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Characterization of Diabetic and Non Diabetic Human Nuclear Cataract using Optical Spectroscopy Techniques

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ABSTRACT

Aim: The aim of present study is to understand the mechanism of calcification and deposition of how protein and carbohydrate deposits in Diabetic and Non Diabetic induced Nuclear Cataract Lenses.

Methods: This Experimental comparative study conducted on Twenty six cataract samples after Small Incision Cataract Surgery (SICS) collected and stored in formalin and saline solution to avoid contamination. The Cataract lens specimens were dried for one week at 25 °C, under specific Relative Humidity (RH) conditions, and then used for spectral investigation to determine its biophysical characteristics and molecular composition by using FTIR spectroscopy and X-ray Diffraction technique.

Results: FTRI technique on the diabetic nuclear cataracts we have found unsaturated aldehydes groups are found at a frequency of 1688(narrow peak) and also aldehydes are found at a frequency of 2823 (medium peak) and XRD technique resulted that there is difference in the presence of electron density in different samples and mainly in the diabetic nuclear cataracts and non diabetic nuclear cataract.

Conclusion: We can understand that if we can be able to reduce aldehydes groups' formation in the diabetic cataract lenses then we can be able to reduce the formation of cataract in diabetic patients. The X-ray Diffraction techniques conclude that Diabetic Lenses have different electron density which is different from Non Diabetic Cataract lenses.

Clinical Significance: The significance of this study explained about molecular bonding and aldehyde groups in the diabetic cataract lenses. Hence we found that this technique & further experiments may shed light and give us the better understanding on the Calcification in diabetic cataractous crystalline lens.

Key Words: FTIR (Fourier transform infrared spectroscopy), XRD (X-ray Diffraction), Spectroscopy, Nuclear cataract, KBr (Potassium Bromide).

INTRODUCTION

Cataract is a painless vision loss with leading cause of blindness in the world. Nuclear cataracts are the most common, typically known as "age-related cataract." Found in the center of the lens, they interfere with the ability to see distant objects [1] . There are various cataract types but nuclear cataract is common in tropical climate countries and there were many studies on treatment and management options for cataract, only few studies reported cataractous lens molecular bonding studies. In the world more than 285 million people are affected by diabetes mellitus. This number is expected to increase to 439 million by 2030 according to the International Diabetes Federation. [2]

Diabetes is epidemic and fast gaining the status in India , approximately more than 62 million diabetic individuals currently diagnosed with the disease. India (31.7 million) topped the world with the highest number of people with diabetes. And india also known as diabetic capital of world from the recent news papers statements [3]

However Cataract is considered a major cause of visual impairment in diabetic patients as the incidence and progression of cataract is elevated in patients with diabetes

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mellitus. The association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies. Due to increasing numbers of type 1 and type 2 diabetics worldwide, the incidence of diabetic cataracts steadily rises. [4] Calcification seems not to influence the secondary structure of lens protein because both IR and Raman spectra for the crystalline lens protein in the no calcified area and calcified plaque were similar. [5] Infrared spectroscopy had found potential application in the study of molecular structure of various biological and chemical samples due to its high resolution, simplicity in direct recording, absorption, and transmission signal with great accuracy. The electromagnetic spectrum which has very broad range including UV-VIS-IR range which is popularly known as optical frequency range. All the chemical and biological molecules have their characteristic finger print absorption in Mid-IR to IR region. These regions provide vital information about the vibration and rotational lines of organic molecules. In addition, Raman absorption bands are also an important part of the vibrational spectroscopy and helpful to confirm the identity of particular compounds in mineralized tissue. [6] Previously reported research work on cataract lenses using FTIR spectroscopy show the changes in conformational structure of lens lipid and protein induced by glaucoma than by senile cataract. And it has been reported that the age-related cataractous lens undergoes major biochemical and biophysical alterations, mainly due to the changes with age in protein and lipids of lens membrane crystalline. However, lens calcification is one of the severe problem causing human cataracts. The pathogenesis and mechanism of lens calcification in ocular diseases are not yet completely understood. Several analytical methods have been used to investigate the mechanism of calcium-phosphate deposition in the calcified CL but their operations are very complicated and take much time. Here the investigators have chosen only senile cataractous lens and identified the calcified plaque and quantified it's chemical composition using FTIR and Raman spectroscopy and this study suggests that both microscopic FTIR (Fourier transform infrared spectroscopy) and Raman spectroscopy's were easy to perform and capable to determine the chemical composition of a calcified Cataract lens. [7] In present study we have focused our attention to understand the chemical mechanisms of calcification in Twenty six cataract samples from different patients in whom twelve patients were Diabetic and Fourteen were Non diabetic. This paper focused about Nuclear sclerotic cataract Lenses and identified the calcified plaque and quantified its chemical composition using different devices such as FTIR, and XRD optical spectroscopy.

