DETERMINATION OF CONCENTRATIONS OF MAGNESIUM AND CALCIUM IN HUMAN BLOOD SERUM USING FLAME ATOMIC ABSORPTION SPECTROSCOPY AND THEIR RELATION WITH HUMAN BEHAVIOUR AND COMMON DISORDERS

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ABSTRACT

Some elements play a highly important role in our human body. We require these elements to keep our human body healthy and active. If the required elements are not taken in sufficient amount, it can cause symptoms of nutritional deficiency and many other disorders. However, our needs for these elements are easily met by eating a variety of foods from the different food groups. Our research was based on two important elements i.e. Magnesium (Mg) and Calcium (Ca)). We studied their importance in the human body as well as the effect they have on human health and behaviour when their maintained concentrations in the blood are misbalanced. For this purpose a group of volunteers were chosen from two geologically diverse environments, one being a natural and village environment called Hunza,located in the mountainous region of Pakistan and the other, Karachi, which is a busy and heavily polluted city located in the heart of Pakistan, which is a third world country. The concentrations of these two elements in their blood serum was measured using Flame Atomic Absorption Spectroscopy and were compared with the data provided by the volunteers related to their symptoms and behaviour. As the world moves towards modernization, the decline in the health of young individuals is increasing alarmingly with the decline in the consumption of natural products and environmental pollutants. This causes medical problems like weakness, hair loss, arrhythmia and many more like these to be prevalent in today's generation at an alarming rate. Furthermore, behavioural changes like anxiety, anger, and depression are also found very commonly in today's generation. This study aims at determining the relationship between these two essential elements and how their increased and decreased concentration in human blood serum affects all these factors. It also includes a detailed statistical analysis done using SPSS software for calculating the Mean, Median and the standard deviation for both the rural and urban region..

A connection was observed between people suffering from low levels of Calcium and Magnesium in their blood serum and disorders like hair loss, insomnia, muscle fatigue, joint pain and hyper activity. Behavioural symptoms like anger, frustration and anxiety was also linked with magnesium and calcium deficiency.

KEYWORDS

Flame Atomic Absorption Spectroscopy, Calcium deficiency, Magnesium Deficiency, Hunza, Karachi, Blood serum Concentration, Environmental factors

1. INTRODUCTION

The study was based on the relationship between Magnesium and Calcium imbalances in the human blood serum and how they related to the commonly occurring human disorders and behaviour. All of these elements have to be present in the human body in a controlled quantity for its healthy functioning. Imbalances in the concentrations of these elements within the human body leads to changed behaviour and a deteriorating health. We studied the difference between two groups of person living in completely different environments. We correlated the difference between their element concentration levels and the effect it had on their behaviour as well as studied their environmental and dietary intake. A study conducted by department of analytical chemistry in the University of Sindh, Jamshoro published a research paper titled "Evaluation of calcium, magnesium, potassium, and sodium in biological samples (scalp hair, serum, blood, and urine) of Pakistani referents and arthritis patients of different age groups" [1].This research compared the levels of Calcium (Ca) and Magnesium (Mg) in scalp hair, blood, serum and urine samples of patients suffering from Rheumatoid Arthritis and normal healthy people [1].All the patients were divided in two age groups of above 40 to 60 years and above 60 to 75 years.A microwave assisted wet acid digestion procedure was used for acid digestion of biological samples. The digests of all biological samples were analyzed for Mg and Ca by flame atomic absorption spectrometry (FAAS) [1].The results indicated significantly lower levels of Mg and Ca in the biological samples of referents suffering from rheumatoid arthritis [1].Another study conducted by the department of internal medicine at Liaquat University Hospital,Sindh,Pakistan titled "Hypocalcaemia in Acute Gastroenteritis" determined the concentration of calcium in serum of patients diagnosed with acute gastroenteritis [2].The analysis of the data was done on SPSS version 11.0 and Hypocalcaemia was observed in 57% of the patients. Most of the patients were taken from urban areas and low serum calcium level was observed in patients suffering from acute gastroenteritis [2].

