



PLASMA COPPER AND ASCORBIC ACID STATUS IN LEPROSY: EFFECT OF ASCORBIC ACID SUPPLEMENTATION

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ABSTRACT

Ascorbic acid plays an important role in the control of inflammation induced by reactive oxygen species as well as improves the immune response against the pathogens. Thus, its decreased level in the body may initiate an increased oxidative stress and complication in infectious diseases including leprosy. Along with other micronutrients. Copper is also involved in free radical metabolism and metabolically related to ascorbic acid. These findings led us to study the interrelationship between copper and ascorbic acid status in tuberculoid and lepromatous leprosy. Besides the change in ascorbic acid/ dehydroascorbic acid ratio, plasma copper with its fractions and ceruloplasmin were also found to be increased in leprosy with a higher level in lepromatous variety in comparison to tuberculoid variety. In a parallel study, these groups were treated with one gm of ascorbic acid orally three times a day for fifteen days and the parameters as described were noted before and after the treatment. Increased plasma ascorbic acid with no change in dehydroascorbic acid level, decrease in ceruloplasmin, total copper and its complex with albumin were observed mainly in lepromatous leprosy after the treatment along with an increased output of urinary ascorbic acid. The daily output of urinary oxalate in these patients did not alter much before and after the treatment. The importance of dehydroascorbic acid in leprosy could be related to its involvement in free radical metabolism.

KEYWORDS: Leprosy, dehydroascorbic acid, copper, urinary oxalate. Ceruloplasmin.

INTRODUCTION

Of the various mechanisms that influence the pathogenesis of leprosy, derangement in the balance between reactive oxygen species and natural antioxidants leading to free radical generation is of utmost importance oxidative stress with a decreased antioxidant vitamin have been observed in lepromatous leprosy.^[1] Copper is initially connected with free radical metabolism, but its metabolic role in leprosy has yet to be clarified. The role of copper in human metabolism include mitochondrial energy production, cross - linking of elastin and collagen fibres, melanin formation, erythropoiesis and development of nerves and bone tissue. Some of these functions are defective in leprosy. Copper is a constituent of number of important enzymes e.g., cytochrome oxidase, tyrosinase, monoamine oxidase, dopamine β - hydroxylase and ceruloplasmin. The existence of abnormal pigment metabolism in leprosy led us to think about the involvement of copper and ascorbic acid in the disease processes in leprosy and some of the epidemiological and immunological aspects of leprosy have been explored successfully. Some biochemical abnormalities have been reported already. It has been

associated with a changed serum protein pattern^[2] hypoferrimia, increased β - glucuronidase activity, over mobilisation of free fatty acids, decreased glucose tolerance and decreased concentration of all the fractions of serum lipids. However, these abnormalities in different form of leprosy could not be correlated with the disease processes precisely. The changes in biochemical parameters could be due to superimposed malnutrition in these cases. In India, severe malnutrition often coexists with leprosy can inhibit human immune responses to mycobacterium leprae infection by virtue of its well documented suppressive effects on cell mediated immunity and complement function though interaction between these two is barely understood.

While decreased level of ascorbic acid was found in all types of leprosy some could find it only in multibacillary leprosy.^[1] Ascorbic acid inhibits the growth of mycobacterium leprae probably by inhibiting the β - glucuronidase enzyme non competitively in mycobacterium leprae which is required for the metabolism of hyaluronic acid.^[3] Increased serum β - glucuronidase activity in leprosy naturally lead us to

examine the probable interrelationship between the ascorbic acid status and the biochemical changes in leprosy. Thus, our objective goal was to study the different components of plasma copper along with ascorbic acid and its reduced form in different forms of leprosy patients and compared it with normal control. The role of ascorbic acid supplementation in the diet of these patients on their copper and ascorbic acid status was also to be explored.

