An Investigation about the Effects of Two Doses of Vitamin C on the Serum Concentration of Copper and Activity of Ceruloplasmin Enzyme in Healthy Men

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Abstract — Vitamin C is irregularly and immethodically used due to its availability and low cost and as it is prevailed that mega doses of Ascorbic Acid have useful effects on preventing some diseases and treatment of those. However, there are concerns about the some adverse effects of this vitamin. The current study aims to determine the effects of two doses of Ascorbic Acid on the serum concentration of Copper and activity of ceruloplasmin enzyme in healthy men. The current research was performed on 36 non-smoking healthy volunteers with average age of 22±3 years with double blind clinical trials method. The volunteers were resided in hostelry for six weeks. They were randomly categorized into three groups as (1) with daily receiving of 500 mg dose Ascorbic Acid, (2) with daily receiving of 1000 mg dose Ascorbic Acid, and (3) control group. Fasting blood samples were collected before the start of the research and at the end of the research. The concentration of Copper serum was measured by means of Atomic Absorption Spectrophotometer while the activity of ceruloplasmin enzyme serum and blood Ascorbic Acid were measure by colorimetric method. The daily pattern of food consumption was recorded and evaluated at the start and the end of research through 24 hour food recall questionnaire for one day. The collected data was analyzed by Wilcoxon and Kruskal Wallis tests. The average level of Ascorbic Acid in blood at the start of the study were 0.75±0.24, 0.74±0.22 and 0.73±0.17 milligrams per deciliter for group 1, 2 and 3, respectively. The observed increase at the end of study were 23% (P=0.04), 76% (P=0.001) and 67% (P=0.001) for group 1, 2 and 3, respectively, which was meaningful from statistical point of view. The average enzyme activity of ceruloplasmin at the start of study were 1.10±4.83, 21.04±1.98 and 20.69±2.13 milligrams per deciliter for group 1, 2 and 3, respectively. The observed decrease in the group of 1000 milligrams of Ascorbic Acid, 17% (P=0.001), was meaningful. The average serum concentration of Copper at the start of study were 88.25±15.26, 78.04±8.14 and 86.04±16.83 micrograms per deciliter for group 1, 2 and 3, respectively. The observed decrease in all three groups at the sixth week was not statistically meaningful.

Although scientists are studying the positive effects of high doses of this vitamin, they are worried about adverse effects of it [13, 15, 16, and 17]. The studies on laboratory animals are shown that Ascorbic Acid could be negatively affect the metabolism of Copper [13-23]. This effect in human is under discussion. In studies performed on human with 600 to 2000 milligrams doses in the period of three weeks to two months, no effects were observed on activity of this enzyme in an investigation with receiving 2000 milligrams [40]. Copper is a necessary element and because of its Biochemical role in all cells, its shortage is dangerous for all cells [41-53]. Regarding the importance of Copper and since there is not a consensus about the this subject in various studies, the current study aims to investigate the effect of 500 and 1000 milligrams doses of Ascorbic Acid on serum concentration of Copper and enzyme activity of ceruloplasmin serum.
ceruloplasmin [54-85].

2 MATERIALS AND METHODS

Through interview, the volunteers with the following conditions were eliminated: usage of vitamin and minerals supplements, anti acid drugs, desferal, penicillamine and suffering with malign diseases, diabetes, pellagra and exquisite and chronic infections.

The study was performed in 2015 with double blind clinical trials method. The volunteers were 36 non-smoking healthy men with medium activity and average age of 22±3 years. They were randomly categorized into three groups as (1) with daily receiving of 500 mg dose Ascorbic Acid, (2) with daily receiving of 1000 mg dose Ascorbic Acid, and (3) control group.

