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Biological Samples as a Tool for Analysis of Metals in Serum of Cataract Patients

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Abstract

There is much evidence suggesting that metallic elements may play a role in the formation or cure of human cataract, a disease that is on the increase due to the growing percentage of elderly persons in the world population.We have evaluated the levels of nickel, copper, cadmium, calcium, magnesium, lead and zinc in hairs and nail samples of cataractic subjects and compared these findings to the levels of healthy controls.A group of patients diagnosed with cataract and a group of healthy controls were chosen as subjects. Serum and hair samples were analyzed by atomic absorption spectrophotometry .Results were statistically evaluated by SPSS/PC software and student t-test.A significant difference was found in mean concentrations of metallic elements in comparison of patient group to control group ($p < 0.05$). Mean concentrations of nickel, copper, cadmium and calcium were found significantly higher among cataractous patients at ($p<0.05$) matched to healthy ones while concentrations of magnesium, zinc and lead were significantly lower among group suffering from cecity. Significant difference was observed among two groups under study regarding zinc, nickel, copper, calcium, magnesium, and lead while for cadmium difference was statistically non-significant. In spite of the fact that this study was carried out on a relatively small sample population, the findings suggest some thought-provoking questions, considering that there has been a good amount of controversy regarding the status of these trace elements and their possible role in cataractogenesis suggesting the need for further studies.

Introduction

Cataract is one of the commonest overt maladies connected in the impairment of vision. Cataract is a progressive clouding of the human lens inside the eye. The lens is a small oval like structure and consists of a thin capsule enveloping a bag of protein. The protein is crystal clear and colorless at birth but discolors and clouds with age: first yellow, then brown, and finally cloudy. When the lens becomes cloudy and interferes with vision, it is called a cataract. The lens no longer transmits or focuses light clearly.

Cataract ,globally accounts for virtually 16 million cases of cecity [1] and 50 million cases of low vision. Visually afflicted peoples in world are solon than285 million, of whom 39 million are, darken and 246 million have mild to intense seeable decay . 90% of the experiences darken charged in a developing land and nine out of 10 of the world's blind live in a developing country [2]. In Pakistan, 66% of the cecity is due to cataract [3] An estimated 2.5 cardinal people are irrational due to cataract in one eye and 1.5 million in both eyes [4]. Varied aspects nominative as senescent, alterations in trace element levels, toxins, medication, UV light exposure, diet, some metabolic disorders, quality of life, cationic pump malfunction and lens metabolism disorder [5] and possibly family history can be involved in senile cataract formation.

The pinpoint pathogenesis is not glorious yet, so research for clarifying its etiology is a necessity to avoid from prox disabilities, for reduction surgical costs and to improve the quality of life. Relation between few serum biochemical elements (such as Ca,Zn,Cu,Na) and cataract jazz have been verified [6-8]. Alteration in either of these ions leads to cation imbalance in lens which in turn results in cataract formation [9]. Changes in normal level of serum trace mineral can induce changes in aqueous electrolytes levels. Since the lens metabolism is associated with aqueous humor [10-11] and this thin fluid itself is produced from blood secretions, serum electrolytes concentration directly affects electrolytes of aqueous humor and in turn lens metabolism but knowledge related to alteration in trace minerals is scanty. These alterations could be resultant of inadequate dietary intake or result from metabolic imbalances produced by antagonistic or synergistic interaction among metals .Some Trace elements also play either curative or preventive roles in combating disease, while others have a catastrophic effect because their use adds insult to disease condition.

Alteration in serum Ca^{++}, Zn and Cu level are among the proposed risk factors for cataract formation [8,12]. the lens ionic imbalance with increased levels of calcium (Ca_2^+) and sodium (Na^+), coupled with decreased levels of magnesium (Mg_2^+) and potassium (K^+), is related to cataract development in human [13] . Zinc (Zn) and copper (Cu) are also among possible causative factors in development of cataract [12,14-15].

