

A Dissertation On

**CHOLESTEROL RING IN IRIS: A NONINVASIVE DIAGNOSTIC
EVALUATION FOR DYSLIPIDEMIA**

Submitted by

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1.0 INTRODUCTION

The eyes are said to be the bridge between the spiritual, mental, emotional and physical aspects of our human nature. Iridology is 3,000 year old art and science which has been practiced since history has been recorded. According to archaeological report from 3000 years ago, there was much attention devoted to the study of the iris and its relation to organs of the body in Egypt, China and India. Iridiagnosis is an alternative pre diagnostic tool and technique which claims that patterns, colours, and other characteristics of the iris can be examined to determine the information about a patient's internal health. Iridologists match their observations to iris charts, which divide the iris into zones that correspond to specific parts of the human body. Iridologists see the eyes as “windows” into the overall body's health [1].

The earliest recorded history of iris analysis extend back as far as ancient Babylon. An ophthalmologist, in 1813, unperceptive of these old views on iris analysis, wrote in his book Textbook of Eye Disease as “Everything that affects the organism of an individual cannot exist without effect on the eye and vice versa.”

In the twentieth century, doctors and scientists from the United States, Europe and Australia brought iridology into worldwide recognition. In the last twenty years, iridology has been widely investigated in sites such as the framework, the sclera and pigmentation, and has been used by many traditional doctors in Germany, Russia and some other countries as a reliable form of diagnosis. This is largely due to its trustworthy in the evaluation of function and disease potential.

In the present period of time, people are in need of prophylactic health care and less complicated methods of examining their condition. Iridology provides a non-invasive, painless and economical means of checking into the body, which may be effective in co-occurrence with any other system of diagnosis available [2].

1.1 IRIDOLOGY

Iridology is an art and science of analyzing the colour and structure of the iris of the eye which provide clear details on the state of health.

The iris a part of the eye that separates the anterior chamber of the eye from the lens and lies in front of the posterior chamber of the eye. Iris muscles have the capacity to contract or relax so making the pupil smaller or bigger. The iris a mixture of muscle cells, connective tissue, blood vessels, nerve endings and pigmented cells, all of which produce various colour patterns.

Iridologists report that all parts of the body are delineated in specific locations in the iris and the changes in the functions of body are reflected in iris. It is most important to bring up that the details from the irides can be obtained quickly, painlessly and inexpensively [3]. Iridology, also known as iris diagnosis or iridodiagnosis / iridiagnosis, is defined as a science that identifies pathological and functional changes within organs via assessing the iris for aberrant lines, spots and discolouration [4].

1.2 HISTORY

The science and practice of iridology is antique. The earliest documents exposed have shown that a form of iris explanation was used in Central Asia (Mesopotamia) as early as 1,000 BC. This details was found in cuneiform writings on tablets of

clay from the Chaldea civilization. The Greeks referred this culture as the crib of knowledge.

In 1400-1392 BC, pictures of iris found on Silver plates in the King Tut's tomb.

Hippocrates, the "Father of Medicine" born approximately in 460 BC in Greece on the island of Kos is well-known that he looked in the eyes of his patients for health information.

In 305-300 BC, Ptholemeus, military leader of Alexander used Principles of Iridology to choose the warriors.

In the Bible, St. Luke writes that Christ said, "The lamp of your body is the eye. When your eyes are sound, you have light for the whole body, but when your eyes are bad, you are in darkness."

In 1514-1564, A.Vesalius, Father of Anatomy, explains the iris of a dying man in his hand book.

In the year 1670, the Physician Philippus Meyens, in his book, Physiognomia Medica, explained clearly the division and marking of the iris regarding the organ regions of the body. He wrote and published a book holding diagnostics on both eyes and eyebrows.

The Viennese ophthalmologist, George Joseph Beer (1763-1821), did not know of these old views on iris analysis. Yet, in his 1813 publication, Textbook of Eye Diseases, he wrote, "Everything that affects the organism of an individual cannot remain without effect on the eye and vice versa. He also mentioned somato-iridological links in organisms."

In 1790, Dr. Mac Leiden, a physician from Holland, made the first narration of the Brain and lungs, and created the manual on iridology which was issued in the University of Portugal. A Hungarian, Dr. Med. Ignaz Péczy (1822-1911), published a book in 1880 entitled, *Discovery in Natural History and Medical Science, a guide to the Study and Diagnosis from the Eye*. This book gained an international fame and he is considered the renaissance father of iridology.

In 1959, Rudolfg Schnebel, London Academy of Sciences, won prize for publishing of a double volume of *Iridology* [5]. During the first half of the 20th century, iridiagnosis was practised in the USA primarily by medical doctors. A quote from Henry Lindlahr, M.D. circa 1919, “The ‘regular’ school of medicine (allopathic), as a body, has ignored and will ignore this science (of iridology), because it discloses the flaws of their favorite theories and practices and because it reveals unmistakable the dreadful results of chronic drug poisoning (pharmaceuticals) and ill-advised operations.”

The iridology teaching was removed from the syllabus, due to increasing political and economic pressure upon medical schools by the emerging pharmaceutical industry. Eventually this was lost within the allopathic medical practice. However, it was still alive by naturopathic practitioners in the latter half of the 20th century. Notably, Bernard Jensen, of the U.S.A. was the champion of this valuable tool of assessment until his age of 93 years. Nowadays, iridology is practiced worldwide, and in Europe it has been used clinically for generations.

As the iris tissue forms embryologically, it takes on the characteristics of the genetic information contained in the sperm and ovum cells beyond iris color and visual clearness. These two cells provide influence pertaining to several generations of

physical, emotional and thought information. There are approximately 7.3 billion humans on earth and there are no two irises alike. It can be said that the trabecular patterns in the irises are unique vibrational frequencies of the soul's consciousness. This information can, in part, be subconscious to the individual's awareness, yet has direct influence on their physical, emotional and thought behaviors. Science, through quantum physics, is showing us that everything in our universe is pure energy on a sub-atomic level. Matter is energy vibrating at different frequencies. Thus, iris tissue is a compilation of multiple frequencies that form the unique patterns for the individual that have been created by the soul's consciousness [6]. All of the various glands, organs and structures of the body have vibrational frequencies that predominately resonate with particular thoughts and emotions. For example, in brief, the destructive emotions of anger and rage have a negative effect on the liver, gallbladder, thyroid and parathyroid. These emotions may be conscious or subconscious and are present before a physical condition will manifest in these tissue structures. The pancreas, in both endocrine and exocrine function, will be adversely affected by emotions of grief and sadness. Keep in mind that these emotions can be, and most likely are, from generational influences and are reflected in various signs in the iris. It is important to highlight that the emotion of unconditional love has a effective and extensive healing effect on every cell of the body.

Each eye gives us unique information. The left eye correlates with the left side of our body, which is that feminine, creative, conceptual and intuitive side of the body. The right eye correlates to the right side of our body, which is that masculine, analytical, linear and practical side. *Epigenetics* can define how information in genes is expressed and used by cells. Considering that environmental factors can

influence gene expression, it is also possible through our beliefs and the ways we eat, drink, feel think, live and love to modify the expression of the natal deoxyribonucleic acid (DNA). Researchers have found a range of possible chemical modifications to the DNA and to proteins called histones that associate tightly with DNA in the nucleus. These modifications can determine when, or even if, a given gene is expressed in a cell or the entire organism.

1.3 HOW IRIDOLOGY WORKS

The iris of the eye is the most complex tissue of the body directly exposed to the environment and all pollutions in the environment. It is an extension of the brain, being incredibly endowed with hundreds and thousands of nerve endings, microscopic blood vessels, muscle and other tissues. The iris is connected to every organ and tissue of the body by way of the brain and nervous system. The nerve fibers receive their impulses by way of their connections to the optic nerve, optic thalami and spinal cord. They are formed embryologically from mesoderm and neuroectoderm tissues. Both sympathetic and parasympathetic nervous systems are present in the iris. This is how eye functioning as a miniature television screen showing the most remote portions of the body by way of its nerve reflex responses.

It is well understood that the eye works two ways; not only it enable us to bring images of the outside world within, it also shows images of what is within to the outside. Nerve fibers in the iris respond to changes in body tissues by manifesting a reflex physiology that corresponds to specific tissue changes and locations in the iris [7].

With regard to human embryology, iris tissue derives from the same embryologic layer as the nervous system: ectoderm. The eyes and thalamus emerge from the

same cerebral vesicle: diencephalon (Fig. 1). The thalamus works as a major relay and integration station of the information that goes to all areas of the cerebral cortex, basal ganglia, hypothalamus and brain stem. It is possible that the eyes (irides) work as an embryologic twin structure to the thalamus [8].

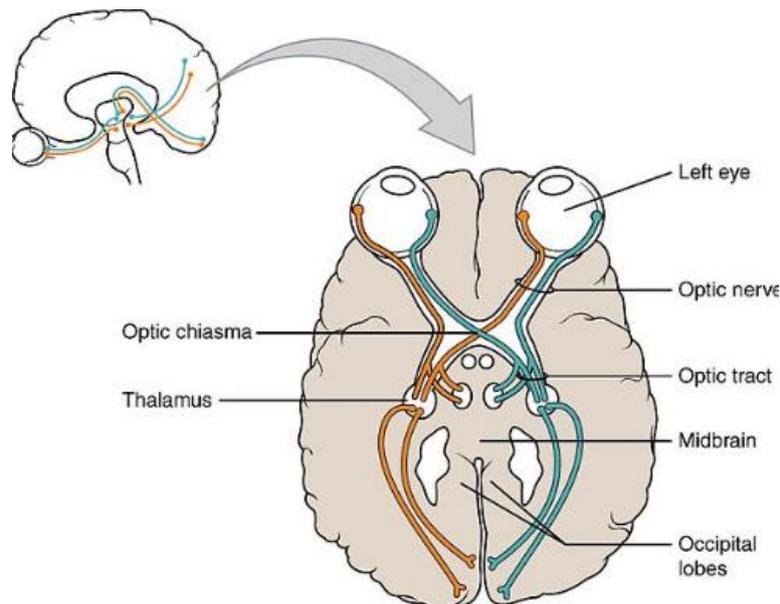


Figure 1: Nerve connection between eyes and thalamus

As sensory organs, the eyes have afferent nerve pathways that carry information to the central nervous system for processing. This information is then sent out via the peripheral nervous system through the efferent nerve pathways to the autonomic nervous system. These nerve impulses innervate the muscles, organs and glands of the body (Fig. 2). The eyes are connected and continuous with the brain's dura mater through the fibrous sheath of the optic nerves, and they are connected directly with the sympathetic nervous system and spinal cord. The optic tract extends to the thalamus area of the brain. This creates a close association with the hypothalamus,

pituitary and pineal glands. These endocrine glands, within the brain, are major control and processing centers for the entire body. Because of this anatomy and physiology, the eyes are in direct contact with the biochemical, hormonal, structural and metabolic processes of the body.

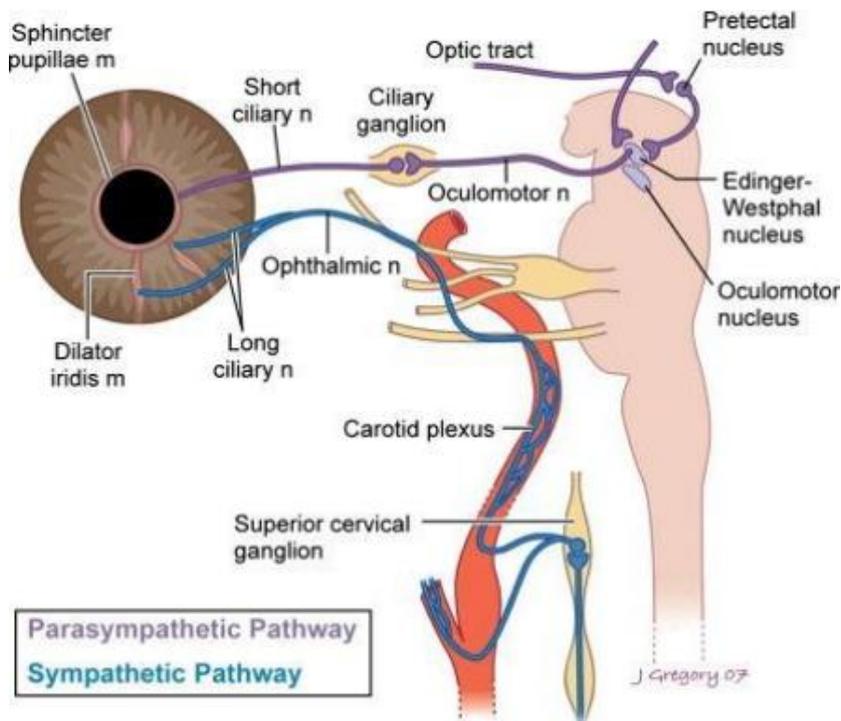


Figure 2: Nerve supply of Iris

This information is recorded in the various structures of the eye, i.e. iris, retina, sclera, cornea, pupil and conjunctiva. Thus, it can be said that the eyes are a reflex or window into the bioenergetics of the physical body and a person's feelings and thoughts [8]. The circular muscle fibres are supplied by the short ciliary nerve branch of motor oculi, coming directly from brain. The other structures are supplied by long ciliary nerve, which is in direct communication with cervical ganglia of the sympathetic nervous system. These nerves travel forward to the iris through the choroid coat of eye ball. Along the attached margin of iris they form a plexus from which nerve filaments are given off to muscle fibres and other structures of the iris.

Some of these nerve filaments also go to form a complete network on the surface of iris immediately underneath the surface endothelium. These are arranged in triangles, the bases of which rest on the outer rim of iris, and whose apices point towards the pupil. The sides of these triangles coincides with the blood vessels, these with the sympathetic nerve supply, and in turn with borders of organ areas. The direct connection of nerve filaments with the surface of iris with the cervical ganglia of sympathetic nervous system explains how impressions (vasomotor changes) from all over the body may be conveyed to the iris [9].

Iris analysis can uncover hereditary predispositions to degenerative conditions and early pathogenesis decades before symptoms occur or conventional diagnostic testing may reveal. Thus, it is a valuable asset for preventive healthcare.

According to Pesek [10], the eyes are connected and continuous with the brain's dura mater through the fibrous sheath of the optic nerves, and they are connected directly with the sympathetic nervous system and spinal cord.

The optic tract extends to the thalamus area of the brain. This creates a close association with the hypothalamus, pituitary and pineal glands. These endocrine glands, within the brain, are major control and processing centres for the entire body. Because of this anatomy and physiology, the eyes are in direct contact with the biochemical, hormonal, structural and metabolic processes of the body. This information is recorded in the various structures of the eye, i.e. iris, retina, sclera, cornea, pupil and conjunctiva. Thus, it can be said that the eyes are a reflex or window into the bioenergetics of the physical body and a persons' feelings and thoughts.

Since iris diagnosis is a noninvasive, quick, painless and inexpensive this has been used as tool to assess the abnormal cholesterol content in the body by observing “cholesterol ring” in the patient’s eyes. Study conducted by Ramlee et al [11] has established the fact that the presence of Arcus Lipidus in iris indicate high cholesterol in blood.

1.4 IRIS ANALYSIS

Iris analysis is extremely accurate in determining tissue weaknesses in the body including [12]:

The primary nutritional needs of the body; inherent weak organs, glands, and tissues; constitutional strength or weakness; what organ is in greatest need of repair and rebuilding? relative amount of toxic settlements in organs and tissues; location and stages of tissue inflammation, whether: acute, sub-acute, chronic, or degenerative; under-activity or sluggishness of the bowel; spastic conditions or ballooning of the bowel; prolapse of the transverse colon; nervous condition or inflammation of the bowel; high risk tissue areas in the body that may lead to disease; circulation level in various organs; nerve force and nerve depletion; hyper-activity or hypo-activity of organs; lymphatic system congestion; poor absorption and assimilation of nutrients; buildup of cholesterol and inorganic salts; results of physical or mental fatigue or stress; need for rest to build up immunity, adrenals; tissue areas contributing to suppressed or buried symptoms; buildup of toxic material before the manifestation of disease; recuperative ability and the level of health of the body; pre-clinical stages of cardiovascular and other diseases; healing signs indicating an increase of strength in any organ; acidity of the body, tendency toward arthritis; healthy balance of HCL (acid) in the stomach; suppression of, or

catarrh (mucous) development; adrenal exhaustion, which may indicate low blood pressure; response to treatment and how well the body is healing.

1.5 IRIDOLOGY SIGNS AND PATTERNS

There are three kinds of signs in the iris: Unnatural colourings. 2. White, dark and black signs: chiefly as dots, radiating lines or wisps 3. Circular signs called 'Contraction rings'.