1.1. Principle of Flame Atomic Absorption Spectroscopy

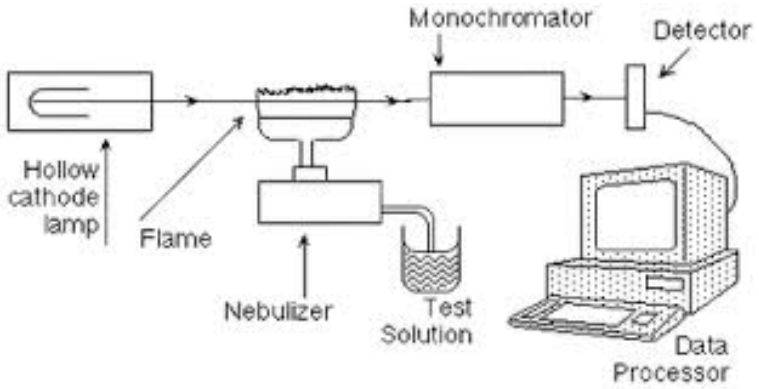

Figure 1. Working Principle of FAAS [3]

Principle of operation of FAAS is that it requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 ^{o}C. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths [4]. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A photomultiplier detects the amount of reduction of the light intensity due to absorption, and this is directly related to the amount of the element in the sample [4].

2. EXPERIMENTAL IMPLEMENTATION

This project started off with a review of previous researches and research papers published regarding FAAS and the elements and their effects in human body. It also included exchanging of ideas and discussions with senior Bio-medical professionals and research officials. Then the members were familiarized with Flame Atomic Absorption Spectroscopy principle and the working and operation of Aurora 1200I.1000 ppm stock/bulk solutions were prepared for Magnesium and Calcium using Aurora 1200I cookbook and a series of standards were set for both elements and their reference calibration curves were set.A research tool in the form of a questionnaire was created to better assist us in getting the desired data and in helping us through the research process and each questionnaire was given an individual and unique identification number. Sites of interests were studied and a plan was devised to successfully collect samples of the targeted audience and bring them safely to the research laboratory..Medical equipment necessary for sample collection were acquired from proper laboratories and approval was taken from the respective heads of the hospitals for the collection of the samples in Hunza and Karachi. The targeted audiences for sample collection were individuals from a rural area called Hunza, which is located in the northern mountainous region of Pakistan and Karachi, which is a main city of Pakistan. The desired blood samples were collected in gel tubes with unique identification numbers, taking the necessary precautions and centrifuged and the serum was collected in eppendorf tubes marked according to their respective gel tube and questionnaire. The blood serum was used to make ample solutions and then the results were measured against the reference calibration curve using Aurora 1200I.Results were obtained and a detailed analysis of the results was done by comparing the concentrations and the information collected from the questionnaires. The last step was to compile the result and draw sound conclusions and represent them.

2.1. Procedure for Making Samples

The samples were collected in gel tubes to prevent them from spoiling. The samples were then centrifuged at 2000 rpm for 10 minutes and the serum was then collected in labeled eppendorf tubes and kept in a maintained temperature in the freezer. Once the sample testing was starting, the serum was taken out from the freezer and then diluted to a level of 1:10 with DI water to get the sample solutions. The beakers containing the sample solutions were labeled according to the respective questionnaire (tool). The samples were then run using Aurora 1200I to get the concentrations of elements in them.

2.1.1. Reagents Used for Magnesium and Preparation of Standard and Bulk Solutions

Magnesium Metal Strip
Hydrochloric Acid (HCl)

Deionised Water
Preparation of Stock / Bulk Solution was done by dissolving 1.00 gm of magnesium metal strip slowly in a minimum volume of 1:1 HCL and diluted to 1 L to get a 1000 ppm Mg solution. Preparation of a series of standards was done and the standards taken were 0.3 ppm.0.5 ppm and 0.8 ppm to get a standard calibration curve of magnesium metal.

