REFERENCE RANGE FOR COPPER IN ADULT POPULATION (20-29 YEARS) OF LAHORE - PAKISTAN

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ABSTRACT

Copper, an essential trace element for health, plays various biological roles in human body. Serum copper reference values are important for assessing copper associated abnormalities and the prevalence of copper deficiency. The aim of this study was to determine reference range of copper in adult population of Lahore. Serum copper concentration was measured by flame atomic absorption spectrometry in 450 healthy adults aged 20 to 29 years, which were randomly selected. After application of exclusion criteria reference values were determined in apparently healthy subjects according to guidelines of International Federation of Clinical Chemistry. Reference value for serum copper concentration ranged between 4.72µmol/L to 31.48µmol/L (mean 17.62 µmol/L ±6.67). The mean serum copper level for males was 18.57µmol/L ± 6.61. The value for male subjects ranged between 4.72µmol/L to 31.7µmol/L. The mean serum copper level in females being 16.52µmol/L ± 6.67. Similarly the values for females range between 4.72 µmol/L to 30.48µmol/L. In conclusion this study presents reference values for serum copper concentration in adult population of Lahore. The results here shows that there is significant difference in serum level of copper among different countries. This difference may be due to racial and genetic difference, dietary habits and socioeconomic and analytical variables. KEY WORDS: copper, reference value, atomic absorption spectrometry, population

ملخص: النحاس من العناصر الاساسية في الصحة ويلعب أدوار بيولوجية مختلفة في جسم الانسان. القيم المرجعية لمستوي النحاس في مصل الدم ضرورية لتحديد المستويات الغير طبيعية للنحاس في المصل ولتحديد معدل انتشار نقص النحاس. الهدف من هذه الدراسة هو تحديد مدى مرجعي لقيم النحاس للبالغين من سكان مدينة لاهور. تم قياس تركيز النحاس في الدم لعدد ٤٥٠ من البالغين الأصحاء الذين تتراوح أعمارهم بين ٢٠ إلى ٢٩ عاما (والذين تم اختيارهم عشوائيا) بواسطة جهاز التحليل الطيفي. بعد تطبيق معايير الاستبعاد تم تحديد القيم المرجعية في الاصحاء وفقا لموجهات الاتحاد الدولي للكيمياء السريرية. القيمة المرجعية لتركيز النحاس في مصل الدم تراوحت بين(٤،٧٢ ميكرو مول /لتر الي ٣١،٤٨ ميكرو مول / لتر) (المتوسط ١٧،٦٢ ميكرو مول / لتر ± ٦،٦٧). كان متوسط مستوى النحاس في المصل للذكور (١٨،٥٧ ميكرو مول / لتر ± ٦،٦١), حيث تراوحت القيمة للذكور بين (٤،٧٢ ميكرو مول / لتر الي ٣١،٧ ميكرو مول / لتر). و متوسط مستوى النحاس في المصل للإناث ١٦،٥٢ميكرو مول / لتر ± ٦،٦٧. وبالمثل، فإن القيم لمجموعة الإناث تراوحت بين(٤،٧٢ ميكرو مول / لتر الي ٣٠،٤٨ ميكرو مول / لتر). الخلاصة: قدمت هذه الدراسة القيم المرجعية لتركيز النحاس في مصل الدم للبالغين من سكان مدينة لاهور. النتائج هنا أظهرت أن هناك فرق كبير في مستوى النحاس بالمصل بين مختلف البلدان, هذا الفرق قد يكون راجعا إلى الاختلافات العرقية أو الجينية, أو للاختلاف في العادات الغذائية أو للمتغيرات الاجتماعية والاقتصادية أو التحليلية.

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INTRODUCTION

The availability of accurate trace element reference values in human is an important indicator to the health status of the general population and the occupational groups which are exposed to trace elements.[1] The key role of the laboratory scientist is to help the clinician in interpreting observed values, by providing relevant reference values in a convenient and practical form.[2] In Pakistan, reference values used in laboratories have been established in the western population. But these can be questioned due to differences in genetic load, lifestyle, and diet.[3]

Copper, an essential trace element is involved in multiple biological processes.[4] The importance of this trace element in health and disease cannot be ignored. To assess the relation of this trace element with different diseases, it is important to have a baseline status of this trace element in the body in the healthy sample of population. So this requires establishment of accurate reliable reference values that can be used in clinical decision.[5]

The recommended daily requirement of copper in our body is 2.5 - 3 mg/day. About 30% of ingested copper is absorbed in the intestine, bound to albumin and transported to the liver, where it is stored. The major circulating form ceruloplasmin is synthesized in the liver.