1.5.1 Unnatural Colourings

The unnatural colourings have their basis in the circulatory fluids of the body. These circulatory fluids (blood and lymph) are affected by external and internal influences, as through medication, or autointoxication, and changes due to uric acid or biliary disturbances. These pathological changes in the lymph are revealed not only by the skin and mucous membranes, but show also in the iris and the sclera, as is evident in jaundice. There are also the deposits in tissues, as may occur in rheumatism and gout.

1.5.2 White, Dark and Black Signs

White signs may also indicate unnatural substances, as with uric-acid crystalline deposits, arteriosclerosis, etc. The next signs to investigate are the inflammation-signs. These appear with acute diseases, and either disappear on recovery, or become darker and darker with the transition to the chronic phase, and ultimately change to black signs with the direct loss of tissue-substance in the organs concerned.

1.5.3 Circular Signs

Circular signs, called Cramp rings (Nerve rings) which appear as shorter or longer segments of arc, are found only in the ciliary zone. These 'ring-furrows' are usually lighter or darker than the remainder of the iris and arise in connection with conditions of continued spasm.

Schnabel ascribes to a slackening or spasm of the sphincters or dilators of the ciliary muscles. Thiel believes that through the continuous regular pull of all the dilator fibres, or at least of a sector of the iris-diaphragm, functioning in the same way as the pupillary margin, that concentric arcs would be formed by circular folds.

According to the opinion of Frau Pastor Madaus, they arise in the true nerve fibre. Dr. Andogsky states that these enter the iris in radial bundles. Thereafter they immediately lose their radial direction and turn parallel to the ciliary border, thus forming the first ring, and thereafter sending several thick radial branches towards the pupillary margin with a number of smaller branching distributions.

1.5.4 Form of Iris-Signs

Lines: Short white lines are usually found lying in contiguity one with another, and are signs of inflammation affecting the organs concerned. Long white lines are those which are not limited to one organ area, but run over several areas. Dark lines in an organ area are indications of nervous weakness.

Flakes and Clouds: They appear as signs of an acute or chronic inflammation of the mucous membranes (catarrh). The signs are usually seen in the form of small/larger flakes in the sectors for lungs, thorax, peritoneum, frontal sinus, etc.

Wisps: White wisps are signs of an extensive tissue-inflammation. Dark wisps appear when the indicated organ has become weak in reaction.

Lacunae: signs of weakness. Lacunae appear wherever the iris fibres diverge in small or large arcs and thus expose the second darker iris layer.

Open lacunae: when the iris fibres do not again converge towards the outer iris rim and join up.



Figure 3: Closed lacunae

Closed lacunae: when the iris fibres reunite towards the outer rim of the iris, thus forming an oval sign (Fig. 3).

Honeycomb Signs: are lacunae in which small white lines provide a honeycomb appearance by running lengthwise and across within the lacunae.

Transverse Signs: ‘Adhesion’ signs, are very fine white lines which run obliquely across the iris structures.

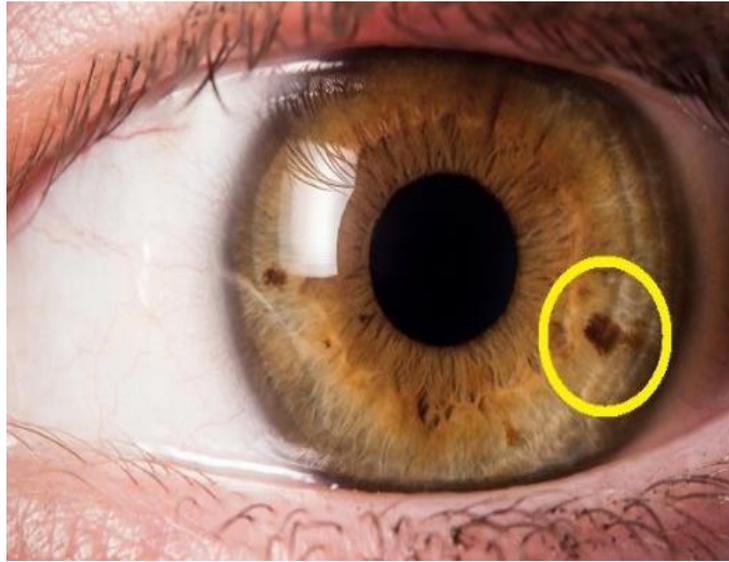


Figure 4: Black dots in iris

Black Dots: oblong or jagged small black lines, suggest tissue disintegration, loss of substance, ulcers (Fig. 4).



Figure 5: Radii Solaris

Radii Solaris: are radiating furrows in the iris tissues which are wider at the base and taper towards the outer rim (Fig. 5).

Wedge Signs: are small black signs which are directed with their bases towards the iris-wreath. If such a sign is seen in the heart area, then the possibility of sudden death.

Contraction rings (Nerve rings): 'Cramp-rings'-are concentric interruptions of the iris fibres which are especially seen in the second and third major zones (Fig. 6). They indicate circulatory disturbances in the tissue, and disturbance of lime metabolism.

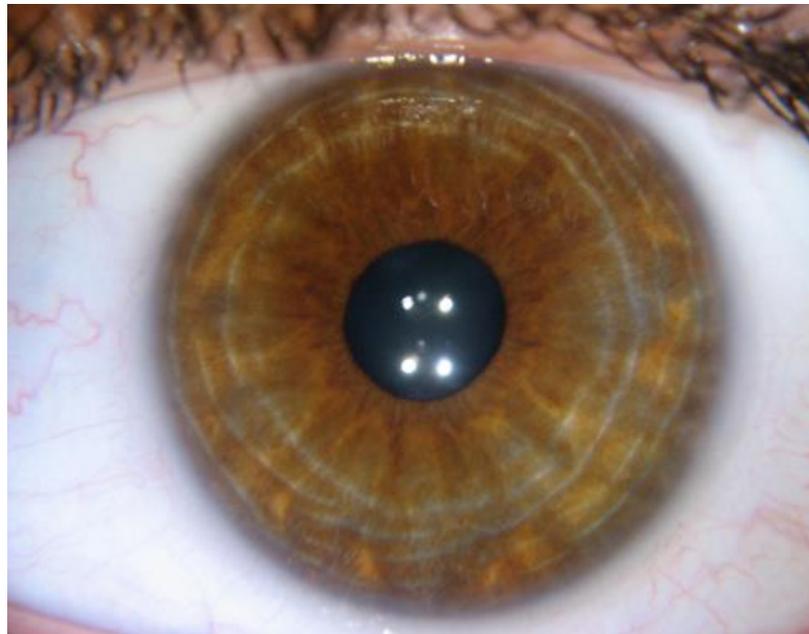


Figure 6: Nerve rings

Signs of Death: Imminent: (a) A black wedge-sign in the heart area (b) Completely solid black scurf rim (c) A perpendicular-oval pupil.

Dark Skin Zone: Indicates a suppressed excretion. A milky-white: scurf rim (arcus senilis) is a sign of arteriosclerosis, also called as cholesterol ring.

1.6 CHOLESTEROL RING

A solid white ring circling the iris at the periphery in zone 7 is called a sodium ring (Fig. 7). Today, it is also recognized as a sign of excessive cholesterol and triglycerides in the body and therefore it is often referred to as a Cholesterol Ring. It varies in its width according to the severity of the condition [7].

It is an indication of elevated cholesterol level which may lead to cardiovascular diseases. It is caused by extracellular lipid deposition in the peripheral cornea, with the deposits consisting of cholesterol, cholesterol esters, phospholipids, and triglycerides. The fatty acids that make up many of the deposited lipid molecules include palmitic, stearic, oleic, and linoleic acids [13].

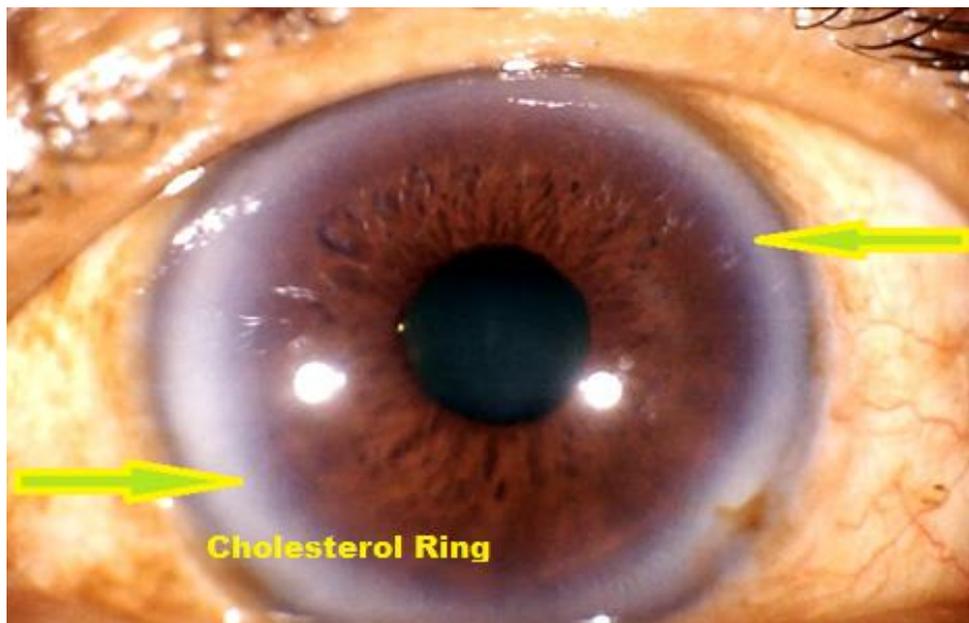


Figure 7: Cholesterol Ring (Image taken from a participant during the study)

1.7 DYSLIPIDEMIA

Dyslipidemia is one of the major risk factors for cardiovascular disease [14]. It has been well known that lipid abnormalities are major risk factors for premature CAD. Recent study revealed the increased prevalence of dyslipidemia is more prevalent in 31-40 year males, suggesting that this group is at increased risk of developing CAD leading to young infarcts [15]. The prevalence of dyslipidemia is considered to be very high in India, which invites attention for urgent lifestyle intervention strategies on prevention and management of cardiovascular risk factors [1]. Early detection and treatment of dyslipidemia can prevent risk for atherogenic cardiovascular disorder. Dyslipidemia is a high level of lipid in the blood poses a significant threat to person's health. The technique presently employed to measure the cholesterol level is by doing blood test known as lipoprotein profile. The lipoprotein profile is an invasive method which causes discomfort amongst many patients. Haq et al. [3] introduced a laser based technology as noninvasive technique to measure blood cholesterol through skin but it is not intended to be used as a screening tool to determine the coronary artery disease (CAD) in general population and level of risk.

AIMS AND OBJECTIVES

2.0 AIMS AND OBJECTIVES

2.1 AIM

The aim of this study was to determine the presence of cholesterol ring in iris of dyslipidemia patients.

2.2 OBJECTIVES OF THE STUDY

2.2.1 Primary Objective: To determine the presence of cholesterol ring in dyslipidemia patients (in- and out-patients) of Government Yoga and Naturopathy Hospital, Arumbakkam, Chennai.

2.2.2 Secondary Objective: To assess the efficacy of iris diagnosis as a noninvasive, quick, painless and inexpensive diagnostic tool.

Thirdly this study tried to develop categorical scale on the morphology of cholesterol ring in iris to identify risk level of cardiovascular diseases.

2.3 IRIDOLOGY CAMERAS

In order to view the eye the following items will be needed. There were different types of cameras available throughout the world for better capturing of iris images and for better analysing. Few of them were listed below.

2.3.1 Basic Model Iridology Camera

Iridologists generally use equipment such as a flashlight and magnifying glass (Fig. 8), cameras or slit-lamp microscopes to examine a patient's irises for tissue changes, as well as features such as specific pigment patterns and "irregular stromal architecture". The markings and patterns are usually compared to an iris chart that correlates specific zones of the iris with specific parts of the body (Fig.9a and 9b).



Figure 8: Iris Light Magnifier

Specifications

10× Magnification

Lens: 38mm in diameter optical glass

Dual Daylight LED's for clearer viewing

Dimensions: 132 mm (L) × 45 mm (W) × 26 mm(H)

Powered by 3 × AAA batteries (not included)

Unit weighs: 50 grams



Figure 9A and 9B: Basic Iridology Camera Model

The details regarding the software used to detect iridology lesions was mentioned in Annexure 1.

2.3.2 Advanced Model Camera

After a quarter century of research and clinical practice, Dr. Pesek has developed a comprehensive system for analyzing the irises and reporting the overall genetic constitution, various conditions and accumulations and the levels of health of the body's systems of detoxification, organs and glands. The 18 brain reflex areas that reflect the conscious and subconscious thought and emotional patterns of the person are also brought to light with this system. Now, this detailed and personalized Holistic Iris Analysis Report is made available to healthcare practitioners with this leading edge, innovative software on CD. This dynamic method utilizes Holistic Iridology®, which encompasses physical, emotional, mental and spiritual aspects to assist in healing the whole person (Fig. 10).



Figure 10: Advanced Iridology Camera Model

This software is most effective when used by the holistic iridologist who has completed a minimum of Level II studies in Holistic Iridology®. Iridology practitioners, with the use of this software, will be able to express their skills in iris assessment and create a report of more than 32-pages with over 140 evaluations for their clients in a clinical and professional manner. Basic guidelines for taking action are included which contain physical, emotional and mental aspects to help the client toward attainment of true health and vitality.

This software allows practitioners to add their own comments and guidelines to the text to further personalize the report. A black and white topography chart of Holistic Iridology® and clear iris chart overlays are included to assist in doing the analysis. This is the leading edge in iris analysis utilizing clinical Holistic Iridology® [Annexure 1].

This is the only iris analysis software of its kind in the world today. **Software is \$895 + S & H.** Website: <http://www.holisticiridology.com/products/software/>

2.3.3 Integrated Iridology Software

Integrated iridology software designed by Toni Miller, a Naturopathic Medical Herbalist and Master Integrated Iridologist with over thirty six years clinical experience. She is Australia's most experienced Iridology teacher, researcher and lecturer and the author of many Iridology resources including the award winning "Integrated Iridology Textbook" (revised 2016). Toni has been teaching Iridology continuously 1984. It is rare indeed to have a teacher who can both explain and offer answers based on the wisdom that comes from nearly 4 decades of professional clinical experience and over 3 decades of ongoing research. Her students graduate with confidence and increased Iridology understanding. Toni was

voted 2014 IIPA Iridologist of the Year and IIPA Instructor of the year and became the first person ever to be awarded these titles simultaneously. Her school – The College of IRIS is the only one specializing in Iridology education in the southern hemisphere.

The software (Iridology Station 5.1 in Annexure 1) produce professional customized reports for the persons taking information from the following options:

- 18 constitutions
- Basic Iridology signs
- Collarette signs. (ANW)
- 30 Sclera signs. (White of the eye)
- Psychological indications seen in the iris
- Variations in the Structure
- 28 Pupil signs

**REVIEW OF
LITERATURE**

3.0 REVIEW OF LITERATURE

3.1 Dyslipidemia

Dyslipidemia is elevation of plasma cholesterol, triglycerides (TGs), or both, or a low HDL cholesterol level that contributes to the development of atherosclerosis. Causes may be primary (genetic) or secondary. Diagnosis is by measuring plasma levels of total cholesterol, TGs, and individual lipoproteins. Treatment involves dietary changes, exercise, and lipid-lowering drugs.

Dyslipidemia is a primary, major risk factor for CAD. Epidemiologic data also suggest that hypercholesterolemia and perhaps coronary atherosclerosis itself are risk factors for ischemic stroke [16].

According to a study conducted in 2016, it was said that Arcus senilis is a grayish or whitish bow shaped or ring-shaped deposit in the cornea. It is also recognized as a sign of hyperlipidemia. The entire process of automated detection of cholesterol presence system was developed using MATLAB coding refers to Mr. Libor Masek's work for iris recognition algorithm. The improvement can be done for detecting the stages of cholesterol presence and to determine the eye problem due to other type of eye diseases such as cataract, glaucoma, diabetic, tumor, etc. The algorithm has been tested on more than 70 samples of normal and abnormal eye images received from database such as CASIA, UBIRIS, MMU and medical web [17].

According to a preliminary study conducted in 2011, to know the real level of cholesterol in the body of on more than 50 samples of normal and abnormal eye images, it was concluded that the threshold boundary of the normal and problem

eye is about 139. This project had shown the entire process of detecting cholesterol presence using automated program (ADCP) [18].

According to a study conducted in 2014, a tool has been developed by using simple and non-intrusive automation system to detect cholesterol presence using iris recognition (image processing). This system applies iris recognition method to isolate the iris area, normalization process and lastly determining the cholesterol presence using thresholds and histogram method to determine the threshold value. The result showed that the incidence of cholesterol was high when Eigen value exceeds a threshold value [19].