MATERIALS & METHODS

Participants and Specimens

All the participants accepted to participate in this study by donating their cataract extraction samples with an age group of 45-75 years. A total Twenty six samples collected in which 14 Males and 12 Female. It also includes Diabetic induced nuclear cataracts (8 male and 4 female) and remaining are Non Diabetic Nuclear Cataract patients which are 14 samples. We had collected the Human Cataract Lens Samples after Small Incision Cataract Surgery (SICS) from Indo Us Hospital, at Hyderabad. Written and verbal consent followed by no objection forms duly signed by the participants was received and this project also conducted tenure to Declaration of Helsinki rules.

Sample collection and storage

All the Twenty six collected cataract samples were stored in formalin saline solution to avoid contamination of sample, generally previous researches reports that human organs are collected or stored in solution to avoid contamination. [8] The Cataract lens samples were dried for one week at 25 °C, under specific Relative Humidity (RH) conditions, and then used for spectral investigation to determine its biophysical characteristics and molecular composition by using FTIR spectroscopy the sample images can be seen in **Figure-1 & 2**

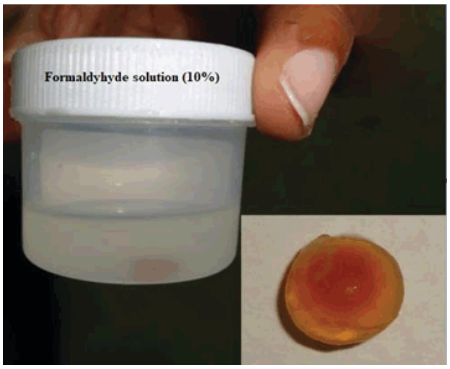

Figure 1: Nuclear cataract extraction lens sample in Formaldehyde (10%) solution.

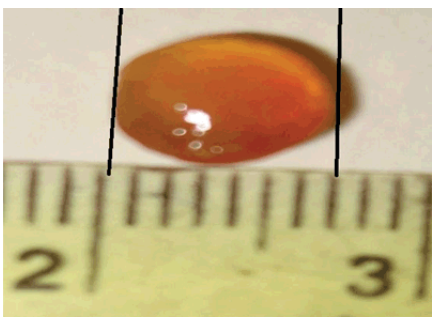

Figure 2: Nuclear cataract extraction lens after dried at 250 C under Relative humidity condition for FTIR analysis.

Preparation of K Br Pallets for IR spectra

For preparing K Br pallet for IR from the solid sample. Here we need the gate mortar and pastel, sample and KBr crystals. This KBr has been dried in the vessel and taken out before we use for KBr pellet preparation. We want to make the sample with the KBr in the proportion of 1:50 ratio it means for 1mg of sample then we added about 50mg of KBr and weighted with electronic weighting machine. Secondly we took KBr in the mortar, pastel with two tip spoons in the spatula and grained it into a very fine powder along with this media; we added the sample in the ratio of 1:50. We took small amount of sample and mixed well and grained. Checked for proper mixing, later finished and converted this into a pellet. We used the carver press to apply the pressure on the sample mixture in the dye and convert this into a fine pallet. Finally placing the pellet on the sample holder and measured the IR spectra of our sample which has been prepared as a KBr pallet.

Instrumentation & Experimental setup

We had studied the extracted nuclear cataractous lens characteristics with below two different types of optical spectroscopy based instruments in our study.