Copper is an integral component of at least 16 essential metalloproteinase, including cytochrome oxidase, monoamine oxidase, lysyl oxidase, tyrosinase and ceruloplasmin. It participates in prostaglandin synthesis, in the formation of connective tissues, in the function of Central nervous system and in hematopoiesis. It is required for red and white cell maturation, cholesterol and glucose metabolism and myocardial contractility. The stability of vein's membrane depends on the adequate amount of copper in the tissues. Copper is involved in the transport of iron and vitamin A.[6] Its deficiency contributes to the development & progression of certain diseases.

The objective of this study was to establish reference range for copper in adult population with 20 to 29 years of age residing in Lahore.

DESIGN AND METHODS

This was a descriptive cross sectional study which was conducted on apparently healthy individual of age range 20 to 29 years from nine different zones of Lahore. A total of 450 healthy individuals comprising of 216 males & 234 females were randomly selected using a multistage sampling method. Five union councils were selected randomly from each zone. From each selected union council ten healthy subjects who were assessed clinically were selected for the study. So a total of 450 samples were collected from randomly selected 45 union councils. The exclusion and inclusion criteria were based on the IFCC guidelines for the establishment of reference values. The study was approved by Ethical Committee. Written consent was taken from all the subjects.

At the time of sampling based on past medical history and physical examination only those individuals were included who did not prove any signs and symptoms of disease.

The samples were collected under aseptic conditions. 7 ml of blood was collected in 2 serum separator vacutainer tubes (SST, BD vacutainer) one for the determination of trace elements and other 3.5 ml blood was collected in SST for determination of albumin, glucose & ALT without venous stasis and without frothing. The sample was transported to University of Health Sciences, Lahore in an ice box containing ice bags.

Serum was separated by centrifugation at 3000 g for 05-10 minutes. Serum separated was then carefully shifted into 1ml nitric acid treated aliquot (for trace elements determination) & the other separated serum was collected in a separate aliquot for ALT, albumin and sugar levels. Hemolyzed and samples having high levels of glucose, albumin or ALT were excluded from this study.

Only nitric acid washed plastic lab ware was used for sample and standard preparation for trace elements. Urine examination for protein & sugar was also performed. Trace element Copper was performed on Hitachi Z-2000 Series Atomic Absorption Spectrophotometer.

Serum copper was measured by flame atomic absorption spectrometry (Hitachi Z-2000) following a one in five dilution with distilled water. The results were multiplied by dilution factor 5.

Statistical analysis: Data was analysed using SPSS version 20. Serum Copper levels were expressed as Mean ± SD.

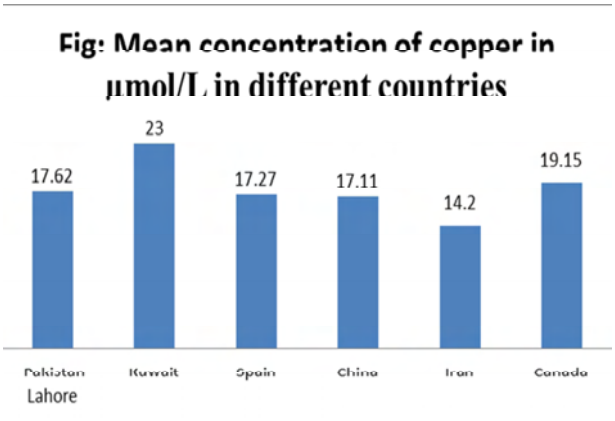
RESULTS

A total of 450 healthy subjects were included in which 216 were males and 234 were females. Only those subjects were included who fulfilled the inclusion criteria. Their health status was assessed after detailed history & physical examination. Blood sugar random, ALT, Albumin, urine for proteins & sugar were performed. The results of these tests were also in the normal range. The blood samples of these healthy 450 subjects were then selected for the estimation of serum copper which was estimated on atomic absorption spectrometer.

The mean age range of these healthy subjects was 25 years. The mean for serum copper was found to be 17.62±6.67 µmol/L. Here the value of serum copper ranged between 4.72 to 31.48µmol/L. The mean serum copper level for males was 18.57±6.61 µmol/L with a range of 4.72 to 31.7µmol/L. The mean serum copper level in females being 16.52±6.67 µmol/L. Here the values for females ranged between 4.72 to 30.48 µmol/L. Minimum & maximum levels of Copper in µmol/L along with 95th confidence interval, also the 2.5th percentile & 97.5th percentile are shown in table 1.

