-3-one + H_2O_2}$$

$$\boldsymbol{H_2O_2 + 4-aminophenazone + Phenol \xrightarrow{peroxidase} H_2O + Quinoneimine}$$

1.8.4.2 TRIGLYCERIDES

The method for this assay is based on a modified Trinder (Barham *et al.*, 1972) colour reaction to produce a fast linear endpoint reaction (McGowan *et al.*, 1983). Triglycerides 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 (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye which is measured at 750nm. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample. The reaction to the determination of triglycerides is shown below

$$\boldsymbol{Triglyceride + H_2O \xrightarrow{lipase} Glycerol + Fatty\ acids}$$

$$\boldsymbol{Glycerol + ATP \xrightarrow{glycerol\ kinase} Glyerol-3-phosphate + ADP}$$

$$Glycerol-3-phosphate \xrightarrow{glycerophosphate\ oxidase} DHAP + H_2O_2$$

$$2H_2O_2 + 4-aminoantipyrine + chlorophenol \xrightarrow{peroxidase} Quinoneimine + 2H_2O$$

1.8.4.3 HDL –CHOLESTEROL

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg^{2+} ions. The cholesterol concentration in the HDL is then determined based on that described by Trinder, (1969).

1.8.4.4 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). The reaction to the determination of LDL-Cholesterol is shown below.

$$LDL-Cholesterol(mmol\ L^{-1})$$

$$= TC(mmol\ L^{-1}) - \frac{TG(mmol\ L^{-1})}{2.2} - HDL(mmol\ L^{-1})$$

1.8.4.5 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 (Lopes-Virella et al., 1977).

1.8.4.6 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. The reaction to the determination of uric acid is shown below.

$$Uric\ acid + O_2 + 2H_2O_2 \xrightarrow{uricase} Allantoin + CO_2 + H_2O_2$$

$$2H_2O_2 + chlorophenol\ sulphonic\ acid + 4AP \xrightarrow{peroxidase} N-(4-antipyryl)-3-chloro-5-sulphonate-p-benzo-quinoneimin$$

1.9 STATISTICAL ANALYSIS

Results are presented as Means ± SEM. 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 odd ratios (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 4

RESULTS

4. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

The general characteristics of the study population stratified by cases and controls are shown in Table 4.1a, b, and c. The incident of senile cataract for women was higher than men. There were 62% female and 38%male with senile cataract. out of the 100 cases studied, there were more females over 71 years presenting with senile cataract than men showing 33(53.23%) and 17(4.74%) respectively (table 4.1a).In the 40 and 70 years age group, the burden increases from 8%, 20%, 22%, and 50% for the various age groups respectively in the patient with cataract (Table 4.1a.).

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} ; $p < 0.0001$(table 4.1b.) but was within 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) according to WHO recommendations.

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) (table 4.1b.). 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 (table 4.1c.). 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 in Table 4d.

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

VARIABLES	CASES (N=100)			CONTROLS (N=100)		
AGE /SEX	**Female N: (%)**	**Male N: (%)**	**Total**	**Female N: (%)**	**Male N: (%)**	**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 (20%)	16 (26.23%)	8 (20.5%)	24 (24%)
61-70	11 (17.74)	11 (28.95%)	22 (22%)	8 (13.11%)	15 (38.46%)	23 (23%)
>71	33 (53.23%)	17 (44.74%)	50 (50%)	29 (47.54%)	12 (30.77%)	41 (41%)
TOTALS	62	38	100	61	39	100

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

VARIABLES	CASES	CONTROL	P value
	Mean ± SD	Means ± SD	
***AGE (years)**	67.58±1.31	62±0.79	**0.0003**
***BMI (Kg/m^2**	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**

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

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

KNOWN DISEASE (%)	CASES	CONTROLS	P-value
Diabetes	9	1	
	(9%)	(1.01%)	0.0000
Hypertension	19	4	
	(19%)	(4.04%)	0.0000
Gout	5	0	
	(5%)	(0%)	0.0000
Unknown	67	94	
	(67%)	(94.95%)	0.0000

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

4.1 BIOCHEMICAL CHARACTERISTICS 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 (Rf). Comparing the serum electrolyte levels, there was a statistically significant difference between the mean serum Na^+ level (Rf=135-145mmol/l) in senile cataract patients (143.2±6.76) and normal individuals (139.3±1.96; p=0.0000).

