Nutritional Status, Lipid Profile and Antioxidant Enzymes in Tribal Population of Zawar (Rajasthan), India

Chandra A,[1] Purabia P,[2] Purabia SL,[3] Singh PP [1]

[1]Professor, Department of Biochemistry, Era's Lucknow Medical College & Hospital, Lucknow, [2] Former Research Scholar, Department of Biochemistry, RNT Medical College, Udaipur, Rajasthan,[3] Former Senior Physician and Medical Superintendant, Zawar Hospital, Zawar, Rajasthan,[1] Dept of Biochemistry, RNT Medical College, Udaipur, Rajasthan and currently visiting Professor, Era's Lucknow Medical College & Hospital, Lucknow

Corresponding Author: Anu Chandra, Professor, Department of Biochemistry, Era's Lucknow Medical College & Hospital, Lucknow; E mail: anuchandra24@gmail.com

ABSTRACT

Tribal living in Arawali Hills are leading an impoverished and primitive life. Zawar is a plain valley (51.78 sq.mt.) encircled by Arawali hills and ridges. Few decades back India's richest Zinc mines have been discovered in Zawar and offers better means of living. Some tribals from Arawali hills have migrated to valley to take jobs in mines. We have measured the intake of calories and nutrients of hill tribals (low socio-economic group) and those living and working in Zawar mines. Simultaneously we also measured their oxidative stress, antioxidant enzymes and nutrient antioxidants. The daily intake of calories, dense nutrients, ascorbic acids and β- carotene was measured by dietary recall method and individual nutrients were also calculated by Indian Council Of Medical Research (ICMR) booklet. Superoxide dismutase (both in plasma and RBC), catalase(RBC),thiobarbituric acid reactive substance (Plasma and Urine),serum retinol, β-carotene, α-tocopherol, ascorbic acid were measured by standard methods. Lipid profile was measured by fully automatic analyser using enzymatic kits. The important observations are: 1) The energy intake was less than half of that recommended by ICMR ii) the diet was highly deficient in carbohydrate, fat, protein, β-carotene and ascorbic acid iii) surprisingly protein intake was low in 1-9 years of age group & 19-50 years of age group but was normal in 10-18 years of age group. This appears to be due to intake of leafy vegetables either grown or uncultivated iv) the situation was almost same in low, lower middle and middle socioeconomic groups v) SOD levels in RBC or plasma and catalase levels were within accepted referral range. As expected the TBARS level was slightly raised indicating mildly raised oxidative stress.vi) the blood retinol and β-carotene levels were within normal range but α-tocopherol and ascorbic acid levels were low. In fat very efficient in more than 50% subjects.vii) the lipid profile was also within normal referral range viii) interestingly serum total protein was within normal limits despite poor diet. It ought to be due to relatively better intake of protein ix) hemoglobin levels were slightly lower in about 30% adults and elderly people. The tribals in Zawar are highly undernourished.The tribals with better means of living are also equally undernourished. The reasons appears of negligence and ignorance about nutritional needs. Serum α- tocopherols and ascorbic acids that are two most important support antioxidant were very low in almost all the subjects.

Keywords: nutrition, calorie, nutrient antioxidants, antioxidant enzymes, oxidative stress

INTRODUCTION

The ancient Indian scriptures and even old literature in the modern medicine say that both in importance and time, health precedes disease so we ought to consider first how health may be preserved and then how best one can cure the disease. WHO refers it "Health is state of complete physical, mental and social well being and not merely the absence of disease or infirmity". Obviously nutrition is an essential arm of normal health and that it helps to prevent or delay the disease. With over 1.20 billion population, it is irony that India with its resources, determination, might and recourse, presents a development paradox.[1] Tribals are too rigid to follow their outmoded customs with the result even today they are nearer to primitive man than the modern man .[2,3,4,5] In fact they abjure the dynamic behavior of social structure and modernization.

Geographically Indian population can be divided into urban and rural. The rural population can further be distinctly sub-divided into tribals and non- tribals. The tribal population is smaller in proportion and more confined to some areas of country especially hilly

regions. This population is distinctly different from non-tribal population in respect to their primitive economic, cultural and living style. They have deep rooted reluctance to accept the tenets of modernization. Most of them live in hills, usually with isolated hutments located quite apart from each other with bare means of living. Only a small percentage of tribals in some areas has migrated to valley for better living. Zawar mine area in Rajasthan represents one such area. Zawar is an industrial semi-urban township in valley with an area of about 58 km and amazingly with higher population of tribals in and around Zawar villages.It is surrounded by festoon of Arawali hills with tribals living scattered. Fortunately, Zawar is a fast developing mining township. It has one of richest zinc mines here and tribal population working in mines can perceptibly be divided into primitive but with better living conditions than hill tribals. Those who live in hills are still extremely poor and have still aversion to accept modern trends of living. The other group has migrated to valley area to take up jobs in mines etc. and learning to accept modernization. Thus Zawar mine area presents an unique opportunity for examining the tribals belonging to different socioeconomic segments.[6]

The present study examines the diet and intake of primary nutrients (proteins, carbohydrates and fats), vitamins A, E,C and provitamin β-carotene in tribals from different age groups. Simultaneously we also examined lipid profile and endogenous antioxidant enzymes. The importance of lipid profile on human health and diseases can never be underscored. In the recent years there is an increasing credence for overriding role of evolutionary conserved endogenous antioxidants over nutrient antioxidants (vit A, E, C and beta-carotene) for maintaining cellular redox homeostasis.[7] Incidentally, besides their other normal physiological duties, Vit A,E,C and beta-carotene also function as support antioxidants[8].