Table 1: Minimum & Maximum levels of COPPER in µmol/L with 95th confidence interval (CI), also the 2.5th percentile & 97.5th percentile

Copper	*Min µmol/L*	*Max µmol/L*	*95th CI*	*2.5th Percentile*	*97.5th Percentile*
Male	3.14	33.05	17.31 -19.83	4.72	31.7
Female	3.14	41.40	15.58–17.78	4.72	30.48
Overall	3.14	42.49	16.84–18.41	4.72	31.48



DISCUSSION

Reference interval refers to the interval between reference limits that includes usually 95% of the reference values.[7] It is also defined as a range comprising between 2.5th & 97.5th percentile of the data distribution from a given reference population.[8] Establishing a normal range of trace elements in the sera of healthy individuals in any geographical area is very

important for interpretation of trace element results and for further studies in that particular region.[9]

Copper, an essential trace element is required in body for performing major functions. Copper is an important component of ceruloplasmin and various enzymes including ferroxidase II, lysyl oxidase, Zn-Cu superoxide dismutase, tyrosinase.[9]

The range for copper varies from 4.72 to 31.48µmol/l with a mean of 17.62µmol/L. These results were in close proximity with another study carried out in Pakistan in 2004 in Islamabad / Rawalpindi area where mean copper concentration was found to be 18.1µmol/L with a range of 9.4 to 30.06 µmol/L.[10] This slight difference in the mean value of copper might be attributed to the difference in the age range of the population included in that study. Another factor contributing to this difference may be the integrated diet mainly consisting of wheat & wheat products.[10]

A study conducted by Manser and Khan[11] in Karachi on normal population found that the mean concentration of copper was 14.71µmol/L which was lower as compared to this study. The main contributing factor for this difference could be the excessive use of the automobiles & pesticides resulting in environmental pollution.

Another factor contributing to the increase in the concentration of copper is the distribution of water for domestic uses, particularly in the system where an acidic pH exists.[12] In this regard, WHO conducted a study in Pakistan on quality of drinking water and found that the concentration of the copper in the drinking water varies widely which is constantly adding copper element to the drinking water. Along with the manufacturing of the commercial appliances like wires & pipes, copper was also used as the copper sulphate pentahydrate for the control of algae in water pipes.

The other factors include the inhalation of copper fumes produced mainly during welding & plumbing increases copper concentration in blood. Copper could also be present in drinking water pipes as it remains in pipes during water supply. The deficiency of iron, zinc, vitamin B and vitamin C also raises copper concentration in human.[13]

Copper is being constantly added in soil form of pesticides, fertilizers, industry & sewage sludge. Similarly melting, grinding & cutting of copper may produce fumes & dusts & exposure & inhalation of these fumes may be the contributors of raised copper levels in our body.[14]

A study conducted in Iran in 2011 showed that the mean value of serum copper in age ranging from 20 to 29 years was 14.2µmol/L. The mean age range being 25 years is comparable to population in Lahore where mean copper concentration was 17.62µmol/L. This was mainly attributed to the lifestyle & habits of the people living in Iran.[15] Urbanization & educational attainments might be the factors contributing to the changes in serum copper levels.

The reference range used by the trace element & Environment Toxicology Laboratory at the University of Alberta had a mean of 19µmol/L for copper which is close to the mean (17.62µmol/L) of our population under study. The author mentioned that the range for a trace element analysis is only suggestive of the usual exposure of this element encountered in daily life.[16]

Similar study conducted in Kuwait determined serum level of copper in normal population. Here mean copper concentration was found to be 23µmol/L.

This large difference in mean concentration of copper is mainly attributed to Desalination plants which are used in Arabian Gulf. These plants were the main source of increased copper levels in the environment. On the other hand industries & oil pollution were constantly adding copper to the environment.[17]

The concentration of copper in our study was comparable to the reported values from Spain and China where mean copper concentration was 17.27µmol/L and 17.11µmol/L respectively.[18] Similarly mean concentration of copper reported from Italy was 19.15µmol/L which was also comparable with the mean concentration of our study.[19]

The mean copper level (17.62µmol/L) of our study was found to be greater than that reported in studies from Bangladesh & Japan that had a mean concentration of 15.38 µmol/L & 12.56µmol/L respectively.[20,21]

Our study did not show a significant difference in mean concentration of copper when compared with mean concentration of copper quoted in reference books ($p = 0.9$).

The mean serum copper concentration of our study compared with various countries is shown in following figure.

CONCLUSION

This study provides data for the establishment of reference range for copper in healthy population of Lahore. Here the mean concentration of copper is 17.62±6.6µmol/L. The results showed that there is significant difference in serum level of copper among different countries. Diet, physical environment and socioeconomic conditions all affect the physiology of a population. These values can be useful for interpretation and clinical management of copper disorders. As there are no established reference ranges for trace elements in Pakistan, these findings can form the basis and reference for any future studies on trace elements in Pakistan.

LIMITATION OF THE STUDY

The study was carried out on only specific age group due to financial constraint faced during the study.