The mean serum K+ level Rf=3.5-5.5mmol/l) of senile cataract patients and normal individual's was 4.21±0.50 and 4.38±0.45; p=0.9965 respectively. There was no significant difference between mean serum Cl^- level (Rf =97-110mmol/l) of cases and control (105.5±3.65 and 105.2±2.35 respectively).

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 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 higher 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 with (311.1±117 µmol L-1) controls (p=1.000). The mean concentration of fasting blood glucose (Rf=3.6-6.4). of the cataract patients (4.92±2.09 mmol L-1) was also lower than the control group (6.01±2.96 mmol L-1, p= 0.9986).

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

VARIABLES	CASES		CONTROL		P value	Normal value 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.

4.2 BIOCHEMICAL VARIABLES ASSOCIATED WITH SENILE CATARACT

Table 4.3 represents 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) mmol/l, ORs at 95%CI were (1.3 (1.O6-1.49 ;p=0.008)), sodium (Na^+) mmol /L 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) 3.17 (1.79-5.61; p=0.000) were significantly 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) 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) 0.88 (0.67-1.15; p= 0.363 and very low density lipoprotein VLDL (mmol/L) 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 sugar(FBS)	1.25(1.O6-1.49)	0.008
Sodium(Na^+)(mmol l-1)	0.58(0.47-0.72)	0.000
Potassium K^+ (mmol l-1)	2.48(1.47-4.72)	0.010
Chloride Cl^- (mmol L^{-1})	0.95(0.87-1.05	0.313
TC (mmol L-1)	1.24(0.95-1.62)	0.115
TG (mmol L-1)	0.58(0.35-0.98)	0.040
HDL –C(mmol L-1)	3.17(1.79-5.61)	0.000
LDL-C(mmol L-1)	0.88(0.67-1.15)	0.363
VLDL (mmol L-1)	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 ratios (ORs) and 95% confidence intervals (CIs) P value defines them level of significance when study population with cataract was compared with 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.

4.3 SERUM BIOCHEMICAL DETERMINANTS OF SENILE CATARACT

The significant and essential biochemical variables related to individual risk of senile cataract are presented in Table 4.4. The trend in the likelihood ratio test indicated a very strong association between serum biochemical elements and the probability of developing the senile cataract in this study. Serum fasting blood glucose (FBG), sodium (Na^+), potassium (K^+), high density lipoprotein-cholesterol (HDL – C) and uric acid (UA) were associated with senile cataract.

The odds ratios for fasting blood glucose and uric acids were under unity (FBG, 1.20; p=0.106) and (UA, 1.01 p=0.011) respectively.

Persons with HDL C abnormalities were twice as likely to develop senile cataract (odds ratio, 2.52 p=0.095).

Exposure to sodium in the absence of other biochemical elements remained the most significant factor associated with senile cataract. ORs (95% CI) 0.60 (0.45 - 0.80), 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	(0.45-0.80)	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 The results are presented as odd ratio (ORs) at 95% confidence intervals (CIs), from log Likelihood-ratio test analysis

CHAPTER 5

DISCUSSION

5. AGE RELATED BURDEN OF SENILE CATARACT

The studies clearly showed that older people aged between 40 -70 years especially women are most likely to develop senile cataract Increasing age increases the burden of suffering for 10 years age interval from 40 via 70 years age groupings (Table 4.1a). This may be due to poor nutritional status and illness of older people as well as physiological, psychological and social changes associate with aging which affect food intake body weight. 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, 2002).