1) Fourier Transform Infrared Spectroscopy.
2) X ray Diffraction

Experiment-1 with Fourier Transform Infrared Spectroscopy (FTIR)

We have used Thermo scientific FTIR. Model: Nicolet 380 for recording the different types of functional groups and vibrational bands present in Cataract Lenses. This instrument mainly works on the principle of Michelson's Interferometer. [9] And after the experimental process the results are collected ,all the results were calculated with a mathematical technique called Fourier Transform. This Fourier Transform helps out to convert the raw data into the actual spectra in Cm^{-1}. FTIR spectroscopy is a technique which is used to obtain an IR spectrum of absorption: (how much light is absorbed by the specimen), transmittance (how much light passes through the specimen) and the area of IR region is very important in investigation the molecules can see in the below **Tabel-1**. Normal IR region is the main IR region, which focuses on investigation of molecules. So first we had taken a sample and put it into the spectrophotometer. IR radiation is passed and a spectra is plot. So for the active molecule, it shows the different types of vibrations.

Table 1: Distribution of IR range according to wavelength

IR Range	Wavelength
Normal IR region	800 nm - 1 micro meter
Mid IR region	1-30 micro meter
Far IR region	30–300 micro meter

Experiment-2 with X Ray Diffraction Spectroscopy

We had used low intensity XRD instrument for the second experiment and done it by powdering the sample which is a solid because the instrument is a powdered low intensity XRD. By doing this technique we had observed that the cataract is in the form of amorphous and also observed couples of peaks are present at same intensity and angle so we were unable to distinguish the electron present. But we have observed a unique thing that Diabetic samples graphs is different from Non Diabetic sample by this we can understand that electron density in Diabetic Nuclear lenses is different from Non Diabetic cataract lens. We had performed this technique on Twelve cataract samples.

RESULTS

All the analysis done based on the spectroscopic analysis and bonding shown under various spectroscopes Here with FTIR technique we have found that in the Diabetic and Non Diabetic induced Cataracts there is difference in chemical bonds and also in functional groups formed and also we have found that there are some functional groups and chemical bonds formed commonly in all cataract lenses. And there are some chemical and functional bonds which are present in Diabetic and not found in Non Diabetic Nuclear Cataracts can see in below **Table-2 and Figure-3.** However in the diabetic nuclear cataracts we have found unsaturated aldehyde groups

Table 2: showing bonds of Non Diabetic induced Nuclear Cataract Patient N=14 samples

Frequency	Bond	Functional Group
330 (weak peak)	N-H stretch	Primary, secondary, amines and amides.
2915 (strong peak)	C-H stretch	Alkenes
1693 (medium peak)	N-H bend	Primary ,amines
1550 (weak peak)	N-O Asymmetric stretch	Nitro Compounds
1073 (narrow peak)	C-N stretch	Aliphatic amines

are found at a frequency of 1688(narrow peak) and also aldehydes are found at a frequency of 2823 (medium peak) can see in **Table-3 and Figure-4 and 5.** With XRD technique we have found that there is difference in the presence of electron density in different samples and mainly in the diabetic nuclear cataracts and non diabetic nuclear cataract. The graphs explain it clearly that the presence of electron density in diabetic cataract lenses is similar and they are also different from the non diabetic cataract lenses which can see in the **Figure 6 and 7** .

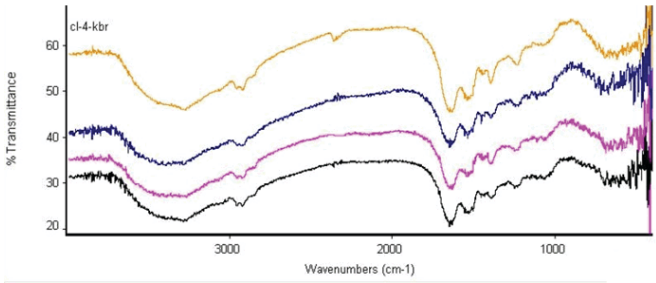

Figure 3: FTIR graph showing bonds of Non Diabetic induced Nuclear Cataract Patient (N=14).