The study done in 2011 applies iris recognition method to isolate the iris area, normalization process and lastly determining the cholesterol presence using OTSU histogram method to determine the threshold value. The result showed that the incidence of cholesterol was high when eigen value exceeds a threshold value [20].

The study conducted in 2006, [21] highlighted the potential of using multispectral iris information in recognition systems. Since most commercial systems use only the near-IR wavelengths for iris analysis, there is lack of literature discussing the benefits of employing multispectral information that includes the visible portion of the electromagnetic spectrum. Depending upon the color of the eye, the nature of iris information presented in different spectral channels can vary. This presents the novel possibility of utilizing user-specific wavelengths for iris image acquisition. Furthermore, the use of multispectral information has the potential to enhance the segmentation and enhancement procedures thereby improving the performance of iris recognition systems. Pixel-clustering based on color information may be used to elicit and examine the various components of the iris.

Although there were many studies to support this study, the images for iris analysis received were from the stored databases and the studies were conducted to find the efficacy of iris algorithm method designed by various Engineering Institutions.

Here, in this study the high quality images of iris were captured directly from the patients visiting Government Yoga and Naturopathy Hospital, Arumbakkam and the images were analysed by qualified Naturopaths using Pro iris software to detect the presence of Cholesterol Ring sign in the iris of the participants. The sample size has been taken as 74.

3.1.1 Prevalence

Lipid abnormalities are major risk factors for premature CAD. The prevalence of dyslipidemia was observed to be higher in males than in females. The increase of prevalence of hypercholesterolemia and hypertriglyceridemia was more prominent in 31-40 age group than in ≤ 30 age group [22]. Recent studies have reported that high cholesterol is present in 25–30% of urban and 15–20% rural subjects. The most common dyslipidemia in India are borderline high LDL cholesterol, low HDL cholesterol and high triglycerides. Studies have reported that over a 20-year period total cholesterol, LDL cholesterol and triglyceride levels have increased among urban populations [23].

3.1.2 Operational Definition

The diagnosis of dyslipidemia will be based on the alterations in the levels of Total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and total triglycerides (TGs).

3.1.3 Pathophysiology

All living organisms utilize lipids for cellular structures, energy, and signalling molecules. Mammals also secrete lipids into milk as a source of energy for infants and onto the skin as a protective coat. Because of this, several tissues have developed specialized systems for lipid secretion, transport, and storage. Lipids such as triglycerides and cholesteryl esters are hydrophobic; these molecules do not remain in solution in the blood. Hydrophobic steroid hormones are transported while associated with specific carrier proteins such as sex hormone-binding globulin and cortisol-binding globulin. Similarly fat-soluble vitamins such as A and D circulate attached to 2 P. Freitas Corradi et al. binding proteins. Similarly, triglyceride and cholesteryl ester move in the blood as components of macromolecular complexes, lipoproteins. Lipoproteins are spherical particles that differ in size, composition, and density but have a common structure. The outer surface of the spheres is composed primarily of phospholipids and apolipoproteins; the word “apo” means “without,” and these proteins are termed apos to indicate the protein without the lipid moieties. Both phospholipids and apolipoproteins are amphipathic, meaning that they have hydrophobic domains that interact with lipids and hydrophilic regions that are charged and allow the particles to interact with plasma, the polar water phase. Apos interact with cell surface receptors and act as cofactors for enzymatic reactions (Fig. 11).

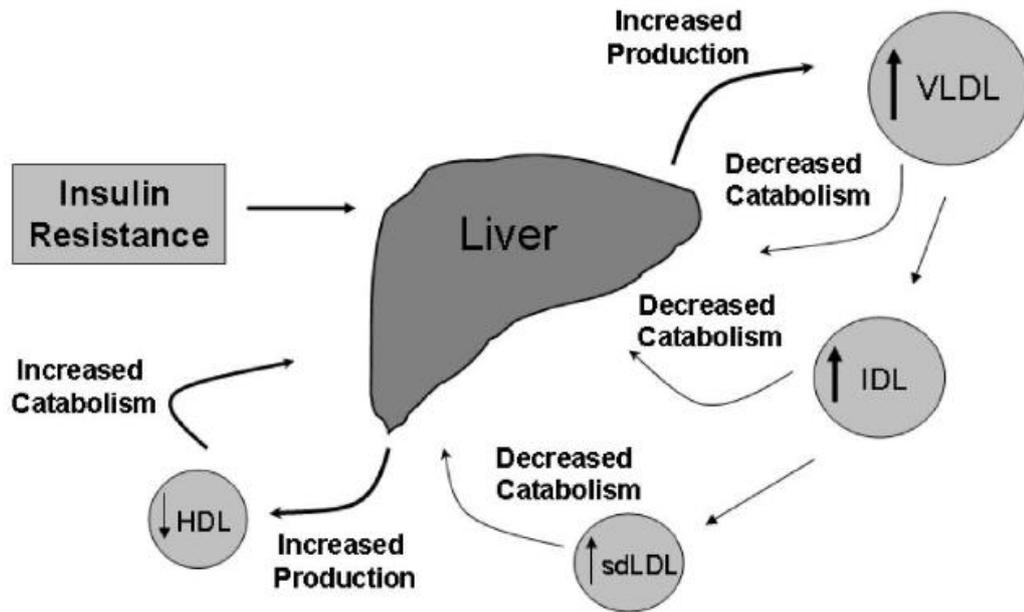


Figure 11: Pathogenesis of Dyslipidemia (BioMed Central)

3.1.3.1 Triglyceride and Chylomicron Metabolism

Triglyceride Metabolism Triglycerides are the major storage form of calories. Aside from providing lipids for cellular structures, they support the energetic requirements of high energy-utilizing tissues such as the heart, diaphragm and other chronically moving muscles, and brown adipose tissue. Other tissues, most notably white adipose, store excess calories and release them during fasting. Triglycerides are either ingested or synthesized by several tissues, most importantly the liver.

Chylomicron Metabolism Chylomicrons are the particles that enable ingested fat-derived calories to enter the body. Following a meal, triglyceride is hydrolyzed to fatty acids that enter the enterocytes and are re-esterified into triglyceride. The triglyceride is associated with a large protein, apolipoprotein B (apoB). This process of chylomicron assembly requires the actions of an intracellular protein termed the microsomal triglyceride transfer protein (MTTP). Another special feature of chylomicrons is that the apoB contained in these particles is formed by

the enzymatic insertion of a nucleotide base change to a stop codon leading the translation of apoB-48, a protein that is 48 % of full-length apoB-100

Chylomicrons are not secreted into the bloodstream, but are conducted away from the gut via the lymphatic system. Peripheral lipolysis of chylomicron triglyceride provides energy to peripheral, i.e., non-hepatic, tissues. Liver uptake of remnants delivers cholesterol and its esters and esters of vitamin A. Once chylomicrons enter the bloodstream via the thoracic duct, they become enriched with several apolipoproteins required for their catabolism. One of these is apoC-II, the activator of lipoprotein lipase (LpL), the endothelial cell surface-associated enzyme that converts triglyceride into free fatty acids. The second is apoE, a ligand to allow association of the partially degraded remnant particle with proteoglycans, heparin-like molecules, within the liver. ApoE is also a ligand for the LDL receptor and LDL receptor-related protein 1 (LRP1), two endocytic receptors within the liver. ApoC-III will prevent liver uptake of remnants likely via blocking lipoprotein interaction with LDL receptors and LRP1. Although the role of LpL as the rate-limiting enzyme in plasma triglyceride metabolism has been known for over 50 years, additional regulatory events have been discovered that affect human physiology and disease. LpL and its genetic cousin hepatic lipase require a complex intracellular assembly. This involves the actions of an intracellular protein termed lipase maturation factor 1 (LMF1). The enzyme association with the endothelial surface, once thought to be a relatively nonspecific binding to heparan sulfate proteoglycans, requires the presence of a binding protein unfortunately named glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1), a protein initially and incorrectly thought to be important for HDL metabolism. Active LpL is a dimeric molecule, and its regulation is affected by the presence of

several members of the angiopoietin-like protein family (ANGPTL3, 4, and 8), which may convert the LpL dimers into inactive forms. Finally apolipoprotein A-V, a relatively minor apoprotein found on triglyceride-rich lipoproteins, is needed for efficient triglyceride metabolism, perhaps because this protein assists with lipoprotein association with the capillary surface. Human deficiency of apoC-II, LMF1, and GPIHBP1 presents with fasting hyperchylomicronemia that is indistinguishable from LpL deficiency. ApoA-V deficiency is associated with less severe hypertriglyceridemia. The loss of ANGPTL3 or 4 leads to reduced circulating triglyceride levels.

3.1.3.2 VLDL/LDL Metabolism

VLDL are produced within the liver and therefore contain triglycerides from three sources: (1) albumin-associated fatty acids primarily released from white adipose tissue, which are reassembled as triglyceride within the liver, (2) fatty acids that are de novo synthesized from carbohydrates during caloric excess, and (3) triglyceride that initially enters the liver as a component of other lipoproteins such as chylomicron remnants. Intracellular triglyceride hydrolysis in white adipose tissue is extremely sensitive to insulin, which inhibits the actions of two intracellular enzymes, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). ATGL is primarily responsible for the release of the first fatty acids from triglycerides, while HSL is the major enzyme that converts newly formed diacylglycerols to monoglycerides. VLDL production is highly dependent on the availability of triglycerides but is also sensitive to insulin actions that drive the de novo fatty acid synthesis pathway. VLDL assembly in the liver parallels to that of chylomicrons, requires MTTP, and utilizes the complete form of apoB, termed

apoB100. Unlike apoB-48 in chylomicrons, apoB-100 contains sequences that allow it to bind to the LDL receptor. After its secretion from the liver, VLDL like chylomicrons interact with LpL. Some VLDL are partially depleted of triglyceride and then internalized by the liver. Other VLDL undergo a more complete depletion of core lipids due to both LpL and hepatic lipase digestion leading to their conversion to LDL.

3.1.3.3 Metabolism of Cholesterol-Rich Lipoproteins

Cholesterol is a component of cell membranes and is the basic molecule used for steroid hormone synthesis. Cholesterol circulates both as an alcohol (cholesterol) and as a more hydrophobic ester (cholesteryl ester). The regulation of cholesterol biosynthesis by intake of dietary cholesterol was one of the earliest proven examples of metabolic regulation. Thus, high levels of cholesterol intake reduce liver de novo cholesterol production. Both cholesterol and its metabolic product – bile – are used to emulsify fats within the small intestines, a prelude to their absorption. As a component of cell membranes, cholesterol uptake is a characteristic of rapidly growing cells. In addition, steroid hormone-producing tissues are especially important sites of uptake of circulating cholesterol. These tissues can also perform de novo cholesterol synthesis. Probably for this reason, plasma cholesterol levels have minor effects on production of adrenal and gonadal hormones. Studies illustrating factors that regulate cellular cholesterol biosynthesis and cellular cholesterol uptake have led to the development of drugs to lower plasma cholesterol and have illuminated basic mechanisms responsible for the interaction of cells with their environment. Cholesterol is synthesized from two carbon acyl groups. The enzymes that control cholesterol production are

coordinately regulated by members of the transcription factor sterol response element binding protein (SREBP) family, some of which also control triglyceride synthesis. SREBPs reside in an inactive form in the endoplasmic reticulum (ER) and are released and modified by the amount of cholesterol within the ER membrane. Once in the nucleus, they bind to the promoters of a number of genes to drive cholesterol biosynthesis and also the uptake of cholesterol from the environment via increased synthesis of the LDL receptor. The production of a circulating inhibitor of the LDL receptor that causes its intracellular degradation, proprotein convertase subtilisin/kexin type9 (PCSK9), is also driven by SREBP, perhaps serving as a damp to prevent excess LDL receptor production. *In vivo*, plasma cholesterol regulation is primarily done by the liver. And the liver is the primary site of cholesterol catabolism as it converts cholesterol into bile.

3.1.3.5 LDL Pathway

LDL are the primary source of circulating cholesterol in primates. Cellular uptake of LDL is accomplished via interaction of apoB-100 with the cell surface LDL receptor. Liver expression of this receptor regulates plasma levels of LDL. All lipoproteins are heterogeneous and vary somewhat in size, composition, and density.

This variation in size has been studied extensively in LDL. Smaller and hence denser LDL are found in the setting of hypertriglyceridemia, either due to a difference in precursor VLDL or via the result of intravascular lipid exchange. In some studies, the presence of small dense LDL is associated with greater cardiovascular risk.

3.1.3.6 Regulation of HDL

The major HDL proteins, apoA-I and apoA-II, are expressed in the gut and liver. Smaller disk like HDL are initially secreted particles. HDL mature by addition of lipid either by acquisition of surface lipid from triglyceride-rich lipoproteins as they are hydrolyzed by LpL or by transfer of cellular cholesterol into HDL by the actions of ATP-binding cassette (ABC) transporters. The cholesterol is then esterified via the actions of the plasma enzyme lecithin cholesterol acyltransferase (LCAT). HDL are catabolized in the liver and kidneys. HDL uptake can occur as whole particle endocytosis or HDL lipid can be metabolized without the accompanying protein.

Lipid uptake requires the scavenger receptor BI. Hepatic lipase and another member of this enzyme family endothelial lipase are involved in this process; these enzymes are phospholipases for HDL surface lipids. Smaller, lipid-depleted HDL and perhaps non-lipid associated apoA-I are filtered and then degraded in the kidney. Acquisition of cellular cholesterol by HDL has been used as a marker for HDL function and has been correlated with cardiac disease risk. In some situations, HDL does not mediate appropriate efflux and is termed dysfunctional HDL.

3.1.3.7 Lipid Transfer

A critical process in regulating the amount and size of HDL and LDL is mediated by cholesteryl ester transfer protein (CETP). This protein transfers cholesteryl ester in the core of LDL and HDL for triglyceride in VLDL. Since core triglyceride, unlike cholesteryl ester, can be hydrolyzed by plasma lipases (LpL and hepatic lipase), these particles can be converted to smaller denser lipoproteins. Thus, hypertriglyceridemia is usually associated with reduced HDL and small dense LDL

both because of defective lipolysis and greater CETP-mediated cholesteryl ester transfer [24].

3.1.4 Etiology

Dyslipidemia may be

Primary: Genetic

Secondary: Caused by lifestyle and other factors

Both primary and secondary causes contribute to dyslipidemias in varying degrees. For example, in familial combined hyperlipidemia, expression may occur only in the presence of significant secondary causes.

Primary causes are single or multiple gene mutations that result in either overproduction or defective clearance of triglycerides and LDL or in underproduction or excessive clearance of HDL.

Secondary causes: 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 used in some processed foods and are as atherogenic as saturated fat.

Diabetes mellitus, alcohol overuse, chronic kidney disease, hypothyroidism, primary biliary cirrhosis and other cholestatic liver diseases, drugs, such as thiazides, beta-blockers, retinoids, highly active antiretroviral agents, cyclosporine, tacrolimus, estrogen and progestins, and glucocorticoids. Secondary causes of low levels of HDL cholesterol include cigarette smoking, anabolic steroids, HIV infection, and nephrotic syndrome.

Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular disease, including CAD, stroke, and peripheral arterial disease. High levels of triglycerides (>1000 mg/dL [> 11.3 mmol/L]) can cause acute pancreatitis. High levels of LDL can cause arcus cornea and tendinous xanthomas at the Achilles, elbow, and knee tendons and over metacarpophalangeal joints. Other clinical findings seen in patients with high LDL (eg, in familial hypercholesterolemia) include xanthelasma (lipid rich yellow plaques on the medial eyelids). Xanthelasma can also occur in patients with primary biliary cirrhosis and normal lipid levels.

Patients with the homozygous form of familial hypercholesterolemia may have arcus cornea, tendinous xanthomas and xanthelasma plus planar or tuberous xanthomas. Planar xanthomas are flat or slightly raised yellowish patches. Tuberous xanthomas are painless, firm nodules typically located over extensor surfaces of joints. Patients with severe elevations of TGs can have eruptive xanthomas over the trunk, back, elbows, buttocks, knees, hands, and feet. Patients with the rare dysbetalipoproteinemia can have palmar and tuberous xanthomas.

Severe hypertriglyceridemia (>2000 mg/dL [> 22.6 mmol/L]) can give retinal arteries and veins a creamy white appearance (lipemia retinalis). Extremely high lipid levels also give a lactescent (milky) appearance to blood plasma. Symptoms can include paresthesias, dyspnea, and confusion [25].

3.1.5 Classification

Dyslipidemia was traditionally classified by patterns of elevation in lipids and lipoproteins. A more practical system categorizes dyslipidemia as primary or secondary and characterizes them by

- Increases in cholesterol only (pure or isolated hypercholesterolemia)
- Increases in TGs only (pure or isolated hypertriglyceridemia),
- Increases in both cholesterol and TGs (mixed or combined hyperlipidemia)

This system does not take into account specific lipoprotein abnormalities (eg, low HDL or high LDL) that may contribute to disease despite normal cholesterol and TG levels.

