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Analysis of Metals in Cataract Fluid of Jordanian Patients by Using the Inductively Coupled Plasma Optical Emission Spectrometry

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The study was completed to determine the level of the following metals (Sodium (Na), Potassium (K), Phosphorus (P), Calcium (Ca), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Zinc (Zn), Selenium (Se), and Cadmium (Cd)) in different cataractous human lenses. Five Samples had collected from the normal individuals healthy people and 25 suffered from cataracts at the Italian Hospital, Al-Karak, Jordan. This study had conducted between November 2020 and October 2021 on 30 individual subjects. Samples fluid from the Cataract sufferers had been gathered, the data was analyzed using Microsoft excels and SPSS software program new version. The trace elements concentration withinside the aspirated fluid were measured with the inductively coupled plasma optical emission spectrometry – Mass (ICP-MS), best controlled with certified standards. The mean values of copper were (12.9±12.4) and (7.4±9.7) for patients and control groups respectively. The average mean values of Cd were found to be (5.9 ±2.4) and (4.6 ± 2.4). The cadium, copper, manganese, magnesium and potassium levels were significantly higher in the catarctous groups. The others elements were lower in the catarctous group than control. Results showed significant change in some metallic elements concentration with cataract. These consequences can be beneficial for understanding and identifying the reasons that can play a function withinside the initiation and improvement of cataract. These factors deficiency may also have some suggestions to diets and diseases via its appropriate concentrations and its consequences on various organs parameters. It appears that the estimation of serum or plasma concentration of these metals may also assist in research and remedy of other diseases in both genders.

Keywords: Cataract; ICP-Mass; Lens; Metals; Trace Elements.

Cataract is the main cause of treatable blindness in the United States, in accordance to the Centers for Disease Control and Prevention. The cataract usually develops after age 55, however presenile cataract can found as well, such as some congenital cataract at birth or in association with ocular or systematic diseases. About 18% of Americans aged forty or older (more than 25 million people) have a cataract in one or both eyes[1, 2, 3].

Cataracts develops in stages, however the development of cataract depends on many factors for instance age, exposure to UV light over a lifetime, genetic elements and some acquired risk factors, such as smoking, excessive alcohol consumption or dietary deficiencies. Diabetic

patients are at greater risk, as well as patients being treated with different medications as corticosteroids or phenothiazine-related medications.

However, snice the cataract develops in stages there may be a preventive or delaying measures that can delay the development of cataract.

The lens is a transparent organ behind the pupil; it is a biconvex structure to add in the function of refraction of the light ray, which is centering the eye for sharp focus of images over the retina. In the early years of life, it has also a very important function of accommodation to change between fan and near vision. The lens is made of crystalline proteins; these proteins undergo some metabolic changes with age that may lead to loss of lens transparency[4, 5].

Relation among few serum biochemical factors (which includes Co, Cd, Zn, Fe, Cu, Mn, Se, Na, K, P, and Ca) and cataract formation had been investigated[6-8]. Alteration in each of those ions ends in cation imbalance in lens, which has destructive results in cataract formation[9]. Changes in degree of serum mineral attention can spark off adjustments in aqueous electrolytes levels. Since the lens metabolism is related to aqueous humor[10-11] and this small amount of fluid itself is created from blood secretions, so serum electrolytes diploma which may additionally moreover have effect on aqueous humor metal diploma and contrary the lens metabolism but Know how associated with alteration in hint minerals is scanty.so investigating its etiology is necessary to decrease the disabilities resulting from the cataract formation to improve the life style for people and decrease its surgical burden on health services.

These alterations may be resultant from insufficient dietary consumption or result from metabolic imbalances produced by adverse or synergistic interaction amongst metals. Some trace elements also play both healing or preventive roles in the disease course, while others have an adverse role in the development of diseases.

Alteration in serum Ca++, Zn and Cu level are among the proposed risk factors for cataract formation[8, 12]. the lens ionic imbalance with stepped forward degrees of calcium (Ca2+) and sodium (Na+), coupled with decreased degrees of magnesium (Mg2+) and potassium (K+), is related to cataract development in human[13]. Zinc (Zn) and copper (Cu) are among viable causative factors in development of cataract[12, 14-15].

The received consequences may be appropriate for information and figuring out the reasons, which can play a function within side the initiation and development of lens.

The objective of the study, that estimation of cataract fluid awareness of those metals may additionally help in studies and treatment of different sicknesses in each genders.