Materials and Methods

Introduction of the study site

Sargodha is the city of Sargodha District, Punjab, Pakistan. It is located in the north-east of Pakistan. It is the eleventh largest city of Pakistan and is known as Pakistan's best citrus-producing area. It is an agricultural trade centre with various industries. Pakistan's largest Airbase is situated in Sargodha and known as 'city of eagles'.

Subjects: Samples were collected from the normal, healthy population and patients of the Sargodha District. Hair and blood samples of patients were collected from different hospitals of Sargodha District including

a. Fatima Hospital, Sgd.

b. District Headquarters Hospital, Sgd.

c. Fatima Memorial Hospital, Sgd.

d. LRBT Hospital, Shahpur.

e. DHQ Hospital, Bhalwal.

Collection and Storage of Samples

Hair

In order to collect the hair samples the patients were asked to wash their hair with medicated soap, followed by drying with clean towel to remove any external contamination. Then with the help of the stainless steel scissors hair were cut close to the scalp in the sub-occipital area of head (1-2cm), making sure that no coloring agent has been used . The hairs collected were stored in the plastic polythene bags.

Blood

With the help of BD syringes 5cc blood was drawn from each patient and blood was stored in the scientific tubes after assigning them codes for further processing. Blood Samples were centrifuge at 10,000 rpm to separate serum. The serum was collected with the help of micropipette and put into Aphon tubes. These tubes were labeled and stored below 4ºc before further processing. For each of the sample same step is repeated.

Wet Acid Digestion

The materials was digested in the Nitric acid and Hydrogen peroxide

Hair

A weighed quantity (100g) of each of the hair sample was taken. Samples were placed in properly washed and labeled Erlenmeyer flasks. 4 ml of nitric acid and 1 ml of hydrogen peroxide was added to each sample. Then it was soaked overnight at room temperature. After that the samples were heated on the hot plate at 250ºc until they were near to dry. Now they were taken away from hot plate, cooled and 2 ml of hydrogen peroxide added to them. These samples were again shifted to hot plate and the procedure was repeated unless the sample becomes water clear. Then 25 ml volume was made up with the help of de-ionized water. The solution is filtered and transferred into marked Teflon bottles.

Serum

0.5 ml serum was taken with micropipette and transferred to flask. Acid digesting solution (HNO3 + H2O2) was added in the ratio 4:1 and left over night for incubation. Samples were heated on hot plate until they were near to dry. Then removed from the hot plate and 2 ml of H2O2 added to them. The process is repeated and finally removed when the sample becomes water clear. The contents of the flask were filtered and collected in 25 ml volumetric flask in order to make the volume with de-ionized water. Solutions were transferred into marked Teflon bottles

Statistical analysis

The significance of difference in trace elements level in samples between two groups was tested using t-test analysis using Microsoft excels and SPSS soft ware 19.0 version. A two sided P value <0.05 was considered statistically significant for the t-test.

Results

Nickel

Ni is the most common skin sensitizer in the population, and totally dominating among women. This might be due to the prevalence of Ni-containing material in the modern environment, including jewelry, buttons, clip coins, and many alloys. We observed that catarcatous pateients have significantly higher levels of nickel in their serum (19.79±0.152mg/L) (Figure 1) and hair (98.502±1.03 µg/g) (Figure 2) samples allegorized to normal one(16.88±.698mg/l)&(75.595±2.88µg/g) respectively..

Zinc

Concentration of zinc was recorded as 124.45±3.45µg/g in hair samples while as 4.51± 0.24 mg/L in serum samples of patients having cecity. While zinc level in hair and serum samples of control group remained as 259.34±16.93 µg/g and 5.70±0.25 mg/L which is statistically higher matched to persons suffering from visual impairment (Table 1 & 2).

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Lead

In comparing cataract patients with control we observed lower level of lead among patients .lead level in hair and serum samples of cataract patients was observed as (42.69±3.15 µg/g) & (.345±0.001mg/L) respectively, while 57.81±2.7 µg/g concentration of lead was observed in hair samples and 1.57±0.06 mg/L in serum samples of control group. Lead was significantly higher among non cataractous group at $p<0.05$ (Table 1 & 2).