Table 3: Showing bonds of Diabetic induced Nuclear Cataract Patient N=12 samples.

Frequency	Bond	Functional Group
330 (weak peak)	N-H stretch	Primary, secondary, amines and amides.
1688 (Narrow peak)	C-O stretch	Alpha and Beta unsaturated aldyhyde ketones
1550 (weak peak)	C-H stretch	Alkanes
2823 (Medium peak)	H-C=O:C-H stretch	Aldehydes
1073 (narrow peak)	C-C stretch in ring	Aromatics

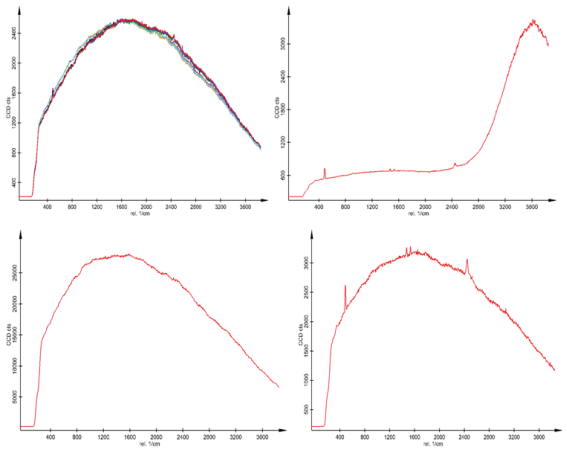

Figure 4: FTIR graph showing a frequency of a various peaks in Diabetic induced cataracts N=12.

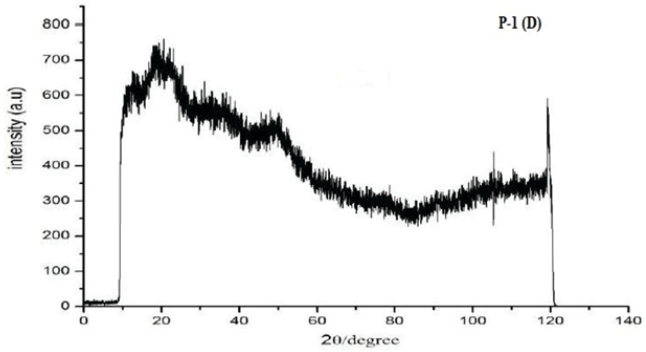

Figure 5: XRD spectroscopy graph showing peaks as electron density in Diabetic Nuclear Cataract Lenses N=12.

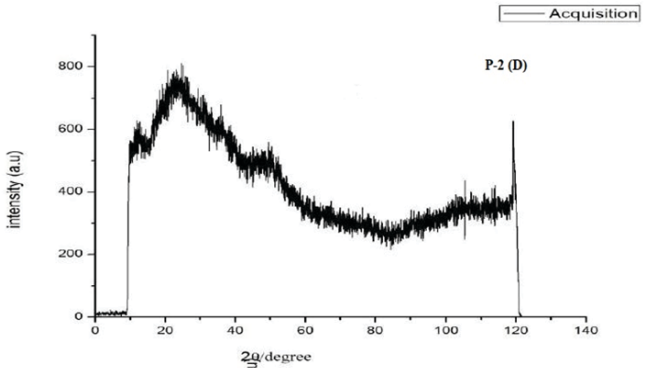

Figure 6: XRD spectroscopy graph showing peaks as electron density in Diabetic Nuclear Cataract Lenses N=12.