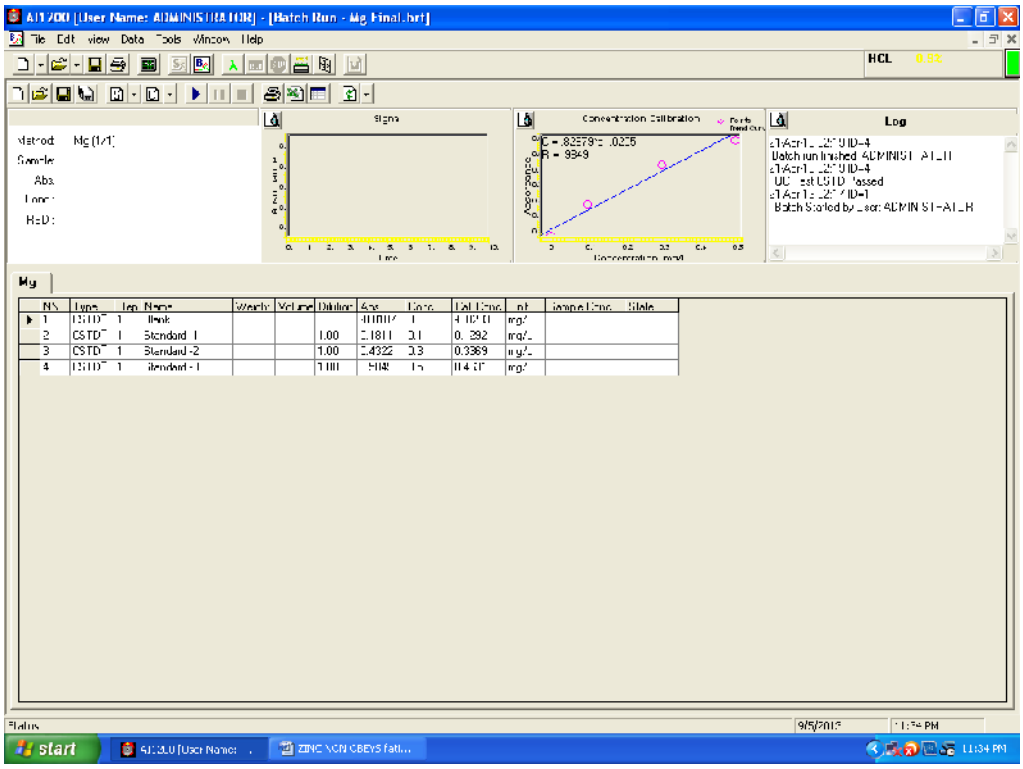

Figure 2. Standard Calibration Curve (Mg)

2.1.2. Reagents Used for Calcium and Preparation of Standard and Bulk Solutions

$CaCO_3$ (Calcium Carbonate)
H_2O
HNO3 (Nitric acid)
Deionised water

Preparation of Stock/ Bulk Solution was done by adding 2.497g of dried $CaCO_3$ to 50ml of H_2O and dissolving the minimum value of 1:4 HNO_3 by adding the acid drop wise and Diluting it to 1 litre to give a 1000 ppm Calcium solution.

Preparation of a series of standards was done and the standards taken were 0.4 ppm.0.8 ppm and 1.6 ppm to get a standard calibration curve of Ca metal.