MATERIALS AND METHODS

Subjects

This study was carried out on 25 patients admitted to the Italian Hospital, Al-Karak, Jordan. This study had conducted between November 2020 and October 2021 on 30 individuals.

Five samples were collected from the healthy people. Fluid of Cataract samples of patients were collected from the same hospitals. Cataract extraction was performed by phacoemulsification using option revolution machine, where the fluids obtained through the surgery was analyzed, phacoemulsification was performed routinely, after the capsulorrhexis all ocular viscoelastic devices and stains were thoroughly washed out, phacoemulsification was completed , fluids from the machine cassette were obtained from each patient separately . While control samples obtained from, patients who underwent clear lens extraction for refractive purposes to correct high myopia, by the same phacoemulsification procedure. The age of patients range was 32-82 years (Table-3).

Statistical analysis

The importance variations in trace elements concentration among experimental groups become examined the use of t-test analysisat evaluation via way of means of the use of Microsoft excels and SPSS software program 19 version. A sided P value <0.05 become taken into consideration statistically extensive for the t-test a look at. The stage of elements have measured via way of means of inductively coupled plasma optical emission spectrometry-MS (ICP-Mass), satisfactory controlled with certified standards. All the imply values have been analyzed via way of means of t take a look at to decide the importance values.

The classification of instrument used for determination elements, solvents, and reagents as follow:-

Microwave digestion of Cataract Fluid for ICP-MS analysis

A one ml of Cataract Fluid sample was digested using (Milestone Ethos 900 Microwave Labstation, Cõ) placed in PTFE bottles, **a** mixture of (HNO_3-H_2O_2) was added in the ratio (v/v) of 8ml HNO_3 (69%) and 2ml H_2O_2(35%) to the sample, then the caped vessels as placed inside the rotor bodies, sealed**,** tightened and subjected to the microwave digestion program, then the solution filtered and washed in 25ml volumetric polyethylene volumetric flask and completed to the mark with deionized water, the used microwave digestion program is summarized in table-1

Digested samples were analyzed for trace elements using an ICP-MS (7500a, Agilent(Japan) operating conditions for this instrument are given in the following table

ICP-MS analysis

Digested samples were analyzed for trace elements using an ICP-MS **(**7500a, Agilent, (Japan) operating conditions for this instrument are given in the following table 2.

Table 1. Microwave program performed to digest the sample

Formula		Ramping time(min)	Power (watt)
$HNO_{38\ ml}$	H_2O_{22ml}	2	250
		2	0
		6	250
		5	400
		5	600

Table 2. ICP-MS conditions

Tune parameters for ICP-MS		
Parameter	Typical Values	Adjustment
RF Power (W)	1300	1200 to 1600
Sampling Depth (mm)	6	4 to 8
Carrier Gas (L/min)	1.2	0.8 to 1.3
Makeup Gas (L/min)	0	0 to 0.4

RESULTS

We follow all conditions of ethical clearance in this recent research, which conducted to minimize the threat of damage to humans and ultimately ensure that the research leads to beneficial outcomes.

Socio-demographic characteristics of participants

The study had carried out on 30 patients admitted to the Italian Hospital, Jordan and five from 30 were control as healthy people. The Patients characteristics given in table-3

Elements Content

The experimental study covered 25 samples of fluid from the anterior chamber of eyes received from sufferers' present process cataract surgery. The twenty-five sufferers with cataract and 5 sufferers as manipulate confirmed at Table – 4. The degrees of elements (Ca, K, Mg, Na, P, Cr, Fe, Cu, Zn, Se, Cr and Cd) inside aspect the control business enterprise have been finished to exclude age as a confounding factor.

Control patients group had significantly higher levels of Ca, Na, P, Fe, Cu, Zn, Se, and Cr compared to patients with cataract. While higher levels of following elements of K, Cu, Mg, Mn, and Cd found in samples from patients with cataract (Table - 4). Most elements confirmed giant versions from affected person to person. The widest variety refers to macro elements consisting of calcium (42.46), sodium (2796.00), potassium (126.22), phosphorus (18.44) and magnesium (17.66). There is a significant group of cataract elements and control (P≥ 0.05). The group of

Table 3. Socio-demographic characteristics of participants

Age years	Number	%
30-50	4	13
51-70	10	33
>70	16	54
Gender	number	%
Male	13	43
Female	17	57
occupation	number	%
Retired	18	60
Working	12	40

Table 4. Comparison between trace elements level in cataract patients and control group

