Original Article

# SERUM ZINC AND NEUTROPHIL FUNCTION IN LOWER, AND UPPER / UPPER MIDDLE SOCIOECONOMIC GROUPS

Uzair Mumtaz, Hamid Javaid Qureshi and Mohammad Shoaib

**Objective:** To assess the serum zinc and neutrophil function levels in different socioeconomic groups.

**Material and Methods:** A total of 100 healthy subjects of 10-30 years of age, both male and female were investigated. Out of these, 50 (25 males and 25 females) belonged to the lower socioeconomic (LSEC) group and 50 (25 males and 25 females) belonged to the upper / upper middle socioeconomic (USEC) group. Serum zinc was determined by colorimetric method and neutrophil function was estimated by the Nitroblue Tetrazolium reduction test (NBT). Both the parameters were compared in the two socioeconomic (SEC) groups.

**Results:** MIt was found that serum zinc was significantly lower in the LSEC group. Similarly NBT percentage phagocytosis was also significantly lower in the LSEC group as compared to the USEC group. NBT percentage phagocytosis did not show significant gender difference in the LSEC group, however it was significantly higher in females than in males in the USEC group.
Generally a significant positive correlation was found between serum zinc and neutrophil phagocytic function.

**Conclusion:** The LSEC class has low serum zinc levels and low NBT percentage phagocytosis and hence is at greater risk of developing infections.

**Key words:** Na.

## Introduction

Zinc, an essential element for all forms of life1, exists in nearly every cell of the body.[2,3] The richest sources of zinc are meat, milk products, shell fish (oyster) and poultry while fruits and vegetables are poor sources.[3,4] Oyster contains the largest content of zinc which is 188.5-341 mg/kg. Human breast milk has higher concentration of zinc[5] (USDA 2011). The quantities of zinc and phytate in the diet are the primary factors determining zinc absorption.[6] Large amounts of phytate and fiber inhibit zinc absorption.[7]

Zinc is important for immunity. The importance of zinc in cell mediated immune responses was revealed by the report of Fortes et al (1998) which stated that zinc supplementation improved cell mediated immune response in older population.[8] Zinc potentiates the effects of antiseptic agents.[9] Zinc supplementation resulted in stronger humoral responses against antigenic challenges particularly in raising immunoglobulin G and immunoglobulin M levels in sheep.[10]

Zinc protects the polymorphonuclear leucocytes against the toxic effects of rosins, which are commonly used in dentistry[11]. It stimulates oxygen radical formation in human neutrophils.[12] Compared with residents who had low zinc levels, people with normal levels had fewer cases of pneumonia, required fewer antibiotic prescriptions for it, and when they did get pneumonia, they had it for fewer days. It is essential for immune system.[3] Neutrophil chemotaxis is impaired in zinc deficiency.[13] Zinc supplementation increases the percentage of phagocytic polymorphonuclear leucocytes and their mean phagocytic activity particularly in subjects with initial low phgocytosis.[14] In zinc deficiency, not only is the total amount of antibodies diminished; even the repertoire of antigens recognized by these antibodies is depressed. Interestingly, this effect is even seen in mild or transient zinc deficiency during pregnancy.[15] Certain developmental steps responsible for B-cell receptor repertoire maturation thus seem to be dependent on zinc.[16] In addition to a negative impact on the specific immune responses, depression of innate immune mechanisms like chemotaxis and phagocytosis of neutrophils has also been found.[15]

Zinc deficiency is associated with diets based on plant foods which are rich in zinc absorption inhibitors. Such diets are habitually consumed in rural areas and in economically poor areas of the cities[17.] Marginal zinc deficiency and suboptimal zinc status have been recognized in many groups of the population in both less developed and industrialized countries. Although the cause in some cases may be inadequate dietary the cause in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are most likely the most common causative factor. Phytate, which is present in foods like cereals, corn, rice and vegetables, has a strong negative effect on zinc absorption from composite meals. Inositol hexaphosphates and pentaphosphates are the phytate forms that exert these negative effects on zinc absorption.[18] Zinc deficiency leads to disturbed body functions, the most important of which is decreased immunity. Its deficiency appears in those individuals who do not take animal food. Majority of poor people in Pakistan do not afford animal foods due to their high prices and hence consume foods of vegetable origin. Food of vegetable origin contains phytates which are zinc absorption inhibitors. Hence the poor population of Pakistan is likely to be suffering from zinc deficiency and immunod-eficiency. The present study was planned to assess zinc levels in LSEC and USEC groups and their effect on the neutrophil phagocytic function.