3.1.7 Diagnostic Criteria

Three key anthropometric measurements are important to evaluate the degree of dyslipidemia – Weight, Body Mass Index, Waist/hip ratio and Lipid Profile.

3.1.7.1 Body Mass Index

The body mass index (BMI), calculated as weight (kg)/height (m)², is used to classify weight status and risk of disease (Table 1).

Table 1: International Classification of Weight According to BMI

Classification	BMI (kg/m ²)	
	Principal cut-off points	Additional cut-off points
Under weight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 – 16.99	16.00 – 16.99
Mild thinness	17.00 – 18.49	17.00 – 18.49
Normal range	18.50 – 24.99	18.50 – 22.99
		23.00 – 24.99

Overweight	≥ 25.00	≥ 25.00
Pre-obese	25.00 – 29.99	25.00 – 27.49
		27.50 – 29.99
Obese	≥ 30.00	≥ 30.00
Obese Class I	30.00 – 34.99	30.00 – 32.49
		32.50 – 34.99
Obese Class II	35.00 – 39.99	35.00 – 37.49
		37.50 – 39.99
Obese Class III	≥ 40.00	≥ 40.00

3.1.7.2 Waist: Hip Ratio

Excess abdominal fat, assessed by measurement of waist circumference or waist-to-hip ratio, is independently associated with higher risk for diabetes mellitus and cardiovascular disease. Measurement of the waist circumference is a surrogate for visceral adipose tissue and should be performed in the horizontal plane above the iliac crest and Hip circumference was measured around the pelvis at the point of maximal protrusion of the buttocks. Cut points that define higher risk for men and women based on ethnicity have been proposed by the international diabetes foundation [26].

3.1.7.3 Lipid Profile

Lipid profiles are commonly used in the routine evaluation of cardiovascular risk, given the high correlations of hypercholesterolemia and hypertriglyceridemia and cardiovascular risk. A standard lipid profile includes determination of serum or plasma total cholesterol (TC), high-density lipoprotein-associated cholesterol (HDL-C), low-density lipoprotein-associated cholesterol (LDL-C), and total triglycerides (TGL).

Blood specimens should be collected after an overnight fast of 10–12 hours. This ensures that chylomicrons are cleared from plasma. In serum, the majority of

cholesterol exists as cholesterol ester. Therefore, in the first step cholesterol ester is hydrolyzed by cholesterol ester hydrolase enzyme. Then cholesterol is oxidized by cholesterol oxidase, generating cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide generated is proportional to serum cholesterol concentration and is measured by its reaction with a suitable compound, for example, 4-aminoantipyrene (reaction catalyzed by peroxidase) to form a colored dye. HDL is usually measured as HDL cholesterol after precipitating out other lipoprotein fractions using poly anions such as dextran sulfate-magnesium chloride, phosphotungstate-magnesium chloride or heparin sulfate-manganese chloride.

For serum triglyceride measurement, lipase enzyme is used, which converts triglyceride into glycerol and free fatty acid. Then glycerol is oxidized by glycerokinase into glycerophosphate. Glycerophosphate is then measured by either its reaction with nicotinamide adenine dinucleotide (NAD, no absorption at 340 nm) to form NADH (absorbs at 340 nm) or its oxidation by glycerophosphate oxidase enzyme, generating dihydroxyacetone and hydrogen peroxide.

Plasma LDL values are typically calculated with the Friedewald formula:

$$\text{LDL cholesterol} = \text{Total cholesterol} - [\text{HDL cholesterol} + (\text{Triglyceride} / 5)]$$

All measurements are in mg/dL. This formula is invalid if triglyceride values are above 400 mg/dL. In such situations direct measurement of LDL is indicated. In addition, this is only applicable for calculating LDL cholesterol in an overnight fasting specimen [26].

The current techniques for measuring the cholesterol level in the human body are by doing blood tests and the test is referred as lipoprotein profile. The lipoprotein profile is an intrusive method which causes discomfort amongst many patients.

So a non-invasive diagnostic procedure has been introduced in older days by looking into the eyes.

3.2 Iris Software

The objective of study is to explain how the presence of cholesterol in blood vessel of human being can be detected by applying iris recognition algorithm. Iris recognition is the today's most widely implemented biometric systems in use. John Daugman has developed the most widely used algorithms and most efficient techniques for iris recognition, but there have been many new methods and algorithms available today [27].

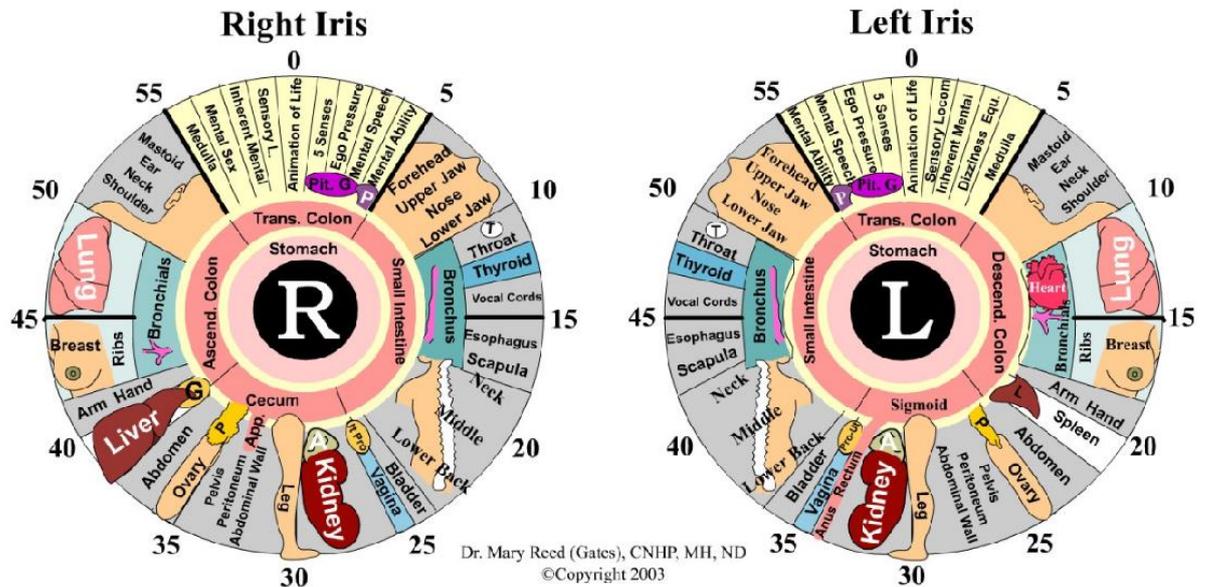


Figure 12: Iridology Chart

Based on the iris recognition methods and iridology chart (Fig. 12), a MATLAB program has been created to detect the present of cholesterol in our body. However, further analysis must be done in order to know the exact range or level of cholesterol in blood vessel [18].

3.2.1 Hough Transform

The Hough transform is a standard computer vision algorithm that can be used to determine the parameters of simple geometric objects, such as lines and circles, present in an image (Fig. 13). The circular Hough transform can be employed to deduce the radius and centre coordinates of the pupil and iris regions. An automatic segmentation algorithm based on the circular Hough transform is employed (Fig. 8). Firstly, an edge map is generated by calculating the first derivatives of intensity values in an eye image and then set the threshold base on the result.

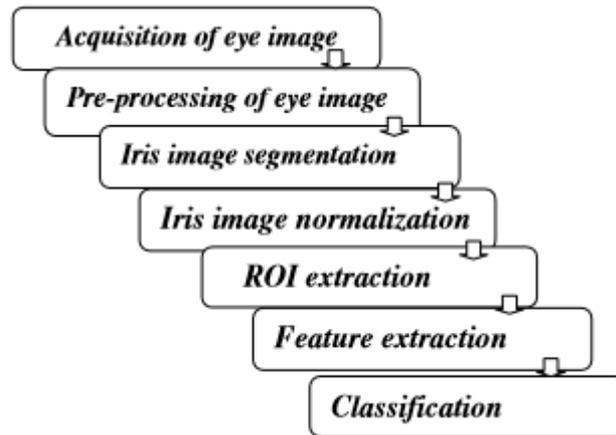


Figure 13: Steps involved in Image Extraction

The location of abnormalities on the iris is associated with the location of the medical condition in the body. The iris of the eye is divided into 60 sectors; each sector is corresponding to an inner organ. The iris is associated via multiple nerve connections to the organs. Depending on the features of the iris classification is done [28].

MATERIALS AND METHODS

4.0 MATERIALS AND METHODS

4.1 SUBJECTS

A total of 74 subjects of both gender with ages ranging between 18 – 35 years participated in the study.

4.2 SELECTION OF SAMPLE

The sample size calculation is done using the formula $4pq/d^2$ and the calculated sample size is 74 subjects using the prevalence as 20% and non-response rate as 10%. The study subjects were recruited from Government Yoga and Naturopathy Medical College Hospital, Chennai, Tamil Nadu, India. The subjects were recruited through screening done to assess diagnostic, inclusion and exclusion criteria. An interactive introductory lecture about the purpose and design of the study was explained. After obtaining the written consent, a clinical examination was performed. Patients with dyslipidemia were asked to do fasting lipid profile blood test. Of the 145 patients, only 74 subjects who reported with blood test reports were recruited. There were no drop outs in this study.

4.3 ETHICAL CONSIDERATIONS

4.3.1 Ethical Clearance: The study was convened after obtaining approval from the Institutional Ethics Committee

4.3.2 Informed Consent

Subjects who fulfilled inclusion criteria were apprised about the purpose of the study and their rights as research subjects. Informed consent form was administered in English and Tamil. Adequate time was given to each patient to go through the information sheet and their queries were answered. Their right to withdraw anytime

from the study and the need for willingness to participate voluntarily in the study was explained. The subjects who expressed their willingness to participate in the study gave a signed informed. A sample information sheet and consent form is enclosed as Annexure 1.

4.4 SCREENING OF SUBJECTS

4.4.1 Criteria for Diagnosis

The diagnosis of dyslipidemia will be based on the serum total cholesterol and triglycerides levels.

Followed Criteria

Inclusion criteria:

- Dyslipidemia patients who had a total cholesterol concentration ≥ 200 mg/dL and triglycerides ≥ 150 mg/dL
- Age group between 35 and 65 years
- Both gender

Exclusion criteria:

- Known to have CAD
- Post traumatic/post surgical condition of the eyes
- Cataract, glaucoma, etc

4.4.2 DURATION OF INTERVENTION

The duration of intervention was 6 months.

4.5 DESIGN

4.5.1 Type of design: Observational Study

4.5.2 Patient Allocation:

The patients were allocated to the study based on convenient sampling technique (N = 74).

4.6 TRIAL PROFILE

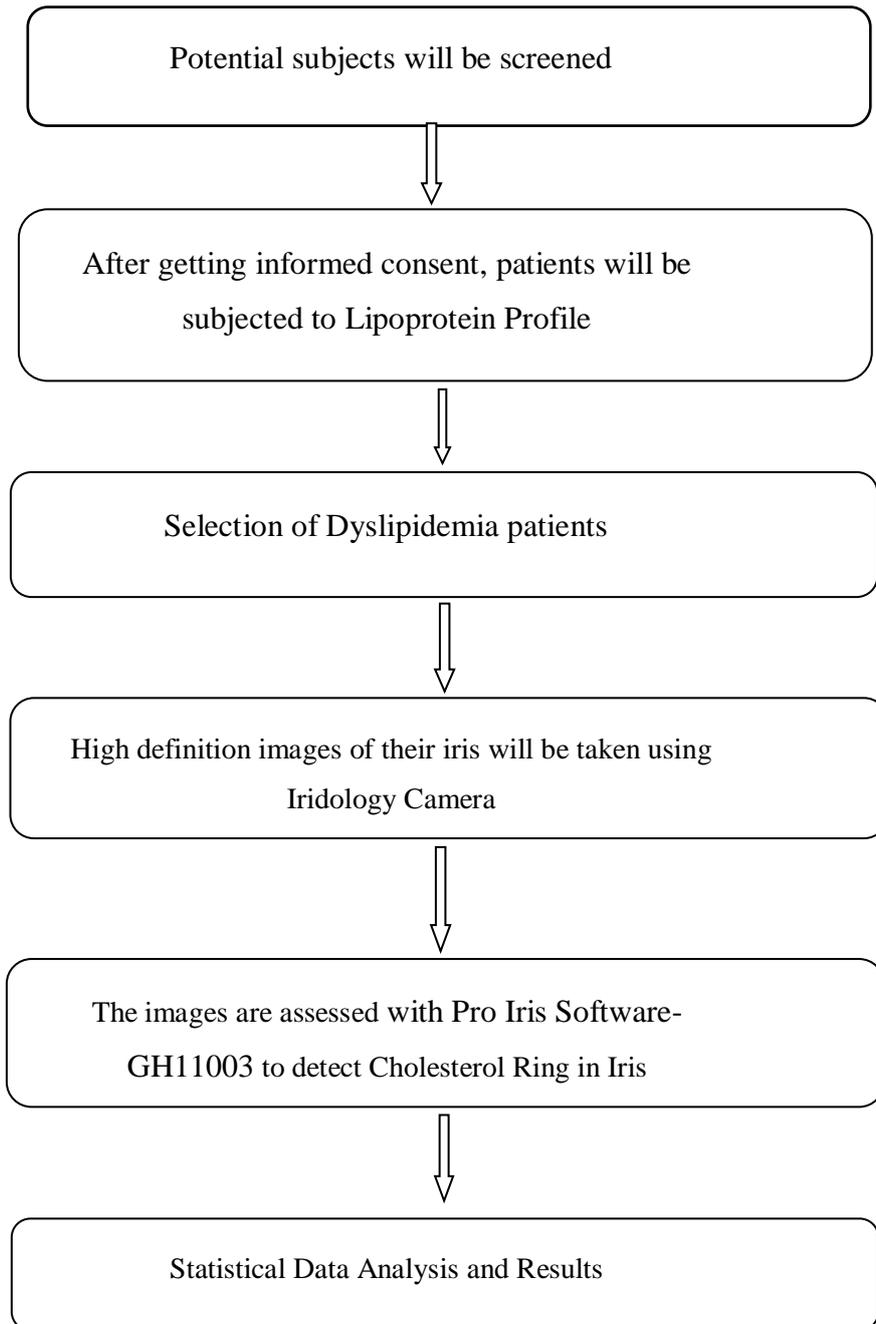


Figure 14: Illustration of the trial profile of the study.

The trial profile of the study is presented as Figure 14 which illustrates the study Plan and data analysis.

4.7 ASSESSMENTS

The baseline assessments will be done for anthropometry measurements. Table 2 demonstrates the primary and secondary outcome variables.

Table 2: List of primary and secondary outcome variables

Primary Outcome Variables	Secondary Outcome Variables
Total Serum Cholesterol	Height
Serum Triglycerides	Weight
	Body Mass Index
	Waist Hip ratio

4.7.1 PRIMARY OUTCOME VARIABLES

Upon analyzing the data, results showed significant changes in the relationship between hypercholesterolemia and the presence of cholesterol ring in iris (strong, mild and very mild); $p < 0.001$ and no significant changes in other parameters as triglycerides, diet pattern, osteoarthritis and hypertension.

4.7.2 SECONDARY OUTCOME VARIABLES

4.7.2.1 Anthropometry

Upon analysing the anthropometric data, age, height, weight and body mass index were normally distributed. Mean and standard deviation were reported accordingly. The frequency of its distribution is given in percentages.



Figure 15: Process of iris image capturing

Anthropometric measurements were recorded by trained interneers of the institute. Both the lab technician and the interneers were blinded to the groups that the subjects were recruited. The iris image capturing and analysis were done by the principal investigator of this study (Fig. 15).

4.8 DATA EXTRACTION

4.8.1 Data Collection Instrument

Data collection will be done using 2014 CE FCC NEW 5.0 MP USB IRISCOPE IRIS ANALYSER Iridology Camera (with Pro Iris Software-GH11003, Germany). This instrument has an automated program (ADCP), developed using MATLAB coding refer to Mr. Libor Masek's work. This algorithm uses a single method to determine the cholesterol sign which is using OTSU method with histogram analysis [6].