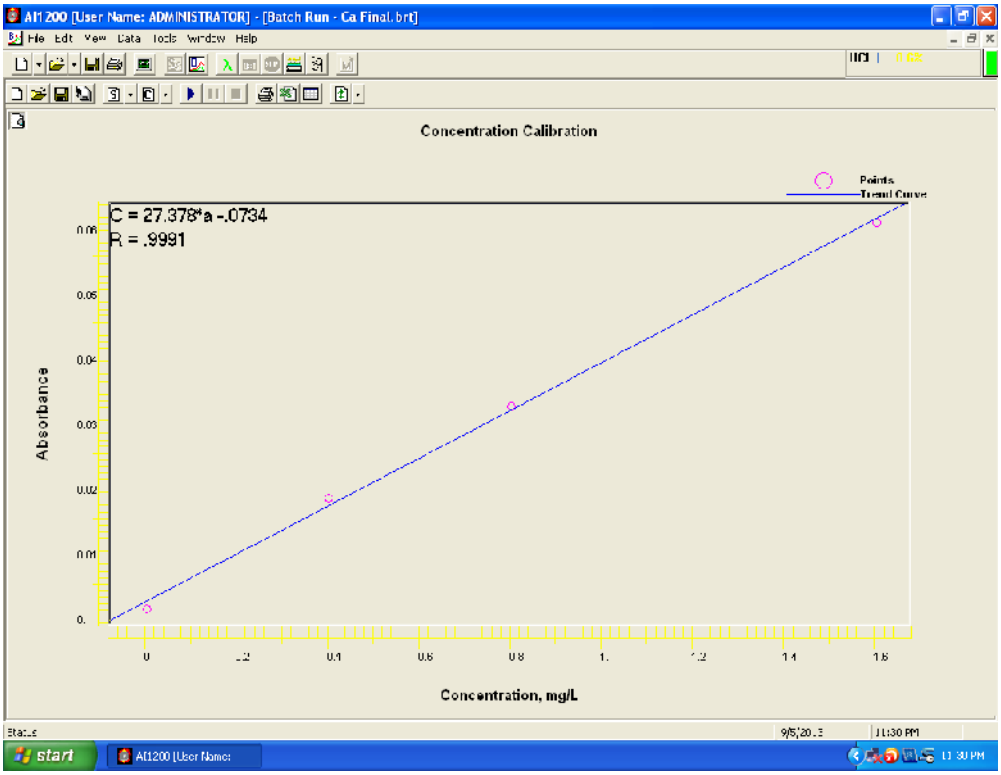

Figure 3. Standard Calibration Curve (Ca)

2.2. Statistical Analysis

Table 1. Mean, Median and Standard Deviation of the Results Obtained from the Rural and Urban Area.

	Magnesium		Calcium	
	Rural	Urban	Rural	Urban
Mean	17.505	16.517	6.2916	4.4136
Median	17.946	17.133	5.5276	4.2547
Std.Deviation	1.170	2.410	2.073	0.741

2.3. Data Analysis

The data was gathered from the questionnaires and the results of FAAS and compiled into a table for accurate results and observations which are displayed in figure Table 2 and Table 3.

]Table 2. Concentrations of Magnesium and Reported Behaviour and Disorders.

ID NUMBER	CONCENTRATION	MALE/FEMALE	DISORDERS	BEHAVIOUR
Mg-01	17.818	Male	Muscle weakness	Angry, Depressed
Mg-02	18.054	Male	Hair loss	Angry, Happy
Mg-03	18.451	Male	Hyperactivity	Angry
Mg-04	15.386	Male	Hair loss, dizziness	Happy
Mg-05	15.659	Male	Muscle fatigue, weakness Weight loss, joint pain, delayed healing	Angry, Sad, Frustrated, Confused
Mg-06	17.897	Male	Weakness	Angry, Happy
Mg-07	18.876	Male	Low BP, Muscle Fatigue , Weakness, Joint pain	Angry, Sad, Confused, Happy
Mg-08	17.995	Male	Joint pain, High BP	Angry, Happy
Mg-09	16.802	Male	Muscle Fatigue ,Weakness, Joint Pain , Low BP	Angry, Sad, Happy, Confused
Mg-10	18.119	Male	Hair loss, Delayed Healing , Tingling sensations in body,	Sad, Happy
Mg-11	16.488	Male	Hair loss, Headache	Angry, Happy
Mg-12	18.402	Female	Muscle Fatigue, Weakness, Weight loss,	Depressed, Sad

			Arrhythmia, Low BP, Headache	
Mg-13	18.411	Male	Hyper activity, Headache	Angry, Depressed, Abusive
Mg-14	15.067	Female	Muscle Fatigue, Weakness, Low BP, Craving for Chocolate, Headache, Paleness, Anxiety, Restlessness, - Dizziness, Burning and tingling	Depressed, Anxious, Happy
Mg-15	18.577	Male	Hairloss	Happy, Frustrated
Mg-16	17.779	Female	Conc. Issues, Weight gain, Hyper activity, Headache	Happy
Mg-17	18.714	Female	Weakness, Low BP, Headache	Sad, Happy
Mg-18	16.026	Male	Hair loss, Headache	Angry, Frustrated, Anxious
Mg-19	14.458	Male	Hair loss, Headache, Insomnia, Delayed healing, Burning and tingling, Hyper activity	Frustrated, Sad, Happy, Abusive, Confused
Mg-20	11.235	Male	Hair loss, Insomnia, Dizziness, Headache	Happy

Table 3. Concentrations of Calcium and Reported Behaviour and Disorders.