Copper

Accumulation of copper in hair and serum samples of person suffering from visual impairment was higher as compared to non-cataractous ones (Table1 & 2)

Calcium

Highly significant difference (Table 1&2) was observed between two groups for serum (184.40±11.37 & 129.059± 1.51mg/L) and hair (1455.79±132.69 & 1069.07±37.097 µg/g) calcium level.

Cadmium

Cadmium concentration in hair samples of cataractous was observed as 2.312±0.13µg/g while same remained as 2.086±0.042 µg/g in normal group. Concentration of this metal remained as 0.416±0.012 &0.342±0.023 among serum samples of cataractous and non-cataractous ones respectively. Difference between two groups for serum samples was statistically significant (Table 1) while for hair samples analysis it remained non significant (Table 2).

Table 1: t-Test statistics on Serum samples.

		t	Sig. (2-Tailed)
Pair 1	Nickel normal - nickel cataract	-4.011	0
Pair 2	Zinc normal - zinc cataract	3.537	0.001
Pair 3	Lead normal - lead cataract	19.028	0
Pair 4	Copper normal - copper catarac	-2.831	0.006
Pair 5	Cadmium normal - cadmium cataract	-2.846	0.006
Pair 6	Magnesium normal - magnesium cataract	7.008	0
Pair 7	Calcium normal - calcium cataract	-4.786	0

Table 2: t-Test Statistics on hair samples.

		t	Sig. (2-tailed)
Pair 1	Nickel normal - nickel cataract	-6.394	0
Pair 2	Zinc normal - zinc cataract	8.828	0
Pair 3	Calcium normal - calcium cataract	-2.848	0.006
Pair 4	Lead normal - lead cataract	3.816	0
Pair 5	Copper normal - copper cataract	-2.206	0.031
Pair 6	Cadmium normal - cadmium cataract	-1.558	0.125
Pair 7	Magnesium normal - magnesium cataract	3.027	0.004

Magnesium

Magnesium level was significantly lower among cataractous patients than normal group .in hair samples concentration of magnesium was observed as 1277.446±41.076 and 1056.140±59.537 µg/g respectively among normal and cataractous group. Concentration of magnesium was recorded as 109.993±1.33 mg/L in serum samples of patients suffering from cecity while in serum samples of normal group it remained as 196.228±12.440 mg/L.

Discussion

Higher levels of nickel was observed in cataractous persons. These higher levels could be responsible factor for development of diseases because along with interfering the metabolism of essential metals such as Fe (II), Mn (II), Ca (II), Zn (II), or Mg (II) it can induce overt cytotoxicity and apoptosis, as well as chromosomal aberrations and morphological transformation in SHE cells [16-18] and 10T1/2 cells [19-22].

Zinc, a trace element that influences cell metabolism through a variety of mechanisms, appears to play an integral role in maintaining normal ocular function [23] The physiological functions for zinc have been studied predominantly in retina and retinal pigment epithelium where zinc is believed to interact with taurine and vitamin A, modify photoreceptor plasma membranes, regulate the light-rhodopsin reaction, modulate synaptic transmission and serve as an antioxidant. Poor Zn intake might result in deficiency and loss of Zn-dependent coenzymes associated with age related cataract.The ocular manifestations of zinc deficiency include altered vision, electroretinograms, and oscillatory potentials, and, if the deficiency is severe, ultra structural changes are detected in the retina and retinal pigment epithelium .we observed this foremost important metal at lower level in patients. Nourmohammadi I & Mirsamadi M [24] also reported significantly higher level of zinc in serum samples of control one(146.84 ± 41.33 µg/dl) matched to cataractous patients (123.39 ± 76.94 µg/dl) . Bhat, et al. [25] in a study in India reported lower zinc level in patients compared to

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controls. Significant difference was observed in serum zinc levels of patients and controls (P < 0.001) by Mahmood, et al. [8] (showing that the patient serum zinc levels (0.896 ± 0.202 vs. 0.478 ± 0.278) were significantly lower than the serum zinc levels of controls. These all finding are in agreement with our findings about zinc.