MATERIALS AND METHODS

Thirty-five normal controls, seventy-nine tuberculoid leprosy patients and one hundred sixteen lepromatous leprosy patients were selected for study. Leprosy patients were visiting the outpatient department of North Bengal Medical College and Hospital, Hathighisa Leprosy Clinic and Jeshu Ashram, a leprosy clinic run by the Missionaries, all in the jurisdiction of Darjeeling District, West Bengal. A thorough base line clinical examination was carried out. Patients who had major ailments along with leprosy were excluded from the study. All the patients were fresh where treatment was not initiated. Based on clinical and pathological findings the typing was made and the patients were divided into tuberculoid and lepromatous groups. All the patients were male with age varying from 20 years to 45 years having similar nutritional and socio-economic status including the normal control group. The project was approved and financed by the Indian Council of Medical Research (ICMR) and passed by the ethical committee of the institution. The goal of the project was explained to the subjects and their consent was obtained.

After a 10 -12 hours overnight fasting, blood samples were collected from all the subjects in the morning and without any delay the plasma samples were analysed for ascorbic acid and dehydroascorbic acid by DNPH method,^[4] copper, both albumin bound and globulin bound and ceruloplasmin was estimated by enzymatic method.^[5,6] 22% sodium sulphite solution was employed

to separate globulin from albumin and copper was determined separately in both the fractions.

In another set of study, 15 normal controls, 15 tuberculoid leprosy patients and 28 lepromatous leprosy patients, who were indoor patients were asked to take one gram (gm) of ascorbic acid three times a day for fifteen days. The vitamin was supplied by us. No other drugs or vitamins were allowed during the study period to the patients. All the parameters determined earlier were again assayed before and after the administration of ascorbic acid. A 24 hours urine collection was analysed for ascorbic acid by DNPH method^[4] and oxalate by colorimetric method.^[7] Since the aqueous ascorbic acid is very unstable at room temperature in the presence of oxygen and the presence of copper ions accelerates the oxidation and autocatalytic.^[8]

Studies with healthy children and adults using supplementation of ascorbic acid of 20 mg/Kg/day and 1 - 3 gm/day respectively have been shown to enhance the capacity of neutrophils and macrophages to eradicate microbes,^[9] Ascorbic acid supplementation of 3 gm/day/patient was thus a safe choice.

RESULTS

Plasma ascorbic acid decreased both in tuberculoid and lepromatous leprosy with concomitant increase in plasma dehydroascorbic acid level ($p < 0.001$). The changes in lepromatous leprosy were more evident in comparison to tuberculoid leprosy ($p < 0.001$).

There was a gradual increase in plasma copper being the maximum in lepromatous leprosy ($p < 0.001$). The albumin bound copper ($p < 0.002$) and the globulin bound copper ($p < 0.001$) reflected the same picture. Plasma ceruloplasmin was found to be increased both in the tuberculoid and lepromatous leprosy ($p < 0.001$). The lepromatous patients showed higher values in comparison to tuberculoid patients ($p < 0.001$) in Table 1.

Table 1: Plasma Changes in Leprosy Patients in Relation to Normal Control.

Condition	No. of Subjects	Ascorbic acid (in mg/dl)	Dehydroascorbic acid (in mg/dl)	Albumin bound Copper (in µg/dl)	Globulin bound Copper (in µg/dl)	Total Copper (in µg/dl)	Ceruloplasmin (in mg/dl)
Normal Control (C)	35	1.08 ± 0.22	0.07 ± 0.08	48 ± 10	108 ± 10	156 ± 12	26 ± 03
Tuberculoid Leprosy (TL)	79	0.72 ± 0.19	0.31 ± 0.11	56 ± 12	125 ± 15	181 ± 23	40 ± 11
Lepromatous Leprosy (LL)	116	0.62 ± 0.18	0.50 ± 0.25	65 ± 22	146 ± 23	211 ± 38	55 ± 12

Values are Mean ± SD

Ascorbic acid treatment increased the plasma ascorbic acid level and its urinary output in all the subjects ($p < 0.001$) without any change in the dehydroascorbic acid level.

Urinary excretion of ascorbic acid in tuberculoid and lepromatous patients, when un-supplemented, was found to be decreased in comparison to normal controls ($p < 0.002$) with no appreciable difference between the two. Ascorbic acid treatment did not alter the daily output of

oxalic acid in these patients depicted in Table 2. There was a slight reduction in total plasma copper in normal controls when treated with ascorbic acid ($p < 0.05$); this

was much more pronounced in lepromatous leprosy ($p < 0.002$), having no appreciable effect in tuberculoid variety.