ID NUMBER	CONCENTRATION	MALE/FEMALE	DISORDERS	BEHAVIOUR
Ca-01	3.7511	Male	Muscle fatigue, weakness, eight loss, joint pain, delayed healing of wound	Angry, sad, confused, frustrated
Ca-02	3.9389	Female	Irregular heartbeat, insomnia, hyper activity, joint pain, weight gain	Angry, happy
Ca-03	4.9132	Female	Muscle fatigue, Hyper activity	Angry, sadness, confused
Ca-04	5.4508	Female	Muscle fatigue, weight gain, joint pain	Happy
Ca-05	4.9473	Female	Hair loss, weight loss, joint pain	Angry, happy
Ca-06	8.5718	Male	Muscle fatigue, nausea, anxiety, paleness, cravings of chocolate, weakness, headache, weight gain	Happy, frustrated, depressed
Ca-07	5.6045	Male	Craving for chocolates	Depressed, happy
Ca-08	9.2939	Male	Weight loss, hyper activity	Happy
Ca-09	8.4972	Female	Weight gain	Angry, depressed, sadness
Ca-10	7.9471	Female	-------	Angry, depressed, sadness, happy
Ca-11	4.1780	Female	Muscle fatigue, weakness	Anxious, depressed
Ca-12	4.0716	Male	Hyper activity, weakness	Happy, frustrated
Ca-13	4.4620	Male	Concentration issues, hyper activity, insomnia	Depressed, happy, frustrated, confused
Ca-14	4.3314	Female	Muscle fatigue, hyper activity, joint pain, weakness, hair loss	Happy, frustrated, confused

Ca-15	4.9401	Male	Irregular heartbeat, high blood pressure, insomnia, palpitation	Angry
Ca-16	3.7853	Female	Muscle fatigue, hyper activity, hair loss, burning sensations	Happy, frustrated, anxious
Ca-17	4.7647	Female	Muscle fatigue, anxiety, high b.p, hyper activity, concentration issues, insomnia, dizziness, headache	Angry, depressed, frustrated, confused
Ca-18	3.9542	Female	Muscle fatigue, nausea, weakness, hair loss, anxiety, joint pain, insomnia, weight gain	Happy
Ca-19	3.5184	Male	Muscle fatigue, headache	Happy
Ca-20	6.1301	Female	Muscle fatigue, headache, restlessness, insomnia, weakness, low b.p	Angry, depressed, anxious

3. RESULTS

3.1. Magnesium Results and Histograms

Volunteers living in the rural regions were reported to have lesser cases of magnesium deficiency having a mean of 17.05 as compared to the volunteers taken from urban areas with a mean of 16.52.People with low magnesium concentration in their human blood serum than the normal range were found to have been suffering from the following common symptoms: Hair loss, Insomnia, Headaches, Muscle Fatigue. They also reported experiencing anger and frustration.

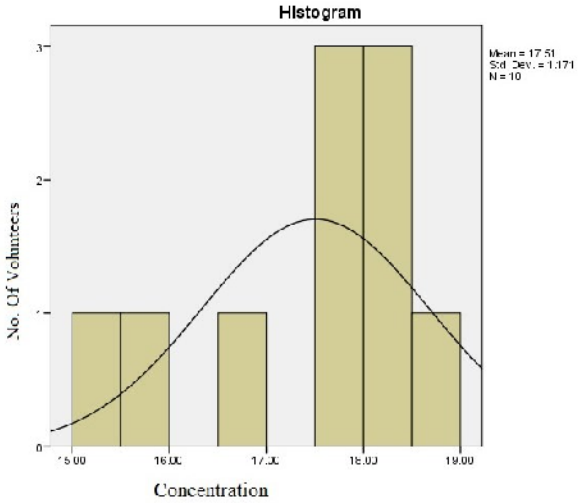

Figure 4. Histogram for serum magnesium levels of rural volunteers.

