CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3699655

Available online at: http://www.iajps.com Research Article

ASSESSMENT OF SERUM CALCIUM LEVELS IN PATIENTS DIAGNOSED WITH CATARACT GLACOUMA KARATOCONUS

[1]Maheen Zafar, [2]Dr. Amber Shehzadi, [3]Fatima Ashraf, [4]Mohsin Maqsood, [5]Aatika Ali, [6]Muhammad Usman Faryad Khan

[1]Kinnaird College for Women, Lahore
[2]Kinnaird College for Women, Lahore
[3]University of Veterinary and Animal Sciences, Lahore
[4]University of Veterinary and Animal Sciences, Lahore
[5]University of the Punjab, Lahore
[6]University of Veterinary and Animal Sciences, Lahore

Article Received: January 2020 **Accepted:** February 2020 **Published:** March 2020

Abstract:

The present study was conducted to investigate serum calcium level in various eye anomalies like cataract, glaucoma, and keratoconus in Pakistani population. Venous blood samples of 68 affected individuals and 30 unaffected individuals were collected. Serum extraction was done by using the standard protocol. The samples were further analyzed through atomic absorption spectrophotometer to calculate serum concentration of calcium ion. The normal range of calcium ion is 8.5-10.2mg/dl. The average value of calcium in patients of cataract, glaucoma and keratoconus were found to be: 10.851 mg/dl, 10.606, 10.60 mg/dl and 10.099 mg/dl respectively. While in case of control samples the average value was 8.5 mg/dl. Statistical analysis of the data showed a significant association between high calcium level and all mentioned eye diseases (cataract= 0.00, glaucoma= 0.00, Keratoconus = 0.00). Therefore, this research predicts that high calcium levels involve in the progression of these diseases. The role of ion imbalance of calcium in these diseases can act as a supportive therapeutic approach in the treatment and prevention of ophthalmic diseases. Calcium channel blockers can be used to prevent the progression of these diseases.

Keywords: Calcium, Glaucoma, Anomalies, Keratoconus, Cataract.

Corresponding author:
Dr. Maheen Zafar,
Kinnaird College for Women, Lahore

QR code

Please cite this article in press Maheen Zafar et al, ***Assessment Of Serum Calcium Levels In Patients Diagnosed With Cataract Glacouma Karatoconus.,*** *Indo Am. J. P. Sci, 2020; 07(03).*

INTRODUCTION:

Human eye is considered as the most complex organ of the body. Eye is an essential body organ as it helps in visualizing the subjects. Human eye absorbs the light that comes from the subjects after refraction, this light is transmitted to the optic nerves where object image is formed (Sridhar *et al* 2018). Lens is the most prominent structure within it. Lens is the transparent structure that present behind iris. The function of lens is to bends light rays so a clear image is formed. Lens is suspended between the aqueous humor Cataract is the translucence of the lens. It is the leading cause of blindness worldwide with 37 million people is affected by it, if percentage is calculated than about 48% of world blindness is caused by cataract (Karen et al). Cataract is the corneal disease; it is clouding of the lens in eyes. It is defined as the opacification of the normally transparent crystalline. Cataract said to be age related because as the age increase proteins clumps together sometimes and forms the cataract (Zhao *et al* 2015). The main symptoms of cataract are dim or blurry vision, seeing halos around light (Gupta et al). Glaucoma is neurodegenerative disease that occurred due to the loss of retinal ganglion cells and their axons (Sushil *et al* 2011). It usually damages the optic nerve (Azadeh *et al* 2016). Glaucoma patients have the high inter ocular pressure (IOP). Risk factors for glaucoma are hypertension diabetes, thyroid disease, genetic and high IOP level (Angela C *et al* 2017).