Table 2: Plasma and Urinary Changes in Normal and Leprosy Patients After Supplementation with Ascorbic Acid in Medicine Wards at a Tertiary Care Hospital in West Bengal, India.

Condition	No. of Subjects	Ascorbic Acid (in mg/dl)	Dehydroascorbic Acid (in mg/dl)	Albumin bound Copper (μ g/dl)	Globulin bound Copper (μ g/dl)	Total Copper (μ g/dl)	Ceruloplasmin (mg/dl)	Urinary Ascorbic Acid (mg/day)	Urinary Oxalate (mg/day)
Normal Control	15	1.00 $\pm$ 0.19	0.08 $\pm$ 0.07	48 $\pm$ 09	107 $\pm$ 08	155 $\pm$ 12	27 $\pm$ 03	33 $\pm$ 16	25 $\pm$ 08
Normal Control with Ascorbic Acid	15	1.97 $\pm$ 0.32	0.10 $\pm$ 0.06	41 $\pm$ 07	104 $\pm$ 11	145 $\pm$ 11	22 $\pm$ 03	124 $\pm$ 31	26 $\pm$ 09
Tuberculoid Leprosy	15	0.76 $\pm$ 0.18	0.33 $\pm$ 0.10	54 $\pm$ 10	125 $\pm$ 14	179 $\pm$ 18	39 $\pm$ 10	17 $\pm$ 07	50 $\pm$ 26
Tuberculoid Leprosy with Ascorbic Acid	15	1.96 $\pm$ 0.21	0.33 $\pm$ 0.08	46 $\pm$ 09	126 $\pm$ 13	172 $\pm$ 19	34 $\pm$ 09	124 $\pm$ 22	51 $\pm$ 26
Lepromatous Leprosy	28	0.67 $\pm$ 0.25	0.53 $\pm$ 0.32	70 $\pm$ 23	143 $\pm$ 18	213 $\pm$ 36	57 $\pm$ 12	14 $\pm$ 08	73 $\pm$ 36
Lepromatous Leprosy with Ascorbic Acid	28	1.82 $\pm$ 0.36	0.44 $\pm$ 0.16	49 $\pm$ 12	139 $\pm$ 13	188 $\pm$ 20	39 $\pm$ 13	130 $\pm$ 23	79 $\pm$ 38

Values are Mean $\pm$ SD

While no change was observed in the globulin bound copper fraction in all three groups treated with ascorbic acid, a decrease in albumin bound copper fraction in normal and tuberculoid patients ($p < 0.05$) with a more pronounced effect in the lepromatous group ($p < 0.001$) was found.

Ascorbic acid treatment reduced the plasma ceruloplasmin content both in the normal control and lepromatous groups ($p < 0.001$) with no appreciable change in the tuberculoid group.

DISCUSSION

Oxidative stress in paucibacillary and multibacillary leprosy and lipid peroxidation in lepromatous leprosy have been reported.^[10,11] Immune enhancing risk of ascorbic acid and zinc has also been stressed.^[12] Selected vitamins and trace elements have been found to support immune function by strengthening epithelial barriers and cellular and humoral immune responses.^[13] A decreased level of antioxidants with increased oxidative stress often complicate the infectious diseases including leprosy. Ascorbic acid seems to control the inflammatory processes induced by reactive oxygen species.^[9] Still the specific role of copper in combating reactive oxygen species vis-à-vis its relation to ascorbic acid is yet to ascertain. Small quantities of copper circulate in the plasma loosely bound to albumin. The firmly bound copper in globulins consisting 80 – 90% of total plasma copper consists of ceruloplasmin. Ceruloplasmin, probably hepatic in origin, requires a normal functioning liver for its formation from albumin copper complex. Hyper-cupremia, noted in diverse inflammatory and