It has been suggested that the incidence of senile cataract rises exponentially with the age after 50 years (Kahn *et al,* 1977). In gender, Weale (1981) reported that, the relative susceptibility to cataract change with age, but so does their response to physical trauma. 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.

5.1 FASTING BLOOD GLUCOSE (FBG) AND SENILE CATARACT

The study established an association between serum fasting blood glucose and senile cataract though the mean concentration of fasting blood glucose of the cataract patients was lower than the control group but within the normal reference ranges

(Table4.3). Conversely, Patterson (1956) reported that there was lenticular opacity when the blood sugar was more than 225 mg/l. The fasting blood glucose was higher in the cataract group than in the control group. Donnelly *et al.,* (2004) in a study on some blood plasma constituents correlate with human cataract also reported that the fasting blood glucose (FBG) level was higher in the cataract group than in the control group. This opposite to our current findings but clearly indicate that glucose play an important role in the biochemical mechanism of cataract formation.

Our findings add to an evolving biochemical and laboratory animal based hypothesis that the lens depends on glycolytic metabolism to produce much of the adenosine triphosphate (ATP) and reducing agents for metabolism. (Winkler *et al*., 1991) The glucose comes from the aqueous humor, the fluid sac between the lens and cornea Energy from glucose is derived from oxidative pathways in superficial lens fiber cells and epithelial cells containing mitochondria. In the presence of normal glucose, Aldose Reductase (AR)-catalyzed reduction represents less than 3% of total glucose utilization, whereas in the presence of high glucose more than 30% of the glucose is used by AR. 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) 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. The accumulation of sorbitol has been shown to cause lens damage, osmotic imbalance and changes in membrane permeability leading to lens opacity (Verma *et al.,* 1977; Kinoshita *et al.,* 1981). 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.

5.2 SERUM SODIUM AND SENILE CATARACT

The most important result of this study is elevated serum Na^+ level in those suffering from senile cataract and persons with lower serum potassium are twice likely to develop senile cataract (table 4.4) consistent with the studies by Donnelley *et al* (1995). Research has demonstrated that Lens has high content of potassium and low content of sodium. Lens K^+ level is 125 mmol/kg of lens water and lens Na^+ is 14-26 mmol/kg of lens water (Delamere *et al*., 2001) These two cations are in balance with each other, which is mainly due to Na+-k+ ATPase pump and lens membrane permeability. Na+ pump activity in lens is as in other cells and it is related to intracellular Na+, extracellular K+ and eventually to serum concentrations of these ions.

Our findings in table 4.3 add to the pump-leak of hypothesis of pathways of solute movement in the lens. (Delamere *et al.,* 2001 and BCSC Section 11 lens and cataract, 2007-2008) Changes in serum electrolytes levels can induce changes in aqueous electrolytes levels and effect on lens metabolism and probably cataract formation. Hence alteration in cation concentration of aqueous humor which is attributed to

alterations in serum cation concentration can be known as a risk factor for cataract formation (Clayton *et al.*, 1990 & Philips *et al.*, 1980).

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 and cataract have been verified (Nourmohammadi *et al.,* 2001). In spite of this, in other studies, such as the Italian American cataract study, (1993), no relation between blood biochemical elements and cataract has been shown. This contrast may be due to nutrition quality and different diets in nations all over the world (Clayton *et al.,* 1982, Philips *et al* 1980 and Shoepheld *et al.,* 1993).

At last, it seems that diets with high Na+ contents leads to high level of serum Na+, which in turn contributes to formation of age-related cataract.