Keratoconus is an eye anomaly in which the cornea that is the outermost avascular and transparent region of eye assumes a conical shape as a result of non-inflammatory thinning and protrusion (Xiaohui *et al* 2012). Minerals are the inorganic substances and are the essential nutrients for the survival of the human body. Minerals act as the charger for the body. At least, 5% of the human body weight is minerals, it is vital for all mental and physical processes. They act as the catalyst for many biological reactions. Calcium is the most abundant macro-nutrient of the human body. Approximately 2% of calcium contributes in total body weight (Hare *et al* 2013). Regulation of the calcium effects in the anomaly of eyes. Imbalance in the calcium levels may lead to development of cataract in the lens. When cataract formed in the eyes level of calcium is increased. The increase in calcium level also causes the glaucoma because as intracellular Ca++ increased it causes the cellular swelling that leads to apoptosis of ganglion cells. (Junyuan *et al* 2004). This happened when Mg is deficient. Mg deficiency causes loss of Na+/K+ ATPase that results in release of Ca++ ions that ultimately causes the cellular swelling and leads towards the progression of glaucoma. High Calcium level is the factor for the progression of keratoconus, evidence is that the patients with diabetes type 2 has keratoconus and diabetes increased the calcium level in body. The present study is conducted to evaluate the serum calcium level in patients of cataract, glaucoma and keratoconous.

MATERIALS AND METHODS:

Study Area

The study was conducted in Lahore, samples were collected from various hospitals of Lahore.

Sample collection

A total of 48 patients 20 of each disease, for keratoconus 8 and 20 control subjects were chosen for this study.

Inclusion criteria

The enrolled patients shall not be affected with following diseases

- Diabetes, hypertension, cardiovascular disease.
- Smokers

The same criteria was applied for the control subjects. Control subjects includes healthy people, who had not any metabolic and eye disease.

QUESTIONNAIRES

Before blood withdrawal consent form was filled by the patients. Questionnaires

(Annexure II) were designed carefully after consultation with ophthalmologist and detailed literature review about variables selected for study. These were administrated to be filled by the subjects at the time of sampling. Details about the age of onset, family and medical history, living standards were asked in the questionnaire. The questionnaire was designed carefully so that, the volunteers do not feel shy in giving us the necessary information to create a solid scenario of patient's disorder.

BLOOD SAMPLING

The sample of patients were collected from various hospitals of Lahore, included, Mayo hospital, Al Ehsan eye hospital, and Lahore General Hospital. The subjects were informed about the aim and purpose of the study, not only to alleviate their anxiety but also to inform them about their health conditions. Almost 3 ml of venous blood was drawn in 3.5 ml serum separating BD vacutainers with the help of 5 ml syringes. The vacutainers were kept at rest for 1 hour at room temperature to let the serum separate.

SERUM EXTRACTION

After the clot formation in the serum separating BD vacutainers, serum was extracted with the help of 100 µL micropipette. The serum was then transferred into carefully labeled Eppendorf. The serum containing Eppendorf were centrifuged in a table centrifuge at 2200rpm for 15-20 minutes. This centrifugation helped to isolate pure serum while the

remaining blood cells settle down at the bottom of the Eppendorf. Then this serum was carefully taken out with the help of a micropipette and placed in another autoclaved, labeled Eppendorf tube. The serum was stored in the freezer and maintained at -20ºC until use.

SERUM SEPERATION

In order to calculate serum ions, serum was first thawed and then treated with HNO3. 0.5 ml of serum was taken in test tube in which 2 ml of HNO3 was added by using measuring cylinder. After that, the serum was left on room temperature for an hour. It was then heated in water bath until the solution turned clear. The test tubes were covered with aluminum foil and again placed in water bath at 65ºC for 1 hour. Following incubation, the test tubes were placed in a fridge to allow the serum to cool. Transferred the sample solution into the Eppendorf and centrifuged the sample at 400rpm for 20 minutes. After the centrifugation, 1 ml of sample mix supernatant was taken into another test tube by using micropipette. Then, 1 ml of sample mix supernatant was diluted with 15 ml of distilled water. The samples were filtered by Whatman filter paper carefully. The diluted solutions were used for analysis by AAS.