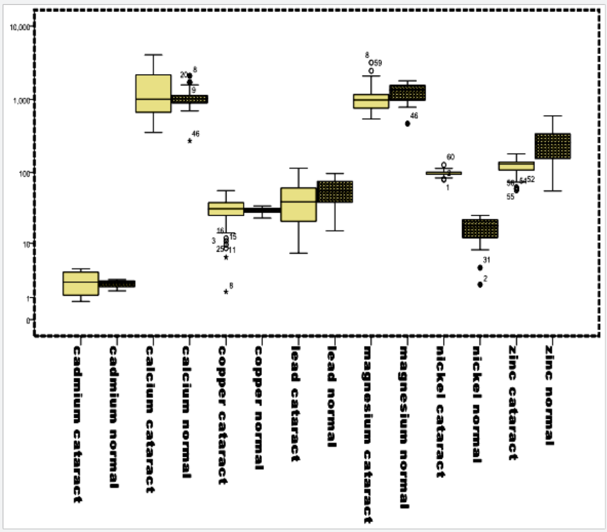

Figure 1: Comparative analysis of metallic elements in hair samples of normal and cataractous patients.

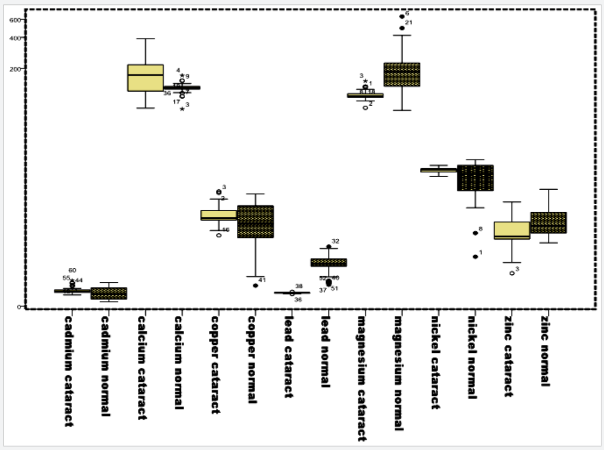

Figure 2: Comparative analysis of metallic elements in serum samples of normal and cataractous patients.

Copper being transitional metal ion-catalyzed formation of hydroxyl radical that could contribute to the protein modification observed in cataract and may play a role in the etiology of age-related cataract [26]. Copper is also known to accelerate lenticular opacification by damaging protein, lipid, and membranous structures. The change in the amount of copper ion in the lens may be the key to revealing the mystery of cataract formation. Akyol, et al. [12] reported slightly higher Cu concentrations (90-160 μg/dl) our observation coincides with Akyol ,as we observed higher concentration of copper in serum as well as hair samples of persons suffering from cataract allegorized to normal ones (Figure 1 & 2).we observed statistically significant difference among two groups regarding serum concentration while for hairs it was non-significant. Jacques, et al. [27] studied serum Cu levels and increased risk of cataract but the association was not statistically significant.

Maintenance of calcium homeostasis is critical to the clarity of the lens [28-30]. The calcium content increases progressively with the onset and maturity of senile cataract has been agreed to by almost all the observers. In some cataractous human lenses, calcium levels have been found to increase as much as 13 times the mean value of the Ca^{2+} concentration of the normal lens [31]. we evaluated the concentration of this metal through analysis of hair and serum samples and observed higher levels of calcium among cataractous patients compared to normal ones.

Our findings related to cadmium level revealed significantly higher concentration in hair and serum samples of cataract ones. Cadmium may hasten cataractogenesis by various mechanisms. It is known to compete with copper in the body and could affect the copper homeostasis of blood and copper containing proteins [32]. Cadmium may directly interact with lens proteins and denature them in cataractogenesis [33].

Conclusion

Findings in this study show a relationship between imbalances of metallic elements with cataract.

However, whether these changes in level of metallic elements are the actual cause of disease or consequences of disease, needs to be studied. Hair and serum provided good picture of this imbalance. Being invasive and easily accessible tools they can be used for such clinical studies.

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