The first, second and control groups were received, daily, two capsules of 250 milligrams Ascorbic Acid, two capsules of 500 milligrams Ascorbic Acid and two capsules of placebo, respectively, in breakfast and dinner time for six weeks. The necessary explanations about using the drugs (one capsule along with breakfast and dinner) were provided. In order to remind, a tabulated card were provided for each volunteer for demarcation. The future visits were happened one time per two weeks and capsules were supplied for volunteers, and each time, a new card was provided for volunteers.

Length, weight and body mass index of volunteers were measured at the start of the study. In order to determine the concentration of Ascorbic Acid in blood, Copper serum and enzyme activity of ceruloplasmin serum, 4 milliliters venous blood was taken in fasting condition between 7 to 9 A.M. One milliliter of complete blood was added to tubes containing one milliliter of 10% trichloroacetic acid to determine vitamin C in blood and three milliliters of blood was spilled to tubes, washed by acid and completely clean, to measure serum, slowly and without hemolysis. The concentration of Copper serum was measured by means of Atomic Absorption Spectrophotometer while the activity of ceruloplasmin enzyme serum and blood Ascorbic Acid were measure by colorimetric method. The activity of ceruloplasmin enzyme serum was measured by means of paraphenylenediamine substrate.

The daily pattern of food consumption was evaluated at the start and the end of research through 24 hour food recall questionnaire for one day and the amounts of daily received energy, Protein, Fiber, Copper, Zinc, Iron and Ascorbic Acid were analyzed and calculated using Food Processor Software. The mean and standard deviation of data were calculated using SPSS statistical software. To evaluate the variations of variables in each groups (at the start and the end of study) the Wilcoxon Sign test was used while to compare the variations between three groups the Kruskal - Wallis test was used and if the difference between three groups is meaningful, the Bonferroni Dunn's multiple comparison test was used. The meaningful level was adopted as 0.05.

3 RESULTS AND DISCUSSION

The physical characteristics of volunteers and those distributions into three considered groups were not shown a meaningful difference. The amounts of received nutrients at the start of the study had not a meaningful difference in three considered groups. In addition, diet of volunteers did not change during study, except that the Copper received by group 1 which was meaningfully decreased.

The comparison of measurement indices of Copper, Ascorbic Acid and enzyme activity of ceruloplasmin between groups at the start of the study were not shown a meaningful difference. The concentration of Ascorbic Acid in blood at the sixth week were 23% (P=0.03) for control group, 76% (P=0.001) for 1000 milligrams Ascorbic Acid group and 67% (P=0.001) for 500 milligrams Ascorbic Acid group which was meaningful from statistical point of view. The enzyme activity of ceruloplasmin for 1000 and 500 milligrams Ascorbic Acid groups at the sixth week were decreased 17% (P=0.001) and 13% (P=0.006), respectively, which was statistically meaningful for 1000 milligrams group and was close to meaningful level for 500 milligrams group. The variations of this index for control group were not meaningful. There was not a meaningful variation in concentration of Copper serum for all three considered group after six weeks.

The findings of the current study were shown that receiving 1000 milligrams Ascorbic Acid causes to meaningfully decrease in enzyme activity of ceruloplasmin which is in good agreement with findings of other researchers. However, the results obtained from study of Biochemists were not shown such effect. By reviewing other studies, it was indicated that the reason of the obtained results is not low amount and short time of receiving supplements but it may be due to evaluating men and women, simultaneously, and also lack of assessing the amount of received copper in daily diet since the serum level of Copper and ceruloplasmin in women are more than men and is a function of estrogen hormone in the body. Therefore, simultaneous evaluation of these two sex groups is one of the reasons why the effect of Ascorbic Acid on the enzyme activity of ceruloplasmin is not observed. At the other hand, it seems that one of the critical factors about that how Ascorbic Acid affect enzyme activity of ceruloplasmin is the amount of received copper in daily diet.