PREPARATION OF STANDARD SOLUTION

Standard solution of calcium was prepared before the serum was analyzed using atomic absorption spectrophotometer.

PREPARATION OF CALCIUM STOCK SOLUTION

Calcium chloride salt was used to make standard stock solution of calcium ion. In order to make 1000ppm solution of calcium chloride, 2.769g of calcium chloride were dissolved in 1000 ml of distilled water.

DILUTION PREPARATION

Dilutions were made from 1000 ppm solution using C1V1=C2V2 formula. Different standard concentrations were taken after estimating the normal level of each ion. Normal value of calcium in serum: 8.5 mg/dL – 10.1 mg/Dl The measurement conditions for atomic absorption spectroscopy (AAS) are given in Table 3.10

Table 3.10: Measurement conditions for Atomic Absorption Spectroscopy

Elements	**Concentration of Calcium**
Slit width (nm)	0.5
Wavelength	422.7
Burner head	Standard
Flame	Air
Oxidant gas pressure	160
Fuel (1/min)	Acetylene
Fuel gas flow	5mL/min
Lamp current (mA)	3mA

STANDARD CALIBIRATION CURVES FOR SERUM IONS

In order to prepare standard calibration curve of calcium ions, the absorbance of each above-mentioned serum ions in respective standard solutions were recorded at their specific wavelength, using the absorbance values, standard calibration curves were plotted by taking known concentrations of ions along x-axis and absorbance along y-axis.

DATA ANALYSIS

Graph pad prism 6 software was used to determine the concentration of ions in serum solution from absorbance value that was recorded. SPSS version 18 was used to assess correlation between various independent variables and dependent variable.

STANDARD CALIBIRATION CURVE FOR CALCIUM ION

The absorbance of calcium in standard solutions recorded by atomic absorption spectrophotometry ASS to make a standard calibration curve.

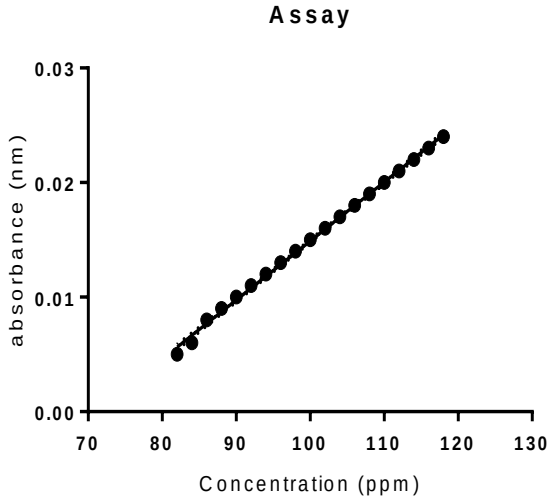

Figure 4.3 Standard calibration curve

CONCENTRATION OF CALCIUM IN SERUM OF CATARACT PATIENTS AND CONTROL

The absorbance of calcium in the serum, both of patients and controls (table 4.3) was recorded at 422.7nm, to find the concentration of calcium in the serum sample. The value of calcium is recorded in their SI unit.