infectious diseases, may result from increased intestinal absorption, an impairment in biliary excretion or defective copper metabolism favouring its hepatic deposition.^[14] It has been claimed that redistribution of copper from liver to blood is probably initiated by a leucocytic endogenous mediator that stimulate liver ceruloplasmin synthesis.^[15] In leprosy, there was an overall increase in the copper content of both the protein fractions indicating thereby the probable normal functioning of the liver which synthesises more ceruloplasmin due to the increased availability of albumin copper complex. The difference observed between tuberculoid and lepromatous variety could be explained in the same light. The increased ceruloplasmin and globulin bound copper in lepromatous leprosy in relation to tuberculoid variety could be due to increased availability of albumin copper complex in the former; it may be also due to the increased absorption of copper from the gut. Thus, increased synthesis of ceruloplasmin incorporating more available copper into bound form is probably a typical feature in leprosy differentiating it from Wilson's disease.

Ascorbic acid decreased binding of zinc and copper by the metallothioneine in the duodenum interfering the absorption of these metals from the gut.^[16] Decreased ascorbic acid in leprosy could account for the increased absorption of copper in these patients. Increase in dehydroascorbic acid in leprosy could be due to the presence of hyper-cupremia in leprosy since copper enhances the oxidation of ascorbic acid to dehydroascorbic acid.^[11] Ceruloplasmin, sometimes referred as copper oxidase, has ascorbic acid oxidase

activity.^[17] Hence high level of ceruloplasmin could be responsible for increased level of dehydroascorbic acid in leprosy. A gradual increase in total copper content including ceruloplasmin in stages of leprosy with concomitant increase in dehydroascorbic acid content and a decrease in ascorbic acid content suggested the above-mentioned explanation. Since the oxidation of ascorbic acid produces one mol of H₂O₂ per mol of dehydroascorbic acid the involvement of free radical metabolism cannot be overruled.^[18] The toxic manifestations in acute infectious disease have been attributed to accumulated dehydroascorbic acid level.^[19] Obviously, this is applicable to leprosy also.

With an intake of 45 mg of ascorbic acid per day, a serum ascorbate level of approximately 0.6 mg/dl would be expected.^[9] Since the ascorbate level in all the subjects of the present study was above this level, the possible effect of low dietary ascorbate on copper metabolism could be ruled out.

High intake of ascorbic acid led to increased excretion of this vitamin in the urine along with the production of oxaluria. In normal subjects about 50% of the daily excretion of oxalic acid is derived from dietary ascorbate. Intake of 4 gm or more daily produced increased oxalic acid excretion.^[20] It was suggested that hyper oxalaemia could lead to the presence of crystalline calcium oxalate in kidney and thyroid of leprosy patients though no pathogenic relationship was established.^[21]

In our study, the increased amount of urinary oxalate in leprosy did not increased further even after the supplementation of excess ascorbic acid proving that the higher oxalate level was not related with the metabolism of ascorbic acid. The increased levels of dehydroascorbic acid were also unaltered with higher intake of ascorbic acid by the leprosy patients. Thus, our data showed a better positive correlation between the disease processes and the magnitude of plasma dehydroascorbic acid and urinary oxalate. Reduction in total copper at the expense of albumin bound copper in the subjects after supplementation of ascorbic acid suggested a decline in the intestinal absorption of copper as well as in copper oxidase activity as reflected by reduced plasma ceruloplasmin level. Thus, the role of ascorbic acid in ameliorating the hyper-cupraemic state found in leprosy is beyond doubt.

It is quite evident that the decreased plasma ascorbic acid level in leprosy was not due to dietary insufficiency; a part of ascorbic acid was converted to its de-hydro form. If the reducing equivalents available from any other source (mainly α – tocopherol) can be exchanged with dehydroascorbic acid, ascorbic acid can be regenerated. Dehydroascorbic acid level does not vary fluctuating the ascorbic status and intimately related with some redox state in pathological processes. Whether the ascorbic acid therapy with or without the conventional therapy will be useful in leprosy may be a matter of conjecture

but its role in ameliorating the hyper-cupremic state in leprosy is beyond doubt.

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Conflict of Interest

The authors declare no conflict of interest in the present study.

Ethical Approval

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Institutional research committee.

Informed Consent

Informed consent was obtained from all the participants individually who were included in the study.

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