## Materials and Methods

This cross sectional analytical study was conducted in the Department of Physiology, Services Institute of Medical Sciences, Lahore on 100 healthy subjects of 10-30 years of age. Out of these, 50 (25 males and 25 females) belonged to the LSEC group and 50 (25 males and 25 females) belonged to the USEC group. The subjects having average monthly income of 0-3200 rupees per capita per month ((US$ 0-1.25 per capita per day) were included in the LSEC group. The subjects having a family income of more than 4000 rupees per capita per month and living in their own house were included in the USEC group19. Informed consent of the subjects was obtained. History taking and general physical examination of the subjects were carried out. Individuals who were diabetic, hypertensive, smokers and those taking any medication or drugs especially steroids, vitamin supplementation or minerals were excluded on history. Five millileters (ml) of blood was drawn aseptically from each subject. Out of this sample, 1 ml of blood was poured in the special siliconised vial containing 20 units of heparin for performing the "Nitroblue Tetrazolium Test" (NBT) to determine the neutrophil function. It was mixed gently, but well, by tilting slightly and "rolling" the vial for approximately 30 seconds. Contact of blood with cap was avoided. The remaining blood was centrifuged at 2500 rpm for 10 minutes at room temperature. The serum was then separated and stored at 4°C. Serum zinc was determined by colorimetric method using the kit manufactured by Spectum20. Neutrophil function was estimated by the stimulated Nitroblue Tetrazolium reduction test (NBT) using the kit manufactured by Sigma21. The test involved incubation of blood with a buffered solution of NBT and a stimulant. Smears were prepared, stained and examined microscopically to determine the percentage of neutrophils showing intracytoplasmic deposits of formazen. Data analysis was carried out with the SPSS version 19 (SPSS, Inc, Chicago, IL, USA). Arithmetic mean and standard deviation (SD) of each parameter were determined. The significance of differences among the groups was analyzed by student's t-test. Pearson's correlation was used to determine correlation between serum zinc and neutrophil percentage phagocytosis. p-value < 0.05 was considered statistically significant.

## Results

**Table 1** shows a comparison of serum zinc and NBT percentage phagocytosis (%) between the two socioeconomic groups. Serum zinc was significantly higher (p = 0.000) in the USEC group as compared to the LSEC group. Similarly, the NBT percentage was significantly higher (p=0.000) in the USEC group as compared to the LSEC group. The serum zinc was significantly higher in the male subjects (p = 0.000) in the USEC group as compared to males in the LSEC group. Similarly, in the female subjects, serum zinc was significantly higher (p=0.000) in the USEC group as compared to the LSEC group. Same is the case with the NBT percentage phagocytosis, which was significantly higher in USEC group as compared to LSEC group (p=0.000) in both males and females (table 2). Table 3 gives gender difference of parameters within USEC group. Serum zinc difference was not significant (p=0.089). NBT percentage phagocytosis in males was significantly less (p=0.000) than in females. Both serum zinc and NBT percentage phagocytosis did not show any significant gender difference the LSEC group (table 4). Serum zinc showed a significant positive correlation with NBT percentage phagocytosis in total subjects of USEC group (r=0.309, p=0.029) and in male subjects of both USEC (r=0.484, p=0.014) and LSEC (r=0.433, p=0.030) groups (Figures 1, 2, and 3). However there was no significant correlation between serum zinc and NBT percentage phagocytosis found in total subjects of LSEC group and in female subjects of both USEC and LSEC group.

## Discussion

Tent study evaluated the serum zinc levels and NBT percentage phagocytosis in the two SEC

**Table-1:** Comparison of serum zinc and NBT percentage phagocytosis in total subjects in the two socioeconomic groups.5

| Parameter | USEC Group (n=50) | LSEC Group (n=50) | P-value |
|---|---|---|---|
| Serum Zinc (ug/dl) | 111.055± 13.66 | 77.28±14.90 | 0.00* |
| NBT percentage phagocytosis (%) | 22.38±7.98 | 8.16±3.29 | 0.000* |

*Values are expressed as Mean±SD * p <0.05 — significant*

**Table-2:** Comparison of serum zinc and NBT percentage phagocytosis (%) in male and female subjects in the two socioeconomic groups.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Parameter | USEC Group (n=25) | USEC Group (n=25) | P-value | LSEC Group (n=50) | LSEC Group (n=50) | P-value |
| Serum Zinc (ug/dl) | 107.76±14.29 | 176.72±8.56 | 0.00* | 114.33±12.42 | 77.83±19.48 | 0.00* |
| NBT Percentage phagocytosis (%) | 18.56±6.77 | 111.055± 13.66 | 0.000* | 26.20±7.33 | 8.208±3.70 | 0.000* |