4.8.2 Data Extraction

Around 74 participants from Government Yoga and Naturopathy Medical College, Arumbakkam, Chennai were participated in the study. The physical examination emphasized measurement of height, weight and blood pressure. Body mass index was calculated by use of body weight (in kilograms) divided by height (in squared meters). The data including demographics (age, gender) and clinical findings (Hypertension, Diabetes, Osteoarthritis, Hypercholesteremia). Blood samples were collected by venipuncture after an overnight fast for 12-14 hours. Venous blood was collected in plain bulbs for measurement of serum lipids. Total Cholesterol and Triglyceride concentrations were measured by routine enzymatic methods. Measurements were carried out in a clinical laboratory that followed the criteria of the WHO Lipid Reference Laboratories followed by taking High Definition Images of Iris of the patients using 2014 CE FCC NEW 5.0 MP USB IRISCOPE IRIS ANALYSER Iridology Camera (with Pro Iris Software-GH11003, Germany). It is vital to isolate this part (iris) from the whole unwanted part in the eye (sample).

This separation or segmentation is the process of removing the outer part of the eye (outside the iris circle), in order to get solid image of iris that is useful for the localization of cholesterol lipid and this will be done by Hough Transform (a standard computer vision algorithm that can be used to determine the parameters of simple geometric objects, such as lines and circles). This process comprises the following actions:

- Eye images of the patients will be acquired using 2014 CE FCC NEW 5.0 MP USB IRISCOPE IRIS ANALYSER Iridology Camera.
- Process of pupil and iris localization and segmentation, to classify the required region.
- Attain normalization iris from circular shape to rectangular shape with full image.
- Crop the normalization iris to 30% from full image
- Analyze the normalization iris to get the histogram value.
- Using OTSU to calculate the optimum threshold to detect Cholesterol presence.
- Cholesterol ring detected or not detected will be displayed in MATLAB window.

The accuracy of an iris analysis is greatly enhanced through the use of advanced instrumentation. Photographic equipment to record the iris is presently improved much of a quality never before available. A photograph is taken of each iris serve as a record to compare with present population and in the future.

There are evidences of research papers regarding the accuracy of the equipment [29] used to capture and record the iris images [6, 13, 17]. Of these, 2D Gabor filter

based texture feature extraction and Histogram using OTSU threshold method has been used for recognition of the irises [29].

After confirmation of diagnosis, patients will be suggested to undergo Naturopathy and Yoga treatments. Using the IRIDOLOGY chart, we assess the progress of tissue strength achieved through naturopathy, the extent of healing signs. Observations will also be made on other changes if any [7]. The data was collected as self-reported observations using primary and secondary outcome variables. The assessment for primary outcome variable was done on the first day. The data was organized in Microsoft Excel Sheets (Version 2010).

RESULTS

5.0 RESULTS

The present study was conducted to determine the presence of cholesterol ring in the iris of dyslipidemia patients in percentages and also to find out the degree of ring grades.

5.1 STATISTICAL ANALYSIS

The statistical analysis was performed using the SPSS (version 16.0). Total Cholesterol and Triglycerides levels were expressed as the mean \pm SD. The normality of the data was checked by the Shapiro-Wilk procedure. As the underlying data distribution is normal. Pearson's chi square test was applied in comparisons of independent and dependent proportions. A p value <0.05 was considered deemed significant. Prevalence of dyslipidemia by means of its determinants was calculated using the prevalence rate formula: number of patients per total number of all subjects at the time of study multiplied by 100. Results were expressed as percentages.

The statistical analysis was performed using the SPSS (version 16.0). Total cholesterol and triglyceride levels were expressed as the mean \pm SD. The data was further categorized according to age group and gender.

For continuous data such as age, the descriptive statistics n, Mean, SD, Median was presented. For categorical data, the number of participants and percentage was presented.

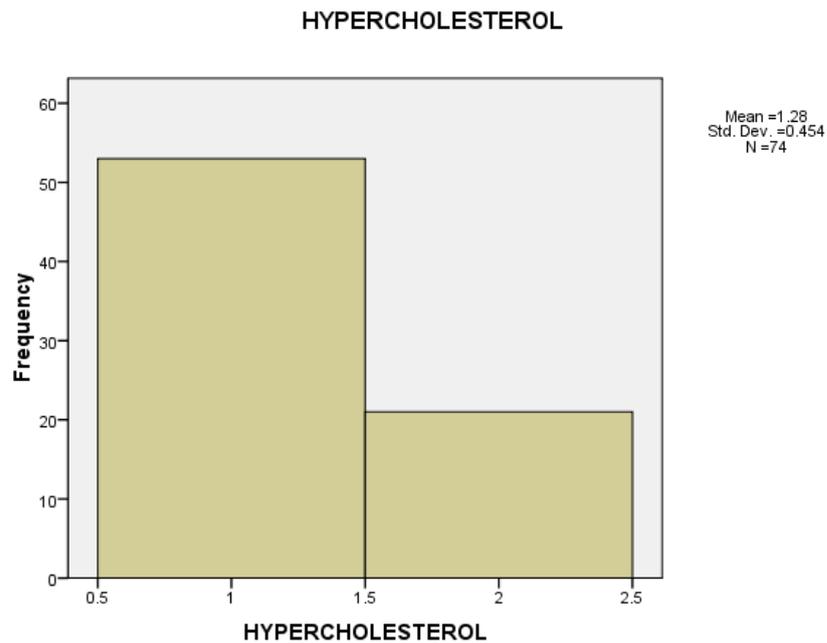


Figure 16: Histogram shows the sample size with no missing values

First, $N = 74$, means that the histogram is based on 74 cases. Since this is the sample size, we conclude that no missing values are present (Fig. 16). SPSS also calculates a mean and standard deviation but these are not meaningful for nominal variables

5.1.1 NORMALITY

The frequencies of Age, sex, height, weight, body mass index and total cholesterol levels were distributed normally and this has been illustrated below.

Normality test was done by summary statistics (Figs 17-21).

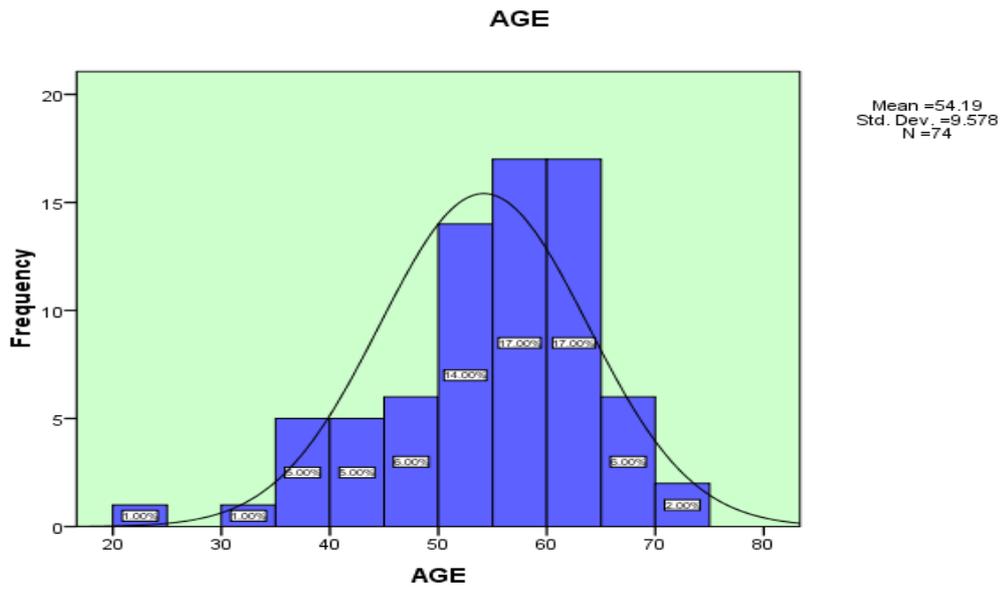


Figure 17: Normal distribution of frequency of age

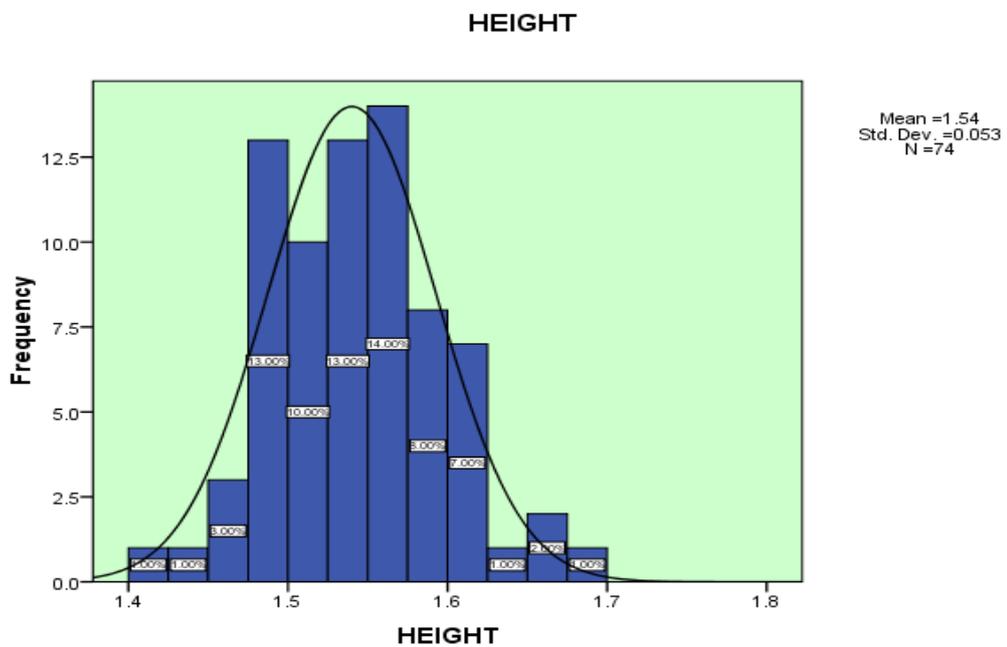


Figure 18: Normal distribution of frequency of height of the participants

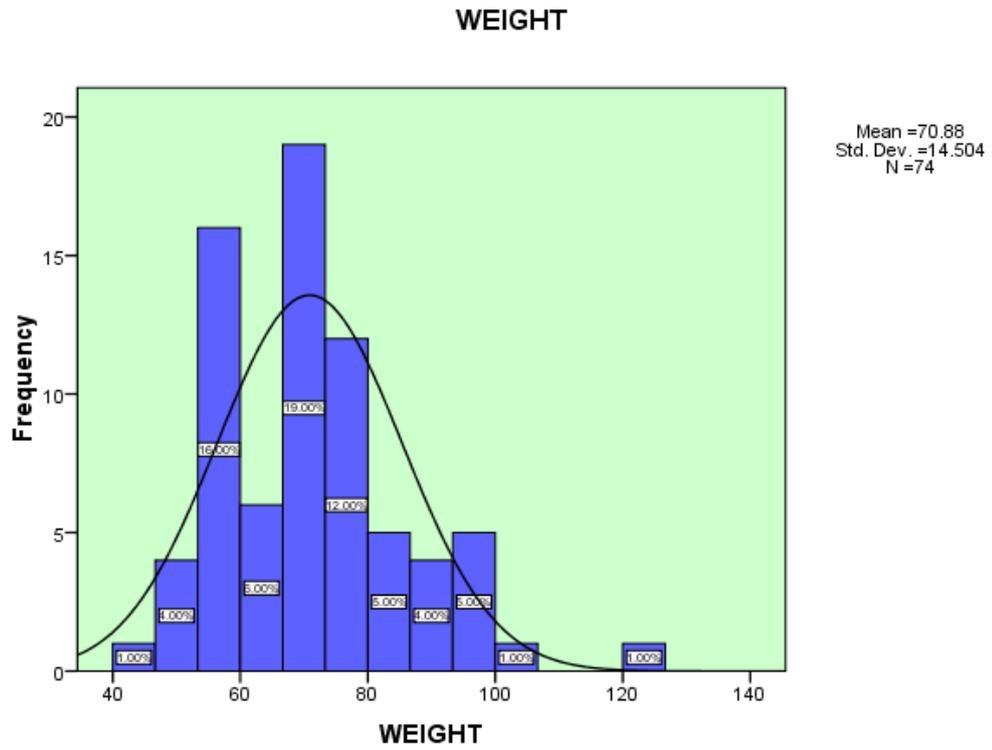


Figure 19: Normal distribution of frequency of weight of the participants

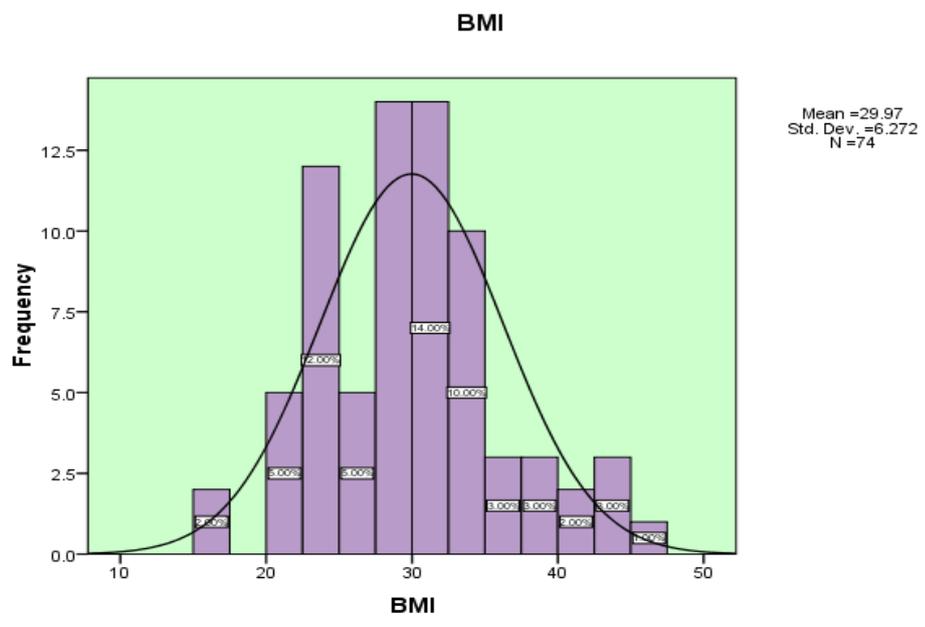


Figure 20: Normal distribution of frequency of body mass index of the participants

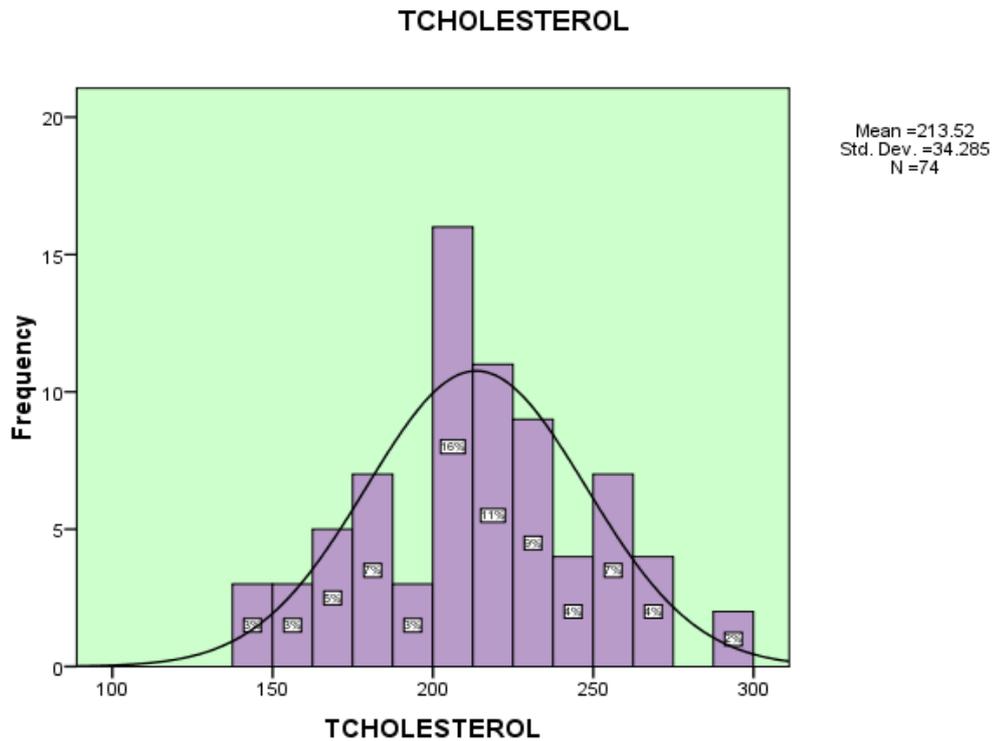


Figure 21: Normal distribution of frequency of Total cholesterol levels of the participants

For continuous data, the descriptive statistics were reported as mean (standard deviation) (Table 3). For skewed data, the summary was reported as percentage for categorical data.

Statistical values are presented as Mean \pm SD, categorical data presented as n (%).