Patients	Cataract	Control	P value
No.,	25	5	
Na ug/ml	2796 ±114.55	3112 ± 132.66	< 0.01
P ug/ml	18.44 ± 3.11	23.66 ± 4.43	< 0.05
k ug/ml	126.22 ± 7.98	116.65 ± 5.66	< 0.05
Ca ug/ml	42.46 ± 4.88	49.50 ± 4.4	< 0.05
Mg ug/ml	17.66 ± 2.10	12.55 ± 2.88	< 0.05
Fe ng/ml	70.13 ± 67.44	81.44 ± 87.66	< 0.01
Mn ng/ml	5.66 ± 3.24	4.12 ± 4.10	< 0.05
Cu ng/ml	12.88 ± 12.44	7.43 ± 9.65	< 0.01
Zn ng/ml	111.32 ± 23.45	123.15 ± 33.41	< 0.01
Se ng/ml	5.44 ± 3.11	9.50 ± 3.44	< 0.01
Cd ng/ml	5.98 ± 2.42	4.64 ± 2.42	n.s
Cr ng/ml	4.22 ± 6.77	6.62 ± 8.66	< 0.05

macro elements covering calcium, phosphorus, potassium, sodium, magnesium, belongs to main elements and these elements occur at level between 17.66 ug/ml for magnesium and 2796 ug/ml for sodium. The remaining elements occur at elements concentrations, though chromium and Zinc occur at about 4 and 111 ng/ml respectively. All other microelements, such as manganese, cobalt, selenium, cadmium, selenium occur only at trace levels.

The group was covering, sodium, potassium, and calcium, belongs to the so-referred to as main elements, and those elements arise at a awareness among 126.22 ppm for potassium and 2796 ug/ml. for sodium. The final factors arise at hint levels, aleven though iron and zinc arise at approximately 70.13ng/ml and 111.32ng/ml respectively. All the opposite metals, in addition to the metal (selenium), arise handiest at ultra-hint levels, e.g., manganese and cobalt with approximately 5.66±3.24 and 12.88 ng/ml, respectively.

DISCUSSION

The function of metals in physiological pastime and pathological reasons of illnesses has been of hobby to scientists for plenty years. According to contemporary works, metals moreover have a crucial function within side the pathogenesis and control of many eye illnesses. Until now, the extraordinary systems of the eyes have studied totally for the content material fabric of selected metals, along with Co, Cd, Zn, Fe, Cu, Mn, Se and Na, K, P, Ca. This working is ready reading a few metals present in concentrations exceeding the detection limitation of the ICP-MSS self-control method, i.e., 0.001 ppm, gift within side the fluid from the catractous lenses in the course of cataract surgical operation procedure.

The evaluation confirmed the presence of a statistically greater sequence of elements concentration at detectable levels. The classification of factors used to be made by means of a hierarchical cluster analysis. Therefore, a substantial concentration in stages aspect affected person should specific interest have to issue impact elements physiological.

This study showed that patients with cataracts elevated levels of K, Mn, Mg and Cd, while the levels of Na, P, Ca, Fe, Cu, Zn, Se, and Cr were lower levels.

In this study, we determined extensively a higher lenticular concentration of P, Fe, Cu, and Pb, as well as that the concentration of K and Mg decreased in cataract sufferers. This finding found to be similar to the findings in previous studies investigating Behcet's disease, retinitis pimentos' and AMD[10, 11, 16, 17]. Zn and Cu had distributions within the neural retina, retinal pigment epithelium and choroid[8]. The essential metals Cu and Zn play main roles in retinal survival and are for the everyday functioning of antioxidant enzymes. Decreasing oxidative stress metals as iron,

promotes formation of free radicals, in particular with the retina[18, 19]. From our data, we observed no proof of ordinary Zn in cataract sufferers, at the least in the population of this study. This is probably because of small pattern length or distinct ethnic groups. Copper has been set up as an acute phase reactant and its concentration in serum was proven to be elevated extensively in various acute and chronic infective, inflammatory, and neoplastic diseases as a result of elevated levels of ceruloplasmin[20].

Increased levels of copper in cataractous lenses with controls because of copper content material became elevated with age progression. The most common form of zinc is zinc oxide; which is unfortunately poorly absorbed. Zinc deficiency related to different forms of macular degeneration. Zinc deficiency in cataractous lenses is one of the top investigated aspects of chelated form of zinc, in comparison to chelated form of magnesium and chromium, which affects cellular metabolism via numerous mechanisms, which seems to play a critical function in retaining everyday ocular function[23]. Poor Zn consumption would possibly bring about deficiency and lack of Zn-established coenzymes related to age associated cataract. The ocular manifestations of zinc deficiency encompass altered vision, depressed electroretinograms, and oscillatory potentials, and, if the deficiency is severe, structural adjustments are detected inside the retina and retinal pigment epithelium[24]. Bhat, et al[25] examined in India and mentioned decrease in zinc level in sufferers in comparison to controls.