*Values are expressed as Mean±SD * p <0.05 — significant*

Table-3: Gender difference of parameters within upper/upper middle socioeconomic group

| Parameter | Male (n=25) | Female (n=25) | P-value |
|---|---|---|---|
| Serum Zinc (ug/dl) | 107.76±14.29 | 114.33±12.42 | 0.089 |
| NBT percentage phagocytosis (%) | 26.20±7.33 | 26.20±3.70 | 0.000* |

*Values are expressed as Mean±SD * p <0.05 — Significant*

Table 4: Gender difference of parameters within Lower socioeconomic group.

| Parameter | Male (n=25) | Female (n=25) | P-value |
|---|---|---|---|
| Serum Zinc (ug/dl) | 76.72±8.76 | 77.83±19.48 | 0.0796 |
| NBT percentage phagocytosis (%) | 8.12±2.91 | 8.20±3.70 | 0.933 |

*Values are expressed as Mean±SD * p >0.05 — Non significant*

**Table-5:** Correlation of serum zinc with NBT percentage phagocytosis in the two socioeconomic groups.

| Correlation of Serum Zinc with | USEC (n=50) | | LSEC (n=50) | |
|---|---|---|---|---|
| | R value | P-value | R value | P-value |
| NBT Percentage phagocytosis (%) | 0.309 | 0.029* | 0.179 | 0.213 |

** p <0.05 — Significant Correlation coefficient (r) and p. value are given.*

**Table-6:** Correlation of serum zinc with ESR, serum lgG, NBT and serum total proteins in male and female subjects in the two socioeconomic groups.

| Correlation of serum zinc with | USEC Group (Male n=25) | | USEC Group (Female n=25) | | LSEC Group (Male n=25) | | LSEC Group (Female n=25) | |
|---|---|---|---|---|---|---|---|---|
| | r value | p value | r value | p value | r value | p value | r value | p value |
| NBT Percentage phagocytosis (%) | 0.484 | 0.014* | -0.848 | 0.821 | 0.433 | 0.0.030* | 0.099 | 0.0.639 |

** p <0.05 — Significant*

groups. In the present study, serum zinc levels were found to be significantly low in the LSEC as compared to the USEC group in the total number of subjects as well as in the male and female subgroups. The deficiency of zinc is associated with diets based on plant origin, which are rich in zinc absorption inhibitors like phytates. All cereals and vegetables contain phytates which can bind zinc and reduce its biological availability [7] (Miller et al 2007). Phytates are co-precipitated with zinc and absorption of zinc is decreasedb[22] (Davies and Nightingale 1975). As the low socioeconomic class of our country can not afford the animal diet and mostly consume the vegetable diet, so they are at the risk of low serum zinc levels due to its decreased absorption as a result of presence of phytates in the diet.

Zinc is necessary for the normal function of the immune system. Even mild zinc deficiency, which is widely spread in contrast to severe zinc deficiency, depresses immunity of humans. The functions of the innate immunity are disturbed by altered zinc levels The functions of the innate immunity are disturbed by altered zinc levels which includes the recruitment of neutrophil granulocytes for phagocytosis in vitro. In vivo, natural killer (NK) cell activity, phagocytosis of macrophages and neutrophils and generation of the oxidative burst are impaired by decreased zinc levels[23].

(Klaus-Helge and Rink 2003) In the current study it was found that NBT percentage phagocytosis was significantly higher ($p < 0.01$) in the USEC group as compared to the LSEC group.

In males, NBT percentage phagocytosis was significantly higher ($p<0.01$) in the USEC group as compared to the LSEC group. Similarly in females, NBT percentage phagocytosis was significantly higher ($p<0.01$) in the USEC group as compared to the LSEC group.

There was a significant positive correlation between serum zinc and NBT percentage phagocytosis in the present study. Also In this study significant gender difference of some parameters was found within the same socioeconomic groups. NBT percentage phagocytosis was significantly more in females as compared to males in the USEC but no such finding was observed in the LSEC.

Serum zinc level is significantly low in the lower socioeconomic class. A significant positive correlation is found between serum zinc and neutrophil phagocytic function.

*Department of Bio-Chemistry*
*University of Health Sciences, lahore*
**theesculapio@hotmail.com**

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