Table 3: Demographic and other characteristics of participants

PARAMETER	MEDIAN	MEAN ± SD (N = 74)
Age (years)	56.00	54.19 ± 9.578
Height (cm)	1.53	1.54 ± .053
Weight (kg)	70.00	70.88 ± 14.504
BMI	29.75	29.97 ± 6.272
Diet (veg/non-veg)	2.00	1.74 ± .440
TGL (mg/dL)	115.00	124.29 ± 76.959
T. Chol (mg/dL)	212.50	213.52 ± 34.285
Diabetes	2.00	1.78 ± 0.414
Hemiplegia	2.00	1.95 ± 0.228
Hypertension	2.00	1.66 ± 0.476
Osteoarthritis	2.00	1.53 ± 0.503

BMI body mass index, *TGL* triglycerides, *T.Chol* total cholesterol, *HChol* hypercholesterolemia, *HTN* hypertension, *OAK* osteoarthritis

5.1.2 FREQUENCIES IN PERCENTILES

In this study, Frequency distributions are portrayed as Pie-charts (Figs 22-29). The study included 74 participants, of whom 20.27% were male and 79.73% were female.

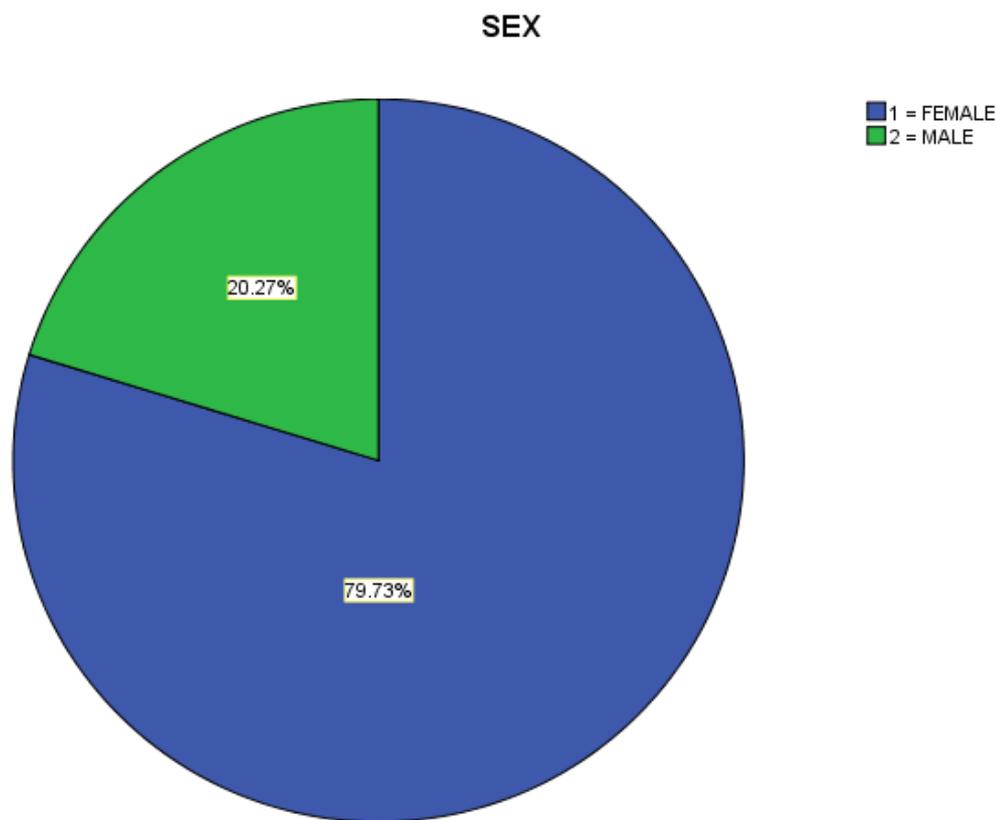


Figure 22: Frequency of sex

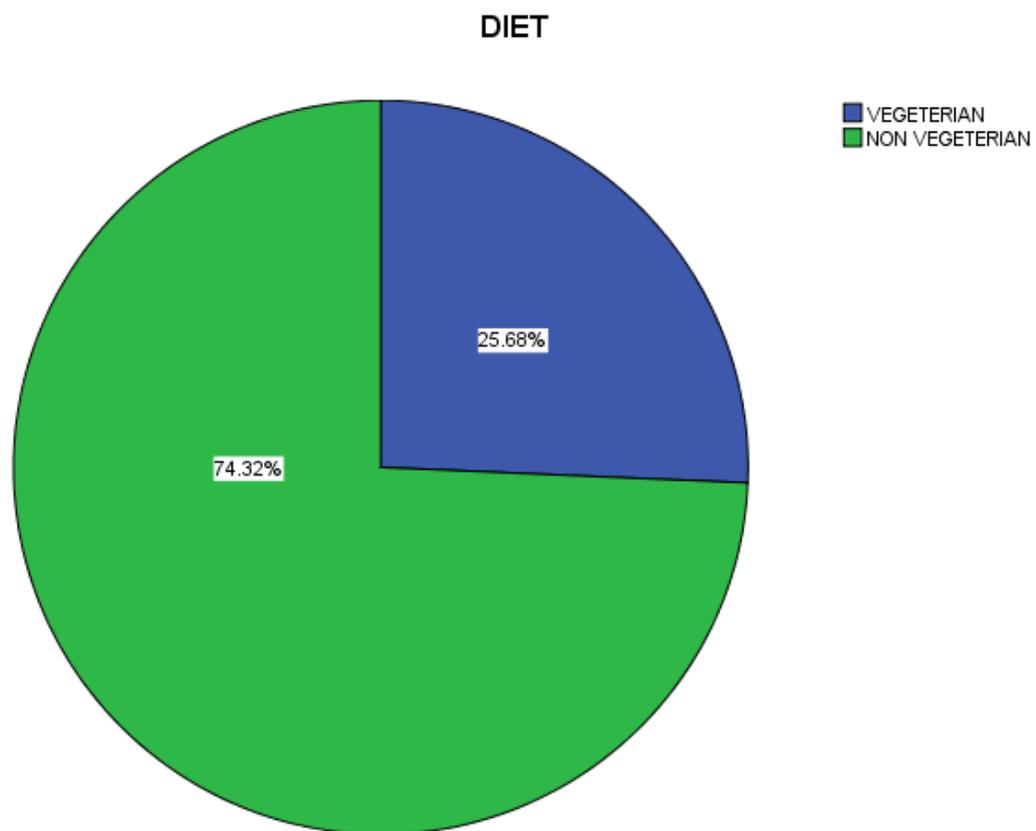


Figure 23: Frequency of veg/non-veg

In the current study, based on the diet the participants were categorised as 25.68% Vegeterians and 74.32% Non-vegeterians.

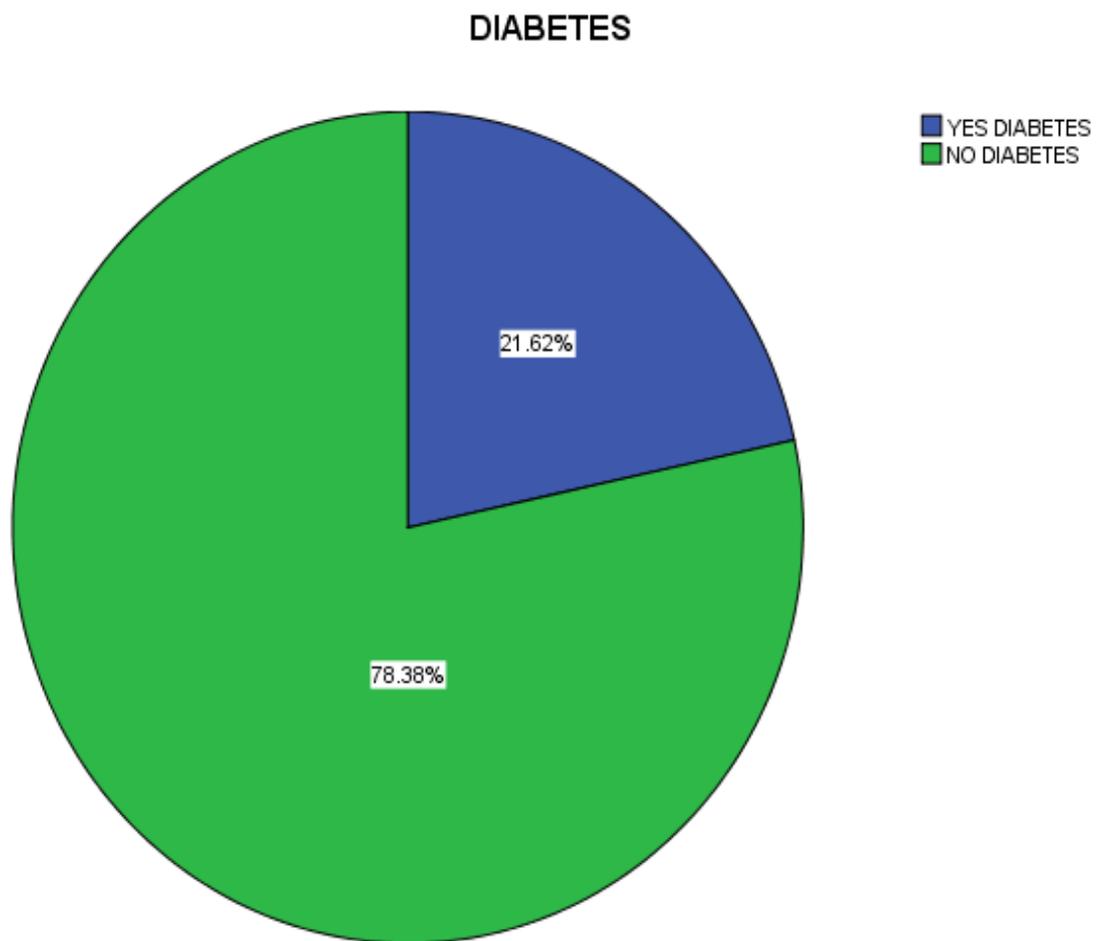


Figure 24: Frequency of diabetes

The collected data showed that among the participants 21.62% were diabetes patients and 78.38% were non-diabetes patients.

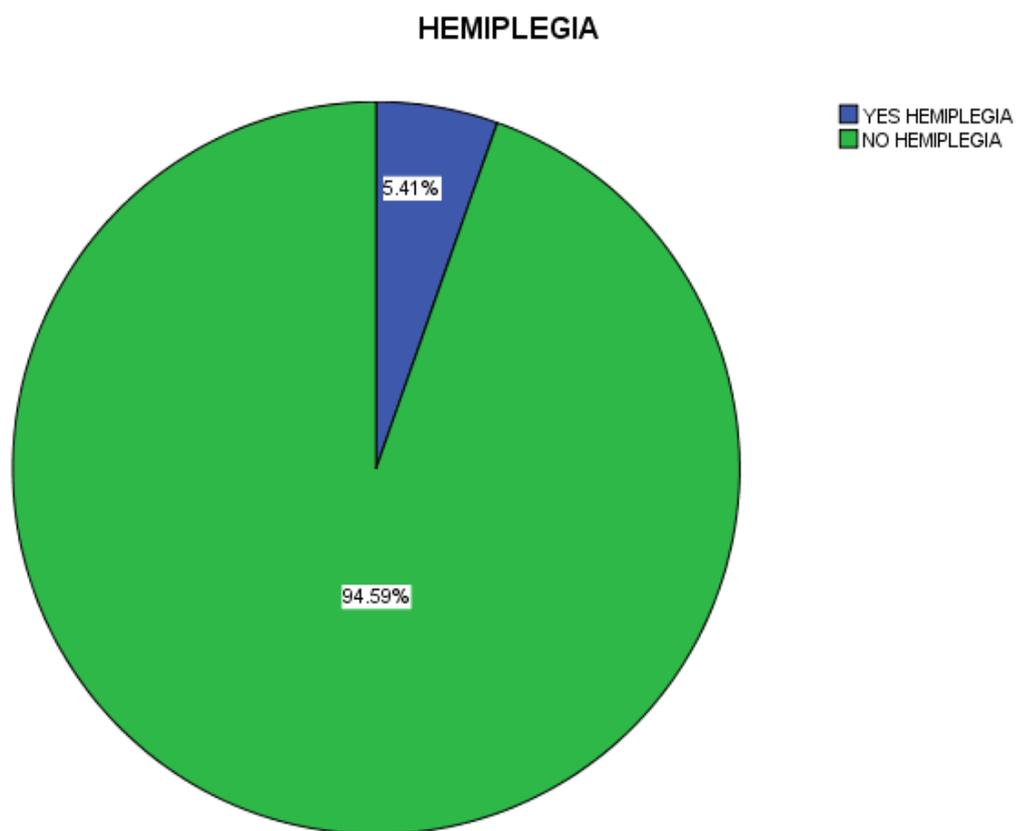


Figure 25: Frequency of Hemiplegia

The collected data showed that among the participants 5.41 % were hemiplegia patients and 94.59% were non-hemiplegic patients.

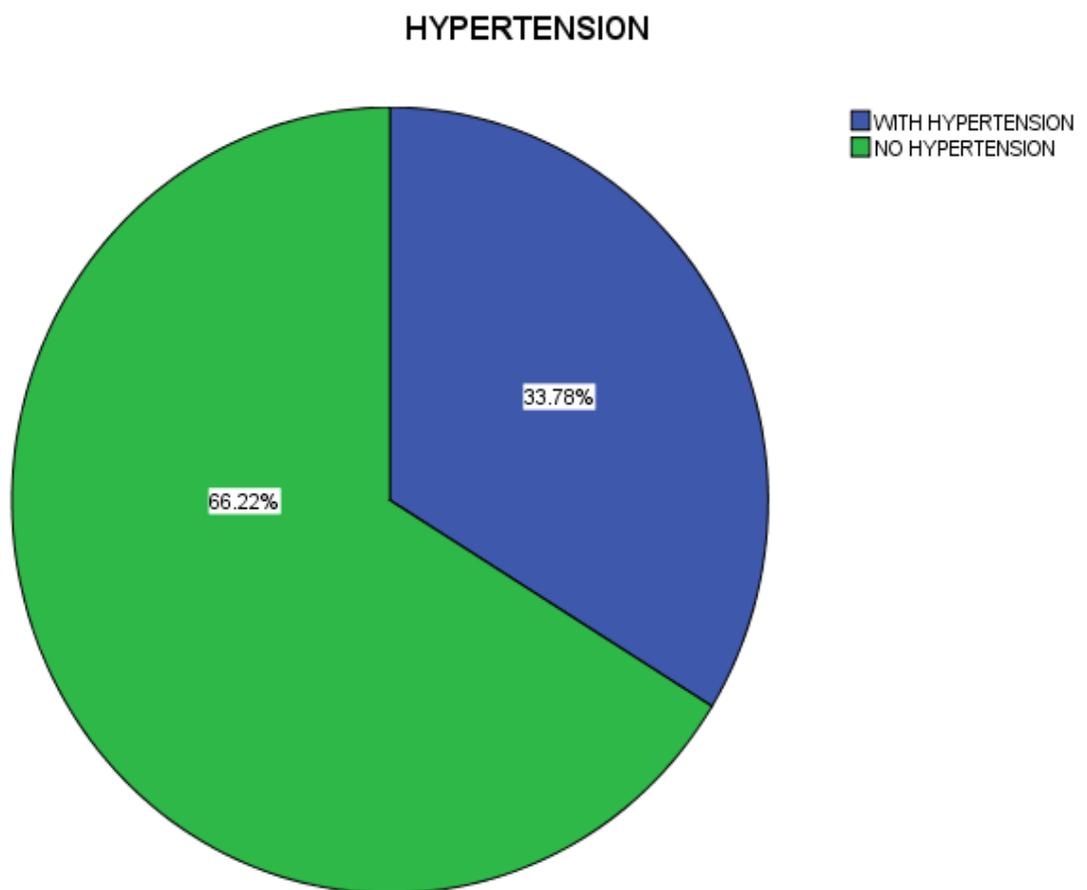


Figure 26: Frequency of Hypertension

The collected data showed that among the participants 33.78 % were hypertensive patients and 66.22 % were non- hypertensive patients.