REFERENCES

1. Goldbeck-Wood S, Dorozynski A, Lie LG, Kučera J, Benckob V, Sabbionic E, Van der Venne MT. Review of trace elements in blood, serum and urine for the Czech and Slovak populations and critical evaluation of their possible use as reference values. Science of the total environment. 1995; 166(1): 211-34.
2. Ashavaid TF, Todur SP, Dherail AJ. Establishment of reference intervals in Indian population. Indian J Clin Biochem. 2005; 20(2): 110–18
3. Mansoor R, Saadat A, Khan FA, Sattar A, Iqbal A. Reference values for serum calcium. Pak J Pathol 2004;15(2):49-53.
4. Lux O, Naidoo D. The assessment of biological variation components of copper, zinc, and selenium. The Journal of Nutritional Biochemistry. 1995; 6(1): 43-7.
5. Rahil-Khazen R, Bolann JB, Ulvik JR. Trace elements reference values in serum determined by inductively coupled plasma atomic emission spectrometry. Clin Chem Lab Med. 2000;38(8):765-72
6. Marjani A, Mojerloo M, Mansorian AR, Golalipour MJ. Serum Zinc and Copper level before and after haemodialysis. Iranian Red Crescent Medical Journal. 2005; 8(1);71-75
7. How to define and determine reference intervals in the clinical laboratory: approved guidelines, NCCLS document C28-A2. 2001; 20(13) http://www.nccls.org/
8. Trujillano J, Muiño JM, March J, Sorribas A. A more flexible parametric estimation of univariate reference intervals: A new method based on the GS-distribution. Clin Chim Acta. 2007;379(1-2):71-80
9. Al-Sayer H, Al-Bader A, Khoursheed M, Asfar S, Hussain T, Behbehani AI, Mathew

A, Dashti H. Serum values of copper, zinc and selenium in adults resident in Kuwait. Med Principles Pract. 2000; 9:139–146
10. Rahman S, Khalid N, Ahmad S, Ullah N, Iqbal ZM. Essential trace metals in human whole blood in relation to environment. Pakistan J. Med. Res. 2004; 43(2):46-51
11. Manser WW, Khan MA. Trace elements studies on Karachi population, part 1: Normal ranges for blood copper, zinc and magnesium for adults. J Pak Med Assoc 1989; 39(2):43-9
12. National standards for drinking water quality. Pakistan Environmental Protection Agency (Pak EPA). Ministry of Environment, Government of Pakistan, Islamabad. 2008. Pak, E. http://scholar.google.com.pk/scholar?q=National+Standards+for+Drinking+Water+Quality+in+pakistan&btnG=&hl=en&as_sdt=0%2C5&as_ylo=2008&as_yhi=2008
13. Ali SFA, Imtiaz N, Mehdi SU, Asif M. Determination of Copper Concentration in Human Blood Serum by Using Flame Atomic Absorption Spectroscopy (FAAS). Paper presented at: International Conference on Chemical Engineering and Pharmaceutical Sciences (ICCEPS'2012) April 13-15, 2012 Pattaya
14. Khan MA, Ahmad I, Inayat ur Rahman. Effect of environmental pollution on heavy metals content of Withania somnifera. Journal of the Chinese Chemical Society. 2007;54:339-343
15. Parizadeh RMS, Kazemi-Bajestani RMS, Moghaddam SA, Mobarhan GM, Esmaeili H, Majidi RM, Emadzadeh A, Safarian M, Nezhad AM, Khodaei HG, Hosseini JS, Parizadeh JMS, Oladi RM, Ferns G. Serum zinc and copper concentrations and socioeconomic status in a large Persian cohort. Asian Biomedicine. 2011; 5 (3): 329-35
16. Guidotti TL, McNamara J, Moses MS. The interpretation of trace element analysis in body fluids. Indian J Med Res. 2008 Oct; 128(4):524-32 .
17. Lattemann S, Höpner T. Environmental impact and impact assessment of seawater desalination. Desalination. 2008; 220(1-3):1–15
18. Shang S, Hong W. Flame atomic absorption spectrometry using a microvolume injection technique for the determination of Cu, Zn, Ca, Mg and Fe in whole blood from healthy infant and mother ears. Fresenius' Journal of Analytical Chemistry. 1997; 357(7): 997-99
19. Minoia C, Sabbioni E, Apostoli P, Pietra R, Pozzoli L, Gallorini M, Nicolaou G, Alessio L, Capodaglio E. Trace elements reference values in tissues from inhabitants of the European community I. A study of 46 elements in urine, blood and serum of Italian subjects. Sci Total Environ. 1990;95: 89-105
20. Khan AH, Khaliquzzaman M, Zaman MB, Husain M, Abdullah M, Akhter S. Trace element composition of blood in adult population in Bangladesh. Journal of Radioanalytical Chemistry. 1980; 57(1):157-167
21. Satoh Y, Yazawa A. Contents of heavy metals in the blood of inhabitants in Yokohama city in Japan. Yokohama E., Ken Nenpo. 1978;17: 63-66.