5.3 LIPOPROTEIN DISORDERS AND THE RISK OF SENILE CATARACT

Although many factors play a part in cataractogenesis, there is considerable evidence that abnormalities in serum lipids and lipids metabolism are important risk factors for the increased incidence of senile cataracts among older persons in the study population (Table 4.3). In most mammalian organs which have been investigated with the objective of membrane characterization, the results have demonstrated an association between cholesterol and phospholipids for the lipid fraction (Deenen *et al.,* 1965). The proteolipid fraction described by Feldman and Feldman, *et al.*, (1965) in human lens is probably the most direct evidence available on the composition of the lipid component of lens fiber membranes. 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 related to posterior subcapsular cataract (PSC) in men (Klein *et al.*, 1997). Likewise in this study, HDL-C and TG were

associated with the development of senile cataracts (Table 4.3). Persons with HDL C abnormalities were twice as likely to develop senile cataract (odds ratio, 2.52 p=0.095) (Table 4.4). The significant type of lipid abnormality observed among the clinically diagnosed senile cataract patients were low high density lipoprotein cholesterol concentrations (HDL-C) (Table 4.4). 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. This specific lipoprotein abnormality e.g., low levels HDL may contribute to disease despite normal cholesterol and TG levels. Low level HDL-C concentration observed in this study may be due to other factors such mutation in ATP-binding cassette transporters'A1 (ABCA1) gene expression in HepG2 cell, familiar HDL deficiency and decreased lipoproteins lipase activity leading to inadequate transport of phospholipids and cholesterol to the extracellular resulting in the hyper catabolism of lipid poor nascent HDL particles. This may result in low circulation HDL-cholesterol levels lower in the cataract group than in the controls. The lower cholesterol might imply a defect in metabolism of cell membrane important in cataractogenesis supporting earlier studies (Phillips *et al.*, 1980; Clayton *et al.*, 1984).

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) and 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 (Malik *et al.*, 1995).

5.4 URIC ACID AND SENILE CATARACT

Reductions in serum uric acid levels are clinically relevant because high levels are often associated with gout. Uric acid level was significantly lower in the senile cataract patients than in the controls (Table 4.2) 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) associated with aging. This current study finding was opposite with Leske *et al.*, (2004), Lens Opacities Case-Control study; Leske found that, higher uric acid levels increased risk for mixed opacities. Kałuzny *et al.*, (1996) investigated the level of uric acid in aqueous humor of cataract patients. The difference was statistically significant. The results suggested uric acid as strong endogenous antioxidant which may play an important role in pathogenesis of cataract as a result of its comparatively high serum concentrations in the eye.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6. CONCLUSION

This case-control study design provides the first report on the serum biochemical risk factors of senile cataract among Ghanaians. The studies clearly established a half (50%) of any study population with the age of ≥70 years especially women are most likely to develop senile cataract. This study strongly established an association between serum biochemical indices and senile cataract. The association exist between low levels of High Density Lipoprotein-cholesterol (HDL) , low levels uric acid, Fasting blood Glucose (FBG), Serum sodium (Na^+), serum potassium (K^+), Triglyceride (TG) and Senile cataract.

The probability of developing senile cataract draws attention to:

Elevated serum Na^+ level in those suffering from senile cataract as a significant factor associated with senile cataract ORs (95% CI) 0.60 (0.45 - 0.80), P=0.001).

Persons with lower serum potassium (K^+) ORs (95% CI) 2.48 (1.47-0.72; p=0.010) are twice likely to develop senile cataract.

Persons with low HDL CHOL abnormalities were twice likely to develop senile cataract (ORs (95% CI), 2.52 p=0.095).

Our findings add to an evolving biochemical and laboratory animal based hypothesis that changes observed in the blood level constituent raised or lowered can be used for diagnostic and prognostic purposes and their detection makes up a large part of routine clinical chemistry especially in an elective cataract surgery procedure. It is essential to have access to such a patient prior to, during and immediately after surgery in order to effectively treat adverse reactions.

6.1 RECOMMENDATIONS

Further studies should be conducted to ascertain whether or not certain dietary habit has a part to play in the aetiogenesis of senile cataract in various regions of Ghana.

Further studies should be conducted to elucidate the association of high Na^+ content foods and induction of cataract in experimental animals.

Large-scale epidemiological research should be conducted to identify risk factors.

We also suggest that further studies should be carried out to ascertain the biochemical derangement or otherwise of the other types of senile cataract on the clinical outcome in the management of senile cataract.

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