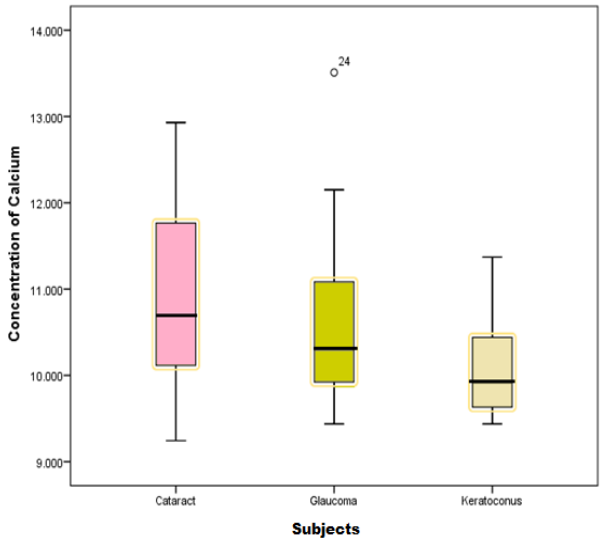

Figure 4.4 Concentration of Calcium in Serum of Cataract Patients and Normal

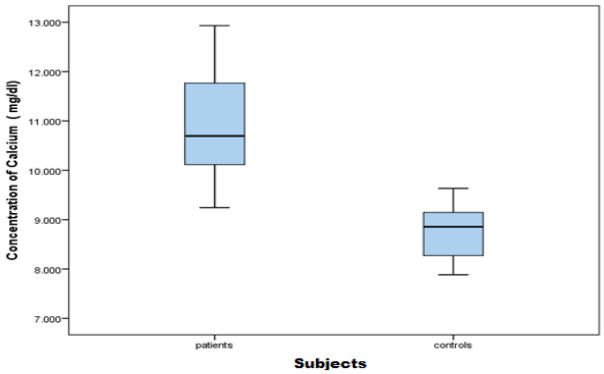

Figure 4.5 Concentration of Calcium in Serum of Glaucoma Patients and Normal

Glaucoma patients has the high level of calcium in their serum.

Statistical analysis of the data

Sr. No	Eye Anomaly	P value of Shapiro-Wilk test for normality	Pearson Correlation value	No of patients		No of control	
				+	–	+	–
1	Cataract	0.965	0.406	20	0	15	5
2	Glaucoma	0.878	0.184	20	0	15	5
3	Keratoconus	0.231	0.000	8	0	5	3

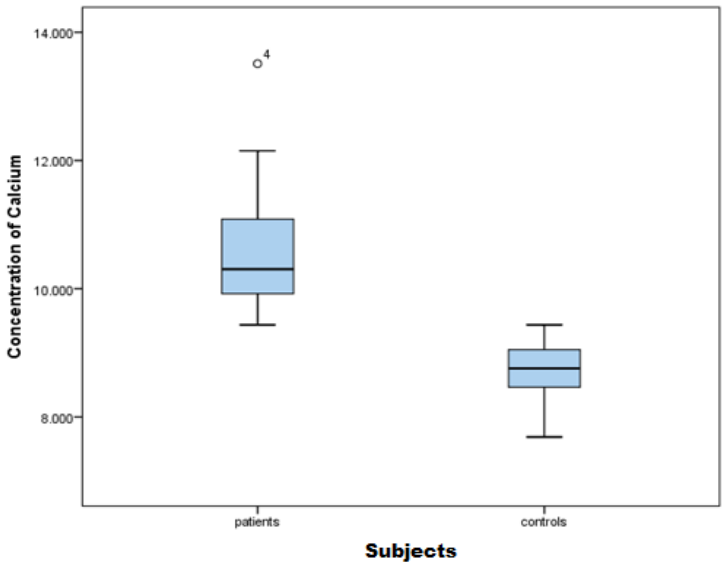

Figure 4.7 Calcium serum level in various eye anomalies.

DISCUSSION:

Minerals are present in small amount in human body and they plays a vital role in the body. An adequate amount of minerals are necessary for the proper metabolic function in the body. Minerals act as the cofactors of the enzyme so their presence in necessary for the proper body functioning. Minerals help in the proper eye development, insufficient amount of minerals (Calcium, Magnesium, and Zinc) can cause the eye anomalies (Tang *et al* 2003). The study was conducted to obtain an insight about role of magnesium in various eye anomalies present in Pakistani population.

Calcium is important for the body functions. Ninety nine percent of body calcium is present in bones rest of the calcium helps in blood clotting, nerve and muscle stimulation and hyoid gland functions. Calcium is the natural tranquillizer. It helps in maintaining the good heart and artery function. The passage of nutrients into and out of cells is reliant on calcium (Teritus *et al* 2004).