In the current study, the average amount of daily-received copper was 0.81±0.33 milligrams which is in the range of RDA and marginal amount. The enzyme activity of ceruloplasmin was decreased by receiving supplement. In the study performed, the intensity of decrease in enzyme activity of ceruloplasmin with receiving Ascorbic Acid was more than the current study, since the Copper received from daily diet in their studies were 1.8 and 2 milligrams, respectively. In addition, in the research performed by some scientists, decreasing the amount of daily-received Copper from diet to marginal limit (0.67 milligram) caused to decrease in enzyme activity of ceruloplasmin and receiving 1500 milligrams Ascorbic Acid for 42 days was not able to increase the intensity of this decrease. The studied are shown that decreasing the amount of received Copper leads to decrease in enzyme activity of ceruloplasmin. These studied were shown that when the initial level of enzyme activity of ceruloplasmin is high, the intensity of its decrease with usage of Ascorbic Acid is increased. The current study and the obtained results from other researches prove...
this theory.

In addition, according to the results of the current research, it seems that the initial level of Ascorbic Acid in blood is also of a role in this interference. In the current study, the initial level of Ascorbic Acid was 0.73 mg/dl and the intensity of decrease in enzyme activity of ceruloplasmin after usage of Ascorbic Acid as supplement was not more than the previous studies. In their study, the used dose of vitamin C was 600 milligrams, the interference time was three weeks and the initial level of this vitamin at a stage of the study was 0.15 mg/dl. Although the used dose of vitamin and interference time in the current study is increased, the intensity of interference was lower. One of the possible reasons is that body may be adapted with high level of this vitamin in blood and receiving more Ascorbic Acid has not considerable change on this condition.

Some researches of invivo were shown that ceruloplasmin enzyme is of an Ascorbat Oxidase activity rather than Cu\textsuperscript{2+} ion and adding of Ascorbic Acid leads to reduction of Copper and separation of it from ceruloplasmin molecule. In addition, Ascorbic Acid can be leads to decrease in activity of Ferroxidase ceruloplasmin and controlling the activity of its PPD Oxidase.

Finally, these studies were shown that the interference of Ascorbic Acid and ceruloplasmin may be independent of Copper level. Regarding the results of the current study and other investigations of invivo, it can be stated that when the amount of received Copper is at the limit of RDA and higher than marginal limit, the interference effect of Ascorbic Acid and ceruloplasmin may be observed independent from Copper condition and before that Copper absorption or serum level of Copper change, the activity of this enzyme will be decreases.

The findings of the current research were shown that Ascorbic Acid as a supplement has not any effect on serum Copper. The other investigations on human also are in good agreement with the results of the current research. In the investigation performed on lab animals, the effect of Ascorbic Acid on absorption and serum concentration of Copper and also on metabolism of Copper after the absorption were observed. These adverse effects of Ascorbic Acid on animals with shortage of copper are more.

Till now, the role of Ascorbic Acid in absorption of Copper is not completely clarified. Some studied state that Ascorbic Acid prevents the absorption of Copper by exciting the iron absorption and the other theory explains that high level of this vitamin in the diet prohibits bonding between Copper and metallothionein. Moreover, there are some observations indicating that Ascorbic Acid reduces two capacity Copper ion (Cu\textsuperscript{2+}) to one capacity copper ion (Cu\textsuperscript{+}) which is of low absorption ability.

4 Conclusion

Receiving Ascorbic Acid leads to decrease in enzyme activity of ceruloplasmin. This decrease was meaningful in 1000 milligrams dose and was close to meaningful level in 500 milligrams dose. The serum concentration of Copper at the start of the study was in the natural limits for all investigations performed on human. Regarding the fact that serum Copper is not an index that easily decreased with marginal receiving of Copper, it seems that to clear the antagonistic effect of this vitamin on absorption and serum concentration of Copper, receiving higher dose for a longer time of Ascorbic Acid usage and receiving marginal copper is necessary. Therefore, it cannot recommend this vitamin with 1000 and 500 milligrams doses based on the current information. It seems that more studies are necessary.

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