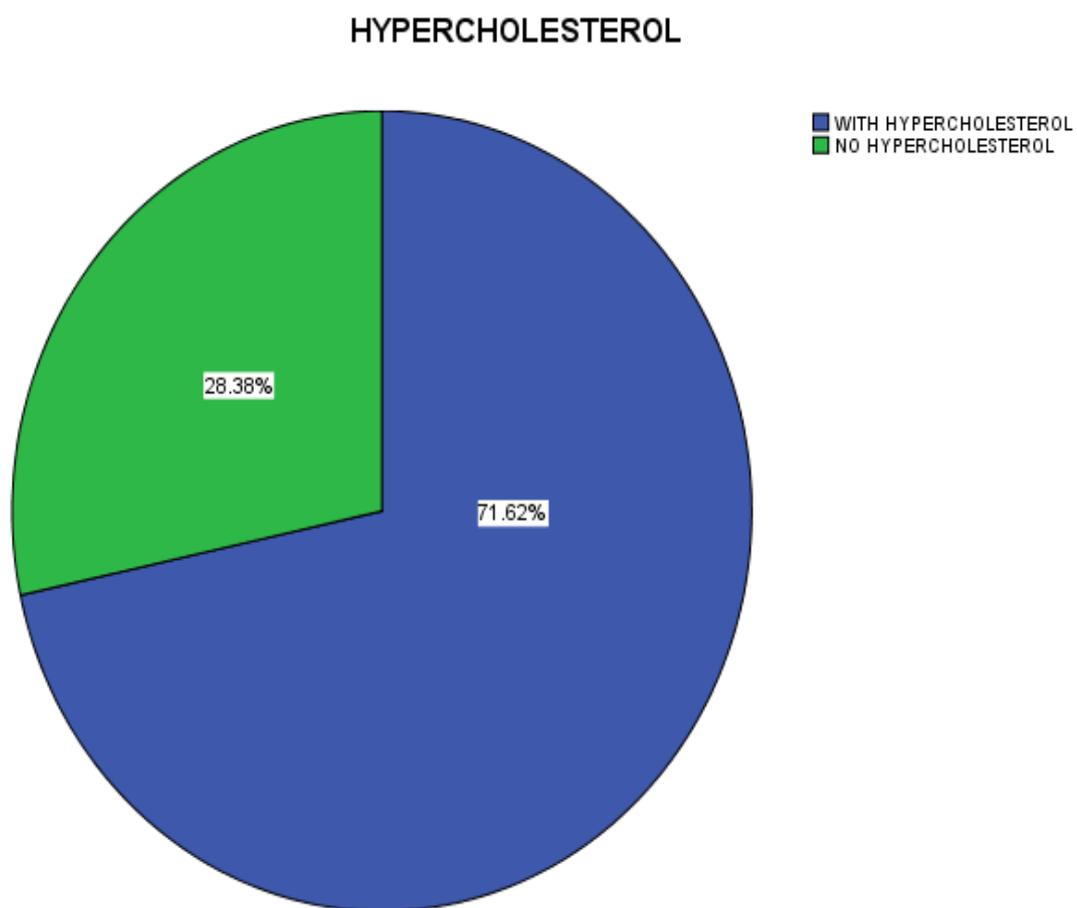


Figure 27: Frequency of Hypercholesterolemia

The collected data showed that among the participants 71.62 % were hemiplegia patients and 28.38 % were non-hemiplegic patients.

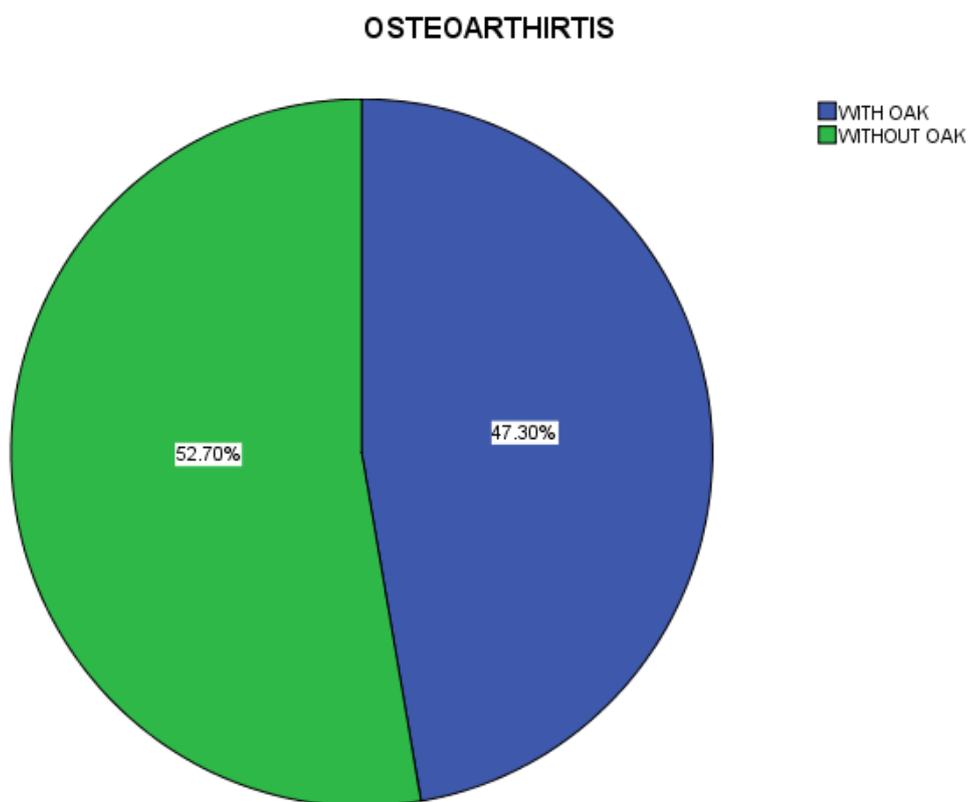


Figure 28: Frequency of Osteoarthritis

The collected data showed that among the participants 47.30 % were osteoarthritis patients and 28.38 % were without osteoarthritis patients.

CHOLESTEROL RING GRADING

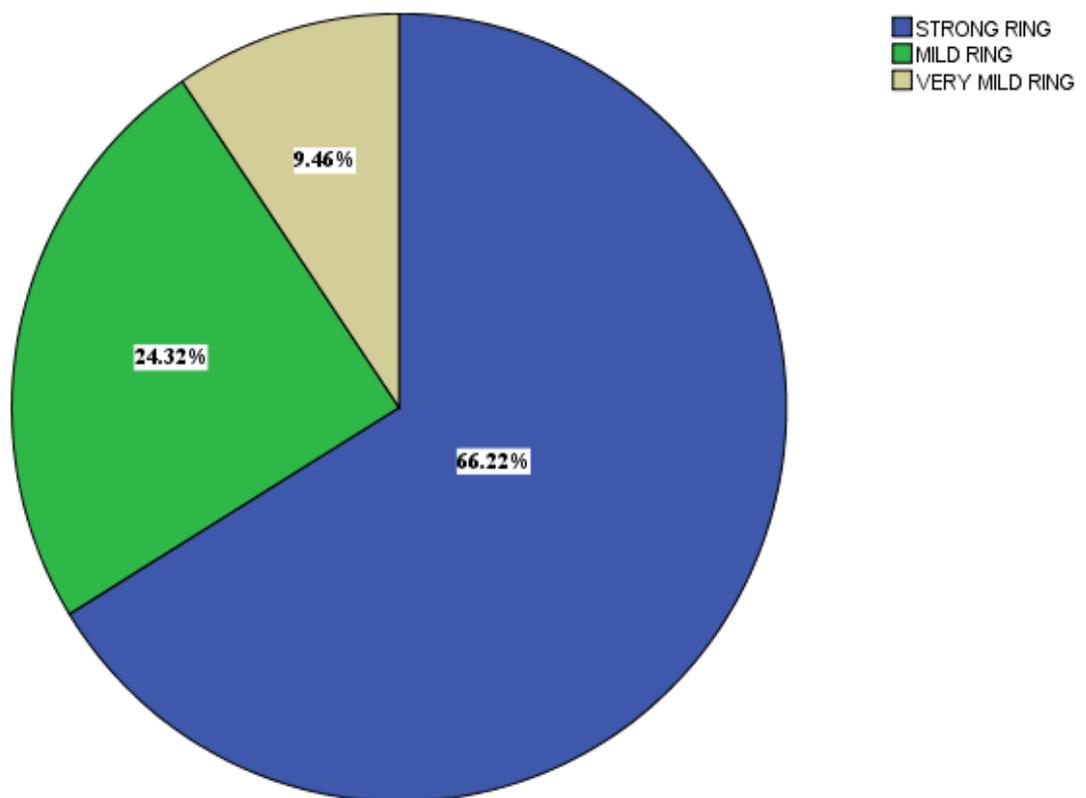


Figure 29: Pie chart showing the frequency of cholesterol ring grade in percentiles.

The collected data showed that among the participants 66.22 % were with strong cholesterol rings in their iris, 24.32 % were with mild cholesterol rings and 9.46% of patients were with very mild rings in the iris.

5.1.3 NON-PARAMETRIC CORRELATIONS

As two-tailed Pearson Correlation does not hold the Assumptions such as interval or ratio level; linearly related; bivariate normally distributed, Spearman's rank correlation (both single tailed and two tailed) has been used in this study (Tables 4 and 5).

Table 4: Two-tailed Spearman's rho Correlation table to show correlation between hypercholesterolemia and Cholesterol rings

Parameters	Spearman's rho Correlation	Hypercholesterol	Ring Grade
Hypercholesterol	Correlation Coefficient	1.000	.206
	Sig. (2-tailed)	.	.078
	N	74	74
Ring Grade	Correlation Coefficient	.206	1.000
	Sig. (2-tailed)	.078	.
	N	74	74

The two tailed Spearman rho correlation coefficient value of 0.206 confirms that there appears to be a positive correlation between the two variables (hypercholesterolemia and cholesterol ring grades).

There is an evidence to show that statistically significant change over the time points for the above mentioned parameters ($p = 0.07$).

Table 5: Correlation between hypercholesterolemia and Cholesterol rings

Spearman's rho Correlations		Hypercholesterol	Ring Grade
Hypercholesterol	Correlation Coefficient	1.000	.206*
	Sig. (1-tailed)	.	.039
	N	74	74
	Correlation Coefficient	.206*	1.000
Ring Grade	Sig. (1-tailed)	.039	.
	N	74	74

*Correlation is significant at 0.05 level (1-tailed).

The single tailed Spearman rho correlation coefficient value of $r = 0.206$ confirms that there appears to be a positive correlation between the two variables (hypercholesterolemia and cholesterol ring grades).

There is an evidence to show that statistically significant change over the time points for the above mentioned parameters ($p = 0.03$, $N=74$).

5.1.4 CROSS TABULATION

In this study, Cross tabulation is done to understand the relationship between two categorical variables (Figs 30-34).

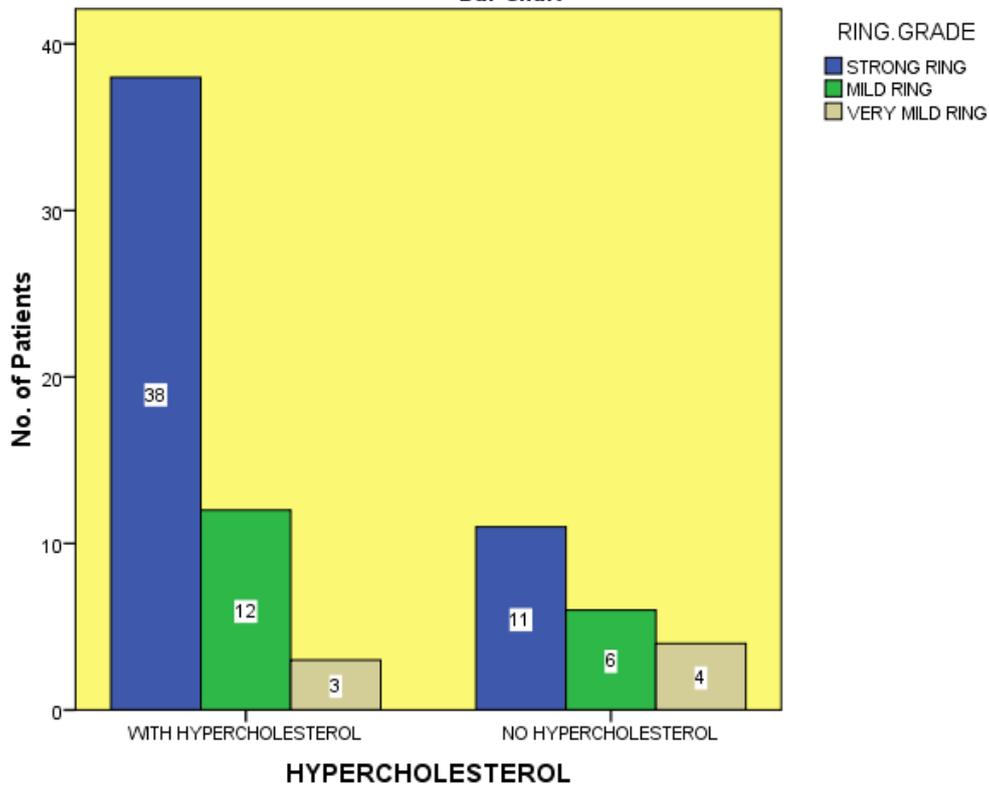


Figure 30: The bar chart illustrates the relationship between hypercholesterolemia and ring grades

The study shows that the number of cholesterol patients with strong white ring is 51.4% (expected value : 35.1 and the observed value is 38); the number of hypercholesterol patients with mild rings is 16.2% (expected value: 12.9, observed value: 12) and the number of hypercholesterol patients with mild rings is 4.1% (expected value: 5, observed value: 3). Finally it was shown that out of 100% hypercholesteremia, 71.6% were noticed with Cholesterol rings in their iris.

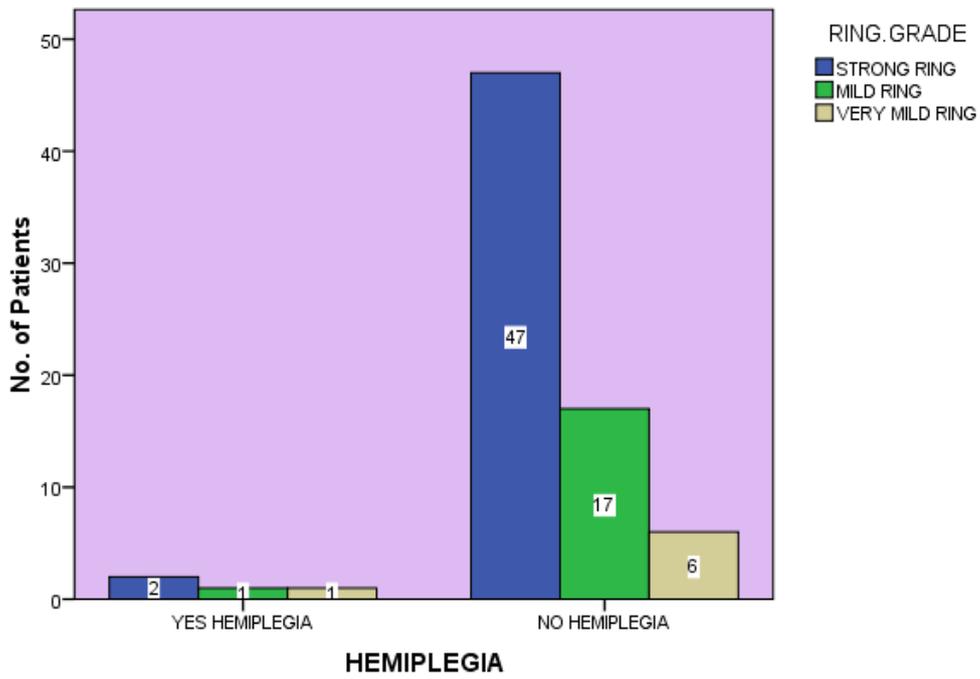


Figure 31: The bar chart illustrates the relationship between hemiplegia and ring grades

There is no enough evidence to show that there is statistically significant change observed between Cholesterol ring and Hemiplegia.