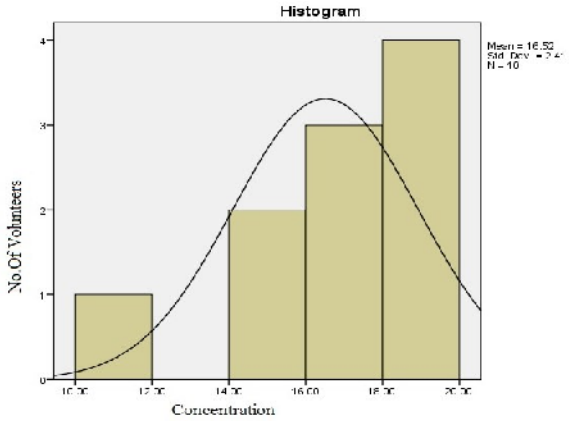

Figure 5. Histogram for serum magnesium levels of urban volunteers.

3.2. Calcium Results and Histograms

Volunteers living in the rural areas were reported to have lesser cases of calcium deficiency having a mean 6.29 of as compared to the volunteers taken from urban areas having a mean of 4.4.Volunteers with low calcium concentration in their human blood serum than the normal range were found to have been suffering from Muscle Fatigue, Joint Pain, Short temperament and Hyper Activity most commonly. They also reported feelings of frustration, depression and anxiety.

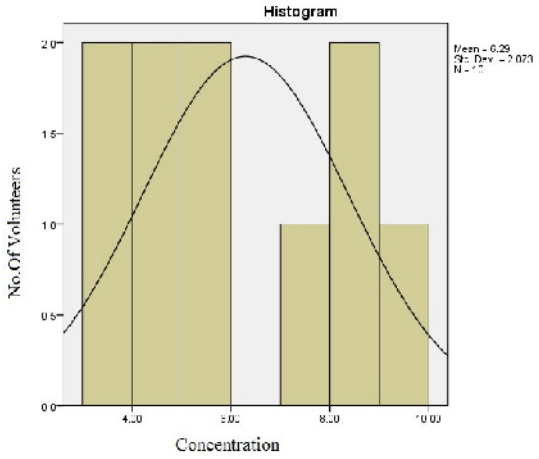

Figure 6. Histogram for serum calcium levels of rural volunteers

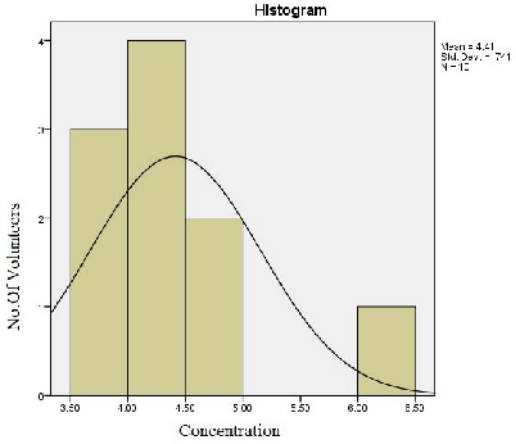

Figure 7.Histogram for serum calcium levels of urban volunteers.

4. DISCUSSION

Volunteers living in the rural regions were reported having lesser cases of magnesium deficiency having a mean of 17.05 as compared to the volunteers taken from urban areas with a mean of 16.52.Volunteers with low magnesium concentration in their human blood serum than the normal range were found to have been suffering from hair loss, insomnia, headaches and muscle fatigue commonly. Behaviourally, they reported feelings of anger and frustration most of the times.

Volunteers living in the rural regions were also reported to have lesser cases of calcium deficiency having a mean 6.29 of as compared to the volunteers taken from urban areas having a mean of 4.4. Volunteers having low calcium concentration in their human blood serum than the normal range reported experiencing muscle fatigue, joint pain and hyperactivity most of the time. Behaviourally, they reported experiencing frustration, depression and anxiety.

5. CONCLUSIONS

Comparison of the results obtained from both urban and rural areas indicated that volunteers living in the urban areas consumed a diet which was deficient in maintaining a healthy concentration of calcium and magnesium in their serum which affected their health and their behaviour in daily life as compared to the volunteers from rural areas where the consumed diet was able to maintain a healthy concentration of these element concentrations in the human blood serum.

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