In the prevailing study, for the sufferers with cataract and glaucoma, we located better copper and decrease zinc concentrations than the values stated above. In general, copper and zinc which mentioned to be metabolically antagonists[26]. This helps the poor correlation, which we have observed among the copper and zinc concentrations within side the aqueous humor of the sufferers with glaucoma[27].

Akyol etal. (1990) found that an excessive copper level together with a low zinc level can due to of the following factors:(1) abnormalities with within the secretion of aqueous humour[28]; (2) troubles inside aspect the outflow of aqueous humour through canal of Schlemmt; and (3) breakdown of the blood-aqueous humour barrier due to trauma, infection, and inflammation[29]. In everyday instances, it determined that the blood-ocular barrier efficaciously excludes a potentially toxic more copper from the eye[30]. Breakdown of the barrier has validated to result in boom of copper tiers in aqueous humour, which is associated with an influx of plasma proteins into the anterior chamber[30].

Copper being transitional metallic ion-catalyzed formation of hydroxyl radical that would contribute to the protein change determined in cataract and might play a function within the etiology of age-associated cataract[31]. Copper is also appeared to enhance up lenticular opacification with the useful resource of lading damaging protein, lipid, and membranous structures. The increase in the concentration of copper ion within the lens can be the important thing to reveal the thriller of cataract formation. Akyol, et al.[12] suggested that Cu concentrations (90-160 ìg/dl) agreed with our statement coincides with Akyol. Maintenance of calcium homeostasis is important to the clarity of the lens[32-34]. The calcium content material will increase regularly with the onset and adulthood of senile cataract has been agreed to with the aid of using nearly all of the observers. In a few cataractous human lenses, calcium ranges observed to grow as a whole as thirteen fold instances that suggest the importance of the Ca2+ concentration of the everyday lens[35]. We evaluated the concentration of this metal through evaluation of samples and determined higher ranges of calcium amongst cataractous sufferers as compared to normal ones. Low level of Cr was determined in cataractous lenses. These decrease ranges may be detectable aspect for improving of the disease because it interferes with the metabolism of important metals including Fe, Mn, Ca, Zn, or Mg, which may set off the cytotoxicity and apoptosis, in addition to chromosomal aberrations and morphological transformation in the cells[16-18].

Our findings regarding cadmium level found out appreciably better attention in hair and serum samples of cataract ones. Cadmium can also additionally hasten cataract development with the aid of using numerous mechanisms. It is understood to compete with copper within the frame and will have an effect on the copper homeostasis of blood and copper containing proteins[36]. Cadmium can also additionally without delay bind with lens proteins and denature them in cataract development[37].

Magnesium is associated with calcium metabolism, so now and again if there's an imbalance with the magnesium, there will be imbalance in the level of calcium. Potassium is some other important element, the right stability of fluid within the body. It facilitates normal muscle contraction and cardiac muscle contraction. It's a key electrolyte. Selenium is a trace mineral that has a strong antioxidant effect. It is also associated with thyroid gland metabolism. It's a key element in thyroid function stability. Chromium is an essential element that is important for glucose metabolism and insulin function. Studies have proven that humans with glaucoma have low chromium level of their red blood cells. Chromium is contained in the optic nerve structure, alongside different dietary ingredients. In the current study, it concluded that during combined cataract formation in the lense the copper concentration will increase with the age progress. This confirms the results of Nath et al. 1969, this increase became because of the inhibition of lactate dehydrogenase enzyme during the formation of cataract. The increase in copper content material in senile lenses became additionally proved by Jain and Nath. 1988. However, copper is essential for diverse metalloenzymes which includes cytochrome oxidase and aldolase, which might be crucial for the preservation of cellular growth[38, 39, 40].

Limitations

Our limitations include a small sample and a selection bias of the participants arriving in our center.

The specifics limitation came to the lack of some devices we have, for example ICP-Mass

CONCLUSION

Findings of this study displayed a relationship between imbalances of metallic factors within cataractous lenses. However, whether or not those changes in level of metallic factors are the cause of disorder or effects of this disorder, which needs to investigated.

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Conflict of interest

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