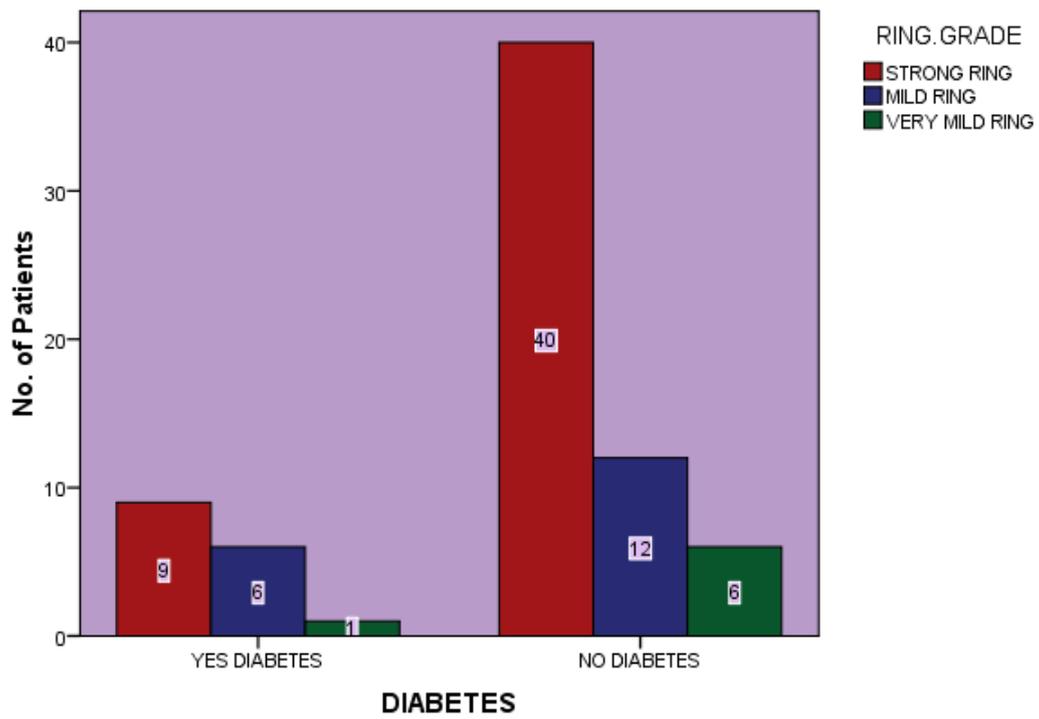


Figure 32: The bar chart illustrates the relationship between Diabetes and ring grades

There is no enough evidence to show that there is statistically significant change observed between Cholesterol ring and Diabetes

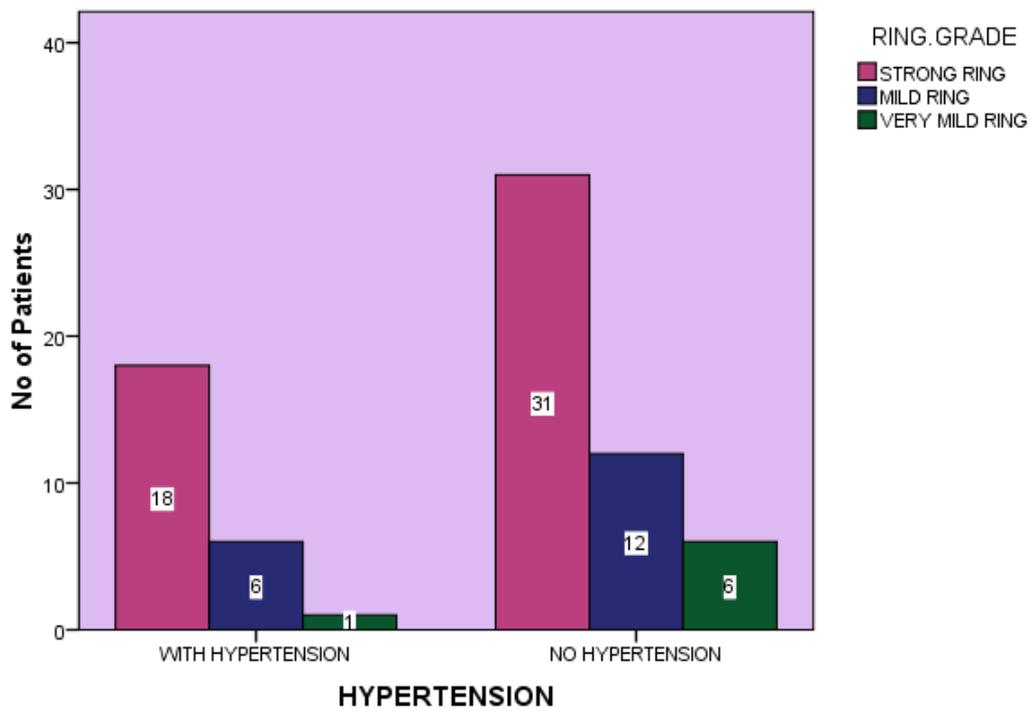


Figure 33: The bar chart illustrates the relationship between hypertension and ring grades.

There is no enough evidence to show that there is statistically significant change observed between Cholesterol ring and Hypertension

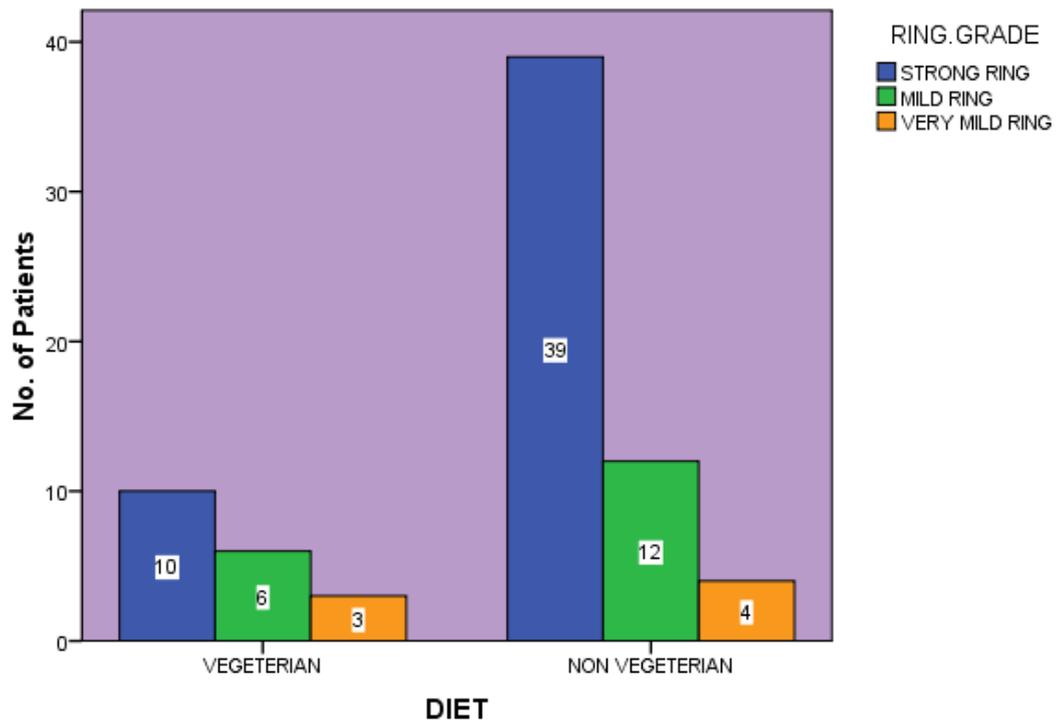


Figure 34: The bar chart illustrates the relationship between Diet pattern and ring grades.

There is no enough evidence to show that there is statistically significant change observed between Cholesterol ring and Diet pattern.

5.1.5 ONE SAMPLE CHI-SQUARE TEST-HYPOTHESIS TESTING

Here we consider hypothesis testing with a discrete outcome variable in a single population (Tables 6a and 6b).

Table 6a: Test statistics using one-sample chi-square test.

	Observed N	Expected N	Residual
STRONG RING	49	24.7	24.3
MILD RING	18	24.7	-6.7
VERY MILD RING	7	24.7	-17.7
Total	74		

Table 6b: Chi square test

	RING.GRADE
Chi-Square	38.459 ^a
Df	2
Asymp. Sig.	.000

- a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 24.7.

Interpretation

HYPOTHESIS

H₀: There is no association between hypercholesterolemia and presence of cholesterol ring in iris.

H1: There is an association between hypercholesterolemia and presence of cholesterol ring in iris.

Critical chi-square statistic value for $p = 0.01$ (95% confidence level) with 2 degree of freedom is 9.12.

Since $38.459 > 9.12$, then REJECT Null hypothesis and so there is strong evidence to say the study is statistically significant ($p < 0.001$).

Under observed N, the observed frequencies are shown and under expected N, shown the theoretically expected frequencies. For each frequency the **Residual** is the difference between the observed and the expected frequency and thus expresses a deviation from the null hypothesis.

There's a 0.1% chance of finding the observed frequencies or a larger deviation from the null hypothesis. We usually reject the null hypothesis if $p < .05$. So we conclude that the observed cholesterol ring grades was significantly different than hypothesized proportion [$\chi^2 (2) = 38.45; p = 0.001$].

DISCUSSION

6.0 DISCUSSION

The study revealed that the presence of cholesterol rings in the iris of dyslipidemia patients is approximately about 71.6%. This study tried to analyse not only the relationship between cholesterol rings and hypercholesterolemia but also between other parameters as osteoarthritis, hypertension, hemiplegia, diabetes and finally serum triglycerides. There is no enough evidence to show the relationship between cholesterol ring in the iris and the other above mentioned parameters in this study. Although dyslipidemia is considered as the alterations in serum triglycerides and total cholesterol level, the study revealed that the alteration in total cholesterol is the only offender that make the appearance of cholesterol ring in the iris of dyslipidemia patients. According to Ramlee et al, it was said that cholesterol rings is caused by extracellular lipid deposition in the peripheral cornea and this has been evidently proved in this study. Thus the iris alert us to the early signs of approaching serious conditions.

6.1 LIMITATIONS

- Fasting lipid profile was not done due to financial issues.
- The sample size is relatively smaller.
- Hence, generalizing the study outcomes to a larger population would not be definitely conclusive.

6.2 DIRECTION FOR FUTURE RESEARCH

- Fasting lipid profile can be taken for complete analysis.
- The study can be performed with larger sample size.
- Since the present study had no control group, further studies with randomized control trials are required to substantiate these results.

- The effectiveness of image acquisition can be done with a software using Graphic User Interface (GUI), where MATLAB has tool to perform it. Result from MATLAB will display the string “Sodium ring is detected” or “no sodium ring is detected” depends on the eye image that be examined.
- The patients can be subjected to Naturopathic and Yogic treatments and the extent of healing signs and any other changes in the iris can be recorded for further study on similar subjects.

CONCLUSION

7.0 CONCLUSION

The present study showed that the presence of cholesterol ring in iris of the participants is an indicators of hypercholesterolemia and the result was 71.6% positive correlation between serum increased cholesterol level and cholesterol ring appearance. However, the other parameters like osteoarthritis, hypertension, hemiplegia, diabetes and finally serum triglycerides does not show positive correlation. Further research with a larger sample size is warranted to reveal more beneficial changes in this field. Today, people are in need of preventative health care and less complex methods of analysing their condition. Iridology provides a non-invasive, painless and economical means of looking into the body, which may be utilized in conjunction with any other system of analysis or diagnosis available.

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ANNEXURE

9.0 ANNEXURE

9.1 INFORMATION SHEET

Investigator: Dr. Y. Rosy Ayda

Name of Participant: _____

Study title: Cholesterol ring in iris: a noninvasive diagnostic evaluation for dyslipidemia

You are invited to take part in this research study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns. You are being asked to participate in this study being conducted in Government Yoga & Naturopathy Medical College & Hospital, Arumbakkam, Chennai – 600 106.

The purpose of the research study is to determine the presence of cholesterol ring in dyslipidemia patients of Government Yoga and Naturopathy Medical College, Arumbakkam.

Study Procedure

This observational study was carried out among 60 adults of both the sexes aged between 35 and 65 years. An interactive introductory lecture about the purpose and design of the study will be explained to Subjects. After obtaining the written consent (bilingual), fasting lipoprotein profile which includes total plasma cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) will be performed to rule out cholesterol level followed by taking

High Definition Images of Iris of the patients using 2014 CE FCC NEW 5.0 MP USB IRISCOPE IRIS ANALYSER Iridology Camera (with Pro Iris Software-GH11003, Germany).

Possible risks to you: Nil

Possible benefits to you: Nil

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, IEC and any person or agency required by law to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision to not participate in the study affect you?

Your decisions to not to participate in this research study will not affect your studies or your relationship with investigator or the institution.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during course of the study without giving any reasons.

However, it is advisable that you talk to the research team prior to stopping the participation.

The results of the study may be intimated to you at the end of the study period.

Signature of investigator

Signature of participant

Date:

9.2 INFORMED CONSENT FORM

Title of the study: Cholesterol ring in iris: a noninvasive diagnostic evaluation for dyslipidemia

Name of the Participant: _____

Name of the Principal Investigator: Dr. Y. Rosy Ayda

Name of the Institution: Government Yoga & Naturopathy Medical College & Hospital, Arumbakkam, Chennai – 600 106

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in the study titled, “**Cholesterol ring in iris: a noninvasive diagnostic evaluation for dyslipidemia**”

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.

4. I have been explained about my rights and responsibilities by the investigator.

5. I have been advised about the risks associated with my participation in this study.

7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

9. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

10. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

11. I have understood that my identity will be kept confidential if my data are publicly presented.

12. I have had my questions answered to my satisfaction.

13. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature of the participant

Name _____

Signature _____

Date _____

Name and Signature of the investigator or his representative obtaining
consent:

Name _____

Signature _____

Date _____

5. □□□□ □□□□ □□□□□□□□ □□□□□□□□ □□□□□□□□□□
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6. எந்தவொரு காரணத்திற்காகவும், என் அனுமதியின்றி எந்த நேரத்திலும், ஆய்வில் இருந்து என் பங்கேற்பு நீக்கப்படும் என்று எனக்குத் தெரியும்.

7. இந்த ஆய்வில் பங்குதாரர்கள், ஒழுங்குமுறை அதிகாரிகள், அரசுக்கு பங்களித்ததன் விளைவாக கிடைத்த தகவலை வெளியிட முதன்மை ஆராய்ச்சியாளருக்கு அனுமதி வழங்கப்படுவதை நான் அறிந்திருக்கிறேன்.

பங்கேற்பாளரின் பெயர் மற்றும் கையொப்பம்:

தேதி-----

முதன்மை ஆராய்ச்சியாளர் கையொப்பம்:

(பெயர்: □□□. □□. □□□□ □□□)

9.3 IRIDOLOGY REPORT SAMPLE

Iridology Analysis Report



Name : S VIJAYA

Sex : Female

Age : 52

Type : Iris

Date : 12/15/2017

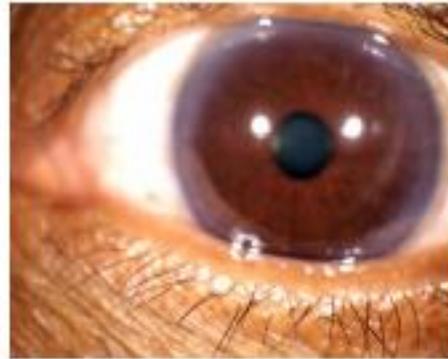
This analysis result is for reference only!

Right Eye : Right Eye20171215104513.jpg

Left Eye : Left Eye20171215104551.jpg

Date : 10:45 15/12, 2017

Date : 10:45 15/12, 2017



Item 1
Eye : Right Eye
Location: Back
Color: Light
Shape: Lacuna
Symptom: Abdomal uncomfort, Constipation, Diarrhea etc. Symptom
Trend: Cervical spondylosis etc. Diseases
Recommend:
Recreation:
Item 2
Eye : Right Eye
Location: Pituitary Gland
Color: Light
Shape: Lacuna
Symptom: Myalgia ,Headache ,Sexual ability deficiency etc. Symptom
Trend: Pituitary function imbalane etc. Disease
Recommend:

Item 1
Eye : Left Eye
Location: Pancreas
Color: Light
Shape: Lacuna
Symptom: Sever pain in upper abdomen & back ,Hyperglycaemia etc. Symptom
Trend: Acute pancreatitis etc. Disease
Recommend:
Recreation:
Item 2
Eye : Left Eye
Location: Adrenal Gland
Color: Light
Shape: Lacuna
Symptom: Menstrual disorder, Sterility change in body shape etc. Symptom
Trend: hyperadrenocorticism etc. diseases
Recommend:

Specification of Iris Camera Used in this Study



About the Product

- Features: * Nice appearance and innovative design * LED illuminator around lens * Imported lens with plated layer * 5.0 Mega pixels high resolution sensor * Special DSP image processor, Optical Image Stabilizer * Single capture button and digital pause capture. * Single/Dual lamp control. * Adjustable focus to give clear image. * Auto white balance and contrast adjustment, Color Temperature
- * Dual image compare function * 3D-Negative capture mode * Compatible with iris lens, hair lens and skin lens. * Deliver clear and accurate images. * Easy to operate. * Maximum resolution: 2560x1920 * OS: Windows XP, WIN2000, 2003, Vista, Win7, Win8
- * Iris analysis system: international technology, unique functions. * Iris analysis system is a medicinal tool that checks the body conditions and prevents diseases from occurring. * We brought in the advanced iris analysis technology from Germany to

lead people to discover sources of illness, and care the body health and spirit in anyways.

- * The instrument can show the body conditions of customers and suggest customers the suitable health food, and the plans to care their bodies. * Support for the mouse fixed image, open source, supports most of the equipment. 1) Advanced auto iris analysis technology to provide the best help for beginners to learn. 2) Software easy to use, help you to command. 3) Recommend to use of 1024×768 resolution, it will be the best. 4) Compatible with Windows XP/2000/Vista/7 32bit and 64bit system.
- This products have passed FCC and CE certification.