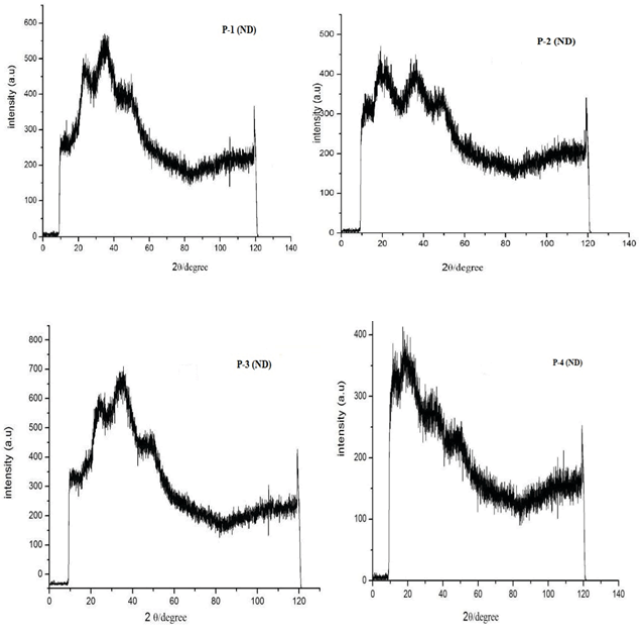

Figure 7: XRD spectroscopy graph showing peaks as electron density in Non Diabetic Nuclear Cataract Lenses N=1.

DISCUSSION

The focus of this study is to understand the difference between in Diabetic and non diabetic induced nuclear cataract calcification with optical spectroscopy techniques, we could not find any supportive study that either agreed with or contradicted. But interestingly in our experiment We had observed that different chemical bonds and functional groups in diabetic and non diabetic cataract lenses. To discuss about this in diabetic nuclear cataracts we had found unsaturated aldehydes groups are at a frequency of 1688 (narrow peak) and also aldehydes are found at a frequency of 2823 (medium peak) in Diabetic induced nuclear cataract lens specimens and in non diabetic specimens no aldehydes groups were found. And there are some commonly found chemical bonds in all the samples which are as follows. In a frequency range of 3331-3380 all the samples have resulted N-H stretch with primary, secondary, amines and amide groups. And also at a frequency range of 2823-2871 all the samples have commonly resulted in the C-H stretch with alkenes functional groups. And also at frequency range of 1517-1577 (N-O) asymmetric stretch with nitro compound functional groups are found. By this we can understand that if we can be able to reduce aldehydes groups' formation in the diabetic cataract lenses then we can be able to reduce the formation of cataract in diabetic patients. There is no earlier studies reported on this topic but we have compared our study with (Chen et.al 2005) He explained that calcification process is still unclear but concluded that many glycoprotein's and phosphor lipids play a role in calcification.[7] Our study found a new results that compounds in all the samples are similar to that of normal lenses but aldehydes groups are found newly and in only Diabetic samples this unique compound was not reported in earlier studies.

However from the graphs of XRD (X-ray Diffraction spectroscopy technique) we had come out with an interesting point that there is difference in electron density in diabetic and non diabetic induced cataract lenses. We had also investigated the compositions from lens specimen it is in amorphous form which are non crystalline. From the graphs extracted from XRD we can observe that diabetic samples graphs peaks are similar to each other .But rest of four non diabetic specimens graphs are showing different graph peaks that can see in Figure- 8. Moreover there was no research studies reported on this technique, so we are unable to compare with previous research studies. This study also supports that by increasing the specimen size we can get a further better results in future studies.

CONCLUSION

The outcome of present study concludes that Using FTIR confirms that the age-related cataractous lens undergoes major biochemical and biophysical alterations, due to the changes with age in protein and lipids of lens membrane, in the structure and proteins of the cytoskeleton, and in the lens crystalline. We also found that there are similar peaks in the same frequency range for Twelve cataract lens. So our results show that the calcification in the lens seemed not to influence the secondary structure of the lens protein. We can understand that if we can be able to reduce aldehydes groups' formation in the diabetic cataract lenses then we can be able to reduce the formation of cataract in diabetic patients.

The X-ray Diffraction techniques conclude that Diabetic Lenses have different electron density which is different from Non Diabetic Cataract lenses. There are no research studies reported on this technique so further studies with this technique can gives us better understanding on the Calcification in crystalline lens.

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Competing Interests

All the authors declare that No competing interests exits.

Authors Contribution

Kalapala A: Conceptulization, Conducted study, collected data, experimental design, wrote manuscript, reviewd.

Ak Chaudhary: Sources, research guidance, reviewed manuscript.

Sr Male: reviewed manuscript , research assistance in experimental design, Literature review.

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