Calcium has vital role in the body but excess amount of calcium in the body is the cause of progression of cataract. Cataract is the disease of the eye lens. About 61.9% blindness is occurred due to the cataract. Calcium sensing receptors present in the eyes, they regulate the extracellular calcium, which results in loss or gain of function, lead to hyperglycemia or hypoglycemia in patients (Sai *et al* 2014). CaSR over activation is cause the hyperglycemia that starts the progression of cataract (Padmanbhan *et al* 2012). Cataract is the most

common cause of blindness and results from the loss of the lens transparency. Maintenance of the unique tissue architecture of the lens is vital for keeping the lens transparent. Membrane transport mechanisms utilizing several magnesium (Mg)-dependent ATPases, play an important role in maintaining lens homeostasis. Therefore, in Mg-deficiency states, ATPase dysfunctions leads to intracellular depletion of K^+ and accumulation of Na^+ and Ca^{2+} (Deepa *et al* 2012). High intracellular Ca^{2+} causes activation of the enzyme calpain II, which leads to the denaturation of crystallin, the soluble lens protein required for maintaining the transparency of the lens. Decrease magnesium levels leads towards the increased calcium levels that cause the cataract progression in eyes (Mustafa *et al* 2014). The present study indicated a positive association between calcium high level and cataract development. The Pearson correlation test value obtained from this study was 0.406 which indicated a positive association between cataract and high calcium level.

Glaucoma is the optic neuropathy that leads to irreversible blindness. In glaucoma intraocular pressure is elevated (Renu *et al* 2014). High calcium level is one of the major causes of the glaucoma. (Koseki *et al* 2008). Calcium level is controlled by taking the calcium channel blockers that alters the intracellular calcium concentration by modifying the calcium flux across cell membranes. CCBs decreased the IOP that decreased the progression of glaucoma. So CCB used to stop the progression of glaucoma (Makoto *et al* 2011). This study indicated a positive association between low magnesium level and glaucoma development and progression. The Pearson correlation test value obtained from this study was 0.184 which proved that calcium was deficient in the serum of glaucoma patients as compared to the controls.

Keratoconus (KC) is a corneal thinning disorder that leads to loss of visual acuity through ectasia, opacity, and irregular astigmatism. It is one of the leading indicators for corneal transplantation. KC usually starts at puberty and progresses until the third or fourth decade; however its progression differs among patients. In the keratoconic cornea, all layers except the endothelium have been shown to have histopathological structural changes (Mariam *et al* 2017). Diabetic type 2 considers is one of the causes of keratoconous. In diabetes calcium level is increased. In present study there is no correlation present between keratoconus and calcium level as Pearson correlation value is 0.00.

The study concludes that the average value of calcium in the serum of patients of cataract, glaucoma and keratoconus were found to be: 10.851 mg/dL, 10.609 mg/dL, and 10.099 mg/dL respectively. While in case of control samples the average value was 8.747 mg/dL. The study concluded that the high level of serum calcium ion showed positive association with the development and progression of the eye diseases (cataract, glaucoma and keratoconus).

CONCLUSION:

The study concludes that the average value of calcium in the serum of patients of cataract, glaucoma and keratoconus were found to be: 10.851 mg/dL, 10.609 mg/dL, and 10.099 mg/dL respectively. While in case of control samples the average value was 8.747 mg/dL. The study concluded that the high level of serum calcium ion showed positive association with the development and progression of the eye diseases (cataract, glaucoma and keratoconus).

REFERENCES:

1. Azadeh D and Shahnin Y. Neuroprotection in Glaucoma. *2016. J Ophthalmic Vis Res.* 2016 Apr-Jun; 11(2): 209–220.
2. Avunduk A, Yardimci S, Avunduk M et al. 1993. Determinations of some Trace and heavy Metals in Rat Lenses aster Tobacco Smoke Exposure and their Relationships to Lens Injury. *Elsevier,* 65(3): 417-423.
3. Choi YS, Joung H, Kim J. Evidence for revising calcium dietary reference intakes (DRIs) for Korean elderly. 2013. *FASEB J,* 28 (27):1065.
4. Deepa K, Manjunatha G, Suma M, et al. Antioxidant and calcium levels in mature and immature diabetic cataract lens. 2012. *IJOLS,* 6 (1).
5. Gupta VB. *et al*. Etiopathogenesis of cataract: An appraisal. 2014. *Indian J Ophthalmol,* 62(2): 103-110.
6. Goodman W, Quarles L. Development and progression of secondary hyperparathyroidism in chronic kidney disease: lessons from molecular genetics. 2008. *Kidney Int,* 74:276–288.
7. Hare B, Richard A, Tony P, Paul M, etl al. Replicatio and Metaanalysis of Canditade loci indentified variations at RAB3GAP1 association with keratoconus. 2013. *Invest Ophthalmol Vis Sci*,54(7): 5132–5135.
8. Ishida, Kyoko M, Tetsuta M et al. Clinical Factors Associated with Progression of Normal-Tension Glaucoma. 1993. *J Glaucoma.*
9. Junyuan G, Xiurong S, Francisco J, Xiaohua G et al. Connection Between connexins, calcium and cataracts in the lens. 2004. *J Gen Physiol,* 124(4): 289–300.
10. Koseki N, Araie M, Tomidoloro A. et al. A placebo-controlled 3 year study of a clcium blocker on visual field and ocular circulation in glaucoma with low normal pressure. 2008. *Epub,* 115 (11): 2049-57.

11. Makoto A, Chihiro M. Use of calcium channel blockers for glaucoma. 2011. *PRE*, 30(1): 54–71.
12. Kosker M, Suri K, Hammersmith KM, Naseef A, Nagra P, Rapuano C. 2014. Another look at the association between diabetes and keratoconus. 2014. Ovid. 33:774–779.
13. Mariam L, Inas H, Michelle D, et al. Molecular and Histopathological Changes Associated with Keratoconus. 2017. *BioMed*. 80 (3):11-15
14. Netland P, Chaturvedi N, Dreyer E. Calcium channel blockers in the management of low tension and open angle glaucoma. 1993. *Am J Ophthalmol*. 15; 115(5):608-13.
15. Padmanbhan P. Fred B, Kenneth T, et al. The role of calcium-independent phospholipase $A_2\gamma$ in modulation of aqueous humor drainage and Ca^{2+} sensitization of trabecular meshwork contraction. 2012. *Am J Physiol Cell Physil*. 302(7): 979–991.
16. Renu A, Igor I, Puneet A. Pathogenic role of magnesium deficiency in ophthalmic diseases. 2014. *Biometals.* 27 (1): 5-18.
17. S.D. Crish, D.J. Calkin. Neurodegeneration in glaucoma: progression and calcium-dependent intracellular mechanisms. 2011. *Neuroscience Forefront.* 176: 1-11.
18. Sai V, Thiagarajan R, Ramar M. Inhibition of diabetic-catarct by Vitamin K1 involves modulation of hyperglycemia-induced alternations to lens calcium homeostasis. 2014. *ELSEVIER.* 128: 73-82.
19. Tang D[1], Borchman D, Yappert MC, Vrensen GF, Rasi V. Influence of age, diabetes and cataract on calcium,lipid-calcium and protein-calcium relationships in human lenses. 2003. *Invest Ophthalmol Vis Sci.* 44(5):2059-66.
20. Vetrugno, Michele C, Giuseppe, Cantatore, Francesco. 2004. *CHAM.* 31(4): 295-302.
21. Wang D, S, Sing K, Lin S. Glaucoma prevalence and intake of iron and calcium in a population based study.2013. *Curr Eye Res.* 38(10):1049-56.
22. Yuan H, Jian G, Joyce T. Mitochondrial defects and dysfunction in Calcium Regulation in Gluacomatous Trabecular Meshwork Cells. 2008. 49 (11):4912-4922.
23. Zhao L., *et al*. Lanosterol reverses protein aggregation in cataracts. 2015. *Nature.* 523 (7562): 607-611.