MATERIALS AND METHODS

In total, 1349 tribals classified as healthy in this study were examined by a physician. They had no clinical sign of any disease, had normal biochemical parameters like blood sugar, lipid profile and creatinine. Modified BG Prasad was used to classify individuals on different scale of socioeconomic status. It is an income based scale and therefore has to be constantly updated to take inflation and depreciation of rupee into account.[9] Tribals belonging to different age groups were included in this study (lower socio economic group 496, lower middle socioeconomic group 298 and middle socioeconomic group 555). 5 ml of blood in overnight fasting condition was collected from each subject by venipuncture with standard collection technique and was transferred to vials containing Ethylene Diamine Tetra Acetic Acid EDTA (10mg), another sample was collected in plain vial for serum. Superoxide dismutase (SOD) and catalase were assayed in erythrocytes (hemolysate) by standard procedures.[10,11] SOD was also assayed in plasma. Retinol, beta – carotene, alpha tocopherol and ascorbic acid were estimated in serum by the standard methods.[12,13] Oxidative stress (OS) was measured as TBARS both in plasma and urine sample by the method of Buege and Aust, (1978).[14] Results of urine sample are expressed on the basis of creatinine. Lipid profile was estimated in serum by fully auto-analyzer using standard enzymatic kits. Statistical significance was determined by students 't' test for unpaired data. The values of significance were evaluated with p values. The differences were considered significant at $p<0.05$.

Table 1: Nutrient intake in low Socio Economic Group

Age	Calories	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Ascorbic acid (mg/day)	B-carotene (µg/day}
1-3 (n=49)	531±106 (100%)	15±3 (100%)	4±2 (100%)	106±22 (100%)	4±1 (100%)	256±116 (100%)
4-6 (n=48)	687±143 (100%)	21±4 (98.86%)	5±2 (100%)	140±32 (100%)	6±3 (100%)	382±195 (100%)
7-9 (n=63)	938±192 (100%)	27±6 (98.86%)	6±2 (100%)	193±42 (100%)	7±3 (100%)	517±266 (100%)
10-18 (n=75)	1255±270	37±8	9±4	255±66	8±3	590±233
19-50 (n=261)	1300±263 (100%)	3 7±8 (97.30%)	9±5 (95.40%)	266±61 (100%)	10±4 (100%)	582±246 (100%)

In bracket- percentage of subjects deficient as per ICMR recommendations
n= number of subjects

RESULTS

The observation of the present study are recorded in Table 1-5, Tables 1-3 present data on the intake of calories, dense nutrients, ascorbic acid and β- carotene in tribals belonging to different socioeconomic groups. Table 4 describes data on antioxidant enzymes, TBARS levels and nutrient oxidants in tribals and non tribals. Table 5 present data on plasma Lipid profile, total protein and hemoglobin in tribals and non tribals. Data reveals several interesting observations.

Table 2: Nutrient intake in lower-middle Socio Economic Group

Age	Calories	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Ascorbic acid (mg/day)	B-carotene (µg/day}
1-3 (n=12)	610±198 (100%)	18±5 (75%)	4±2 (100%)	124±43 (100%)	6±3 (100%)	347±142 (100%)
4-6 (n=22)	753±178 (100%)	21±5 (83.3%)	6±4 (100%)	157±43 (100%)	8±3 (100%)	348±170 (100%)
7-9 (n=46)	952±203 (100%)	27±6 (97.8%)	8±4 (100%)	194±44 (100%)	10±4 (100%)	616±244 (100%)
10-18 (n=69)	1269±33	34±8	10±4	261±78	13±7	780±286
19-50 (n=149)	1319±310 (97.90%)	37±8 (97.31%)	11±5 (92.61%)	268±72 (99.30%)	14±8 (97.30%)	787±300 (100%)

In bracket- percentage of subjects deficient as per ICMR recommendations; n= number of subjects

Table 3: Nutrient intake in Middle Socio Economic Group

Age	Calories	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Ascorbic acid (mg/day)	B-carotene (µg/day}
1-3 (n=12)	588±135	19±4	4±1	119±30	8±4	461±200
4-6 (n=32)	784±163 (100%)	23±5 (81.25%)	7±4 (100%)	156±35 (100%)	11±6 (100%)	569±227 (100%)
7-9 (n=79)	1101±206 (100%)	32±6 (89.8%)	10±5 (98.73%)	219±48 (100%)	17±9 (98.73%)	858±341 (100%)
10-18 (n=138)	1409±259 (100%)	42±8 (100%)	14±7 (100%)	280±58 (100%)	22±12 (100%)	1118±437 (100%)
19-50 (n=295)	1483±293 (100%)	44±9 (88.13%)	13±7 (84.40%)	296±67 (100%)	21±10 (90.16%)	1078±434 (100%)

In bracket- percentage of subjects deficient as per ICMR recommendations; n= number of subjects

In India ICMR prescribes the requirement of nutrients.[15] The intake of calories are nearly half and sometimes less than half. Fat intake was almost 25-30% of recommended intake. The intake of ascorbic acid was amazingly low in low socioeconomic group. It was in the range of 4±1 to 10±4 mg/day as against recommended intake of 40 mg/day. It was somewhat better in other groups but still very low. Surprisingly the intake of β- carotene was still poor. Protein intake was low in all the tribals but marginally better than fat and carbohydrates. These results obviously show gross under nutrition. However, it was that despite this poor nutrition all tribals had no diseases apparently normal and performing normal activities. At present the only conceivable reasons seems to be genetic adaptation.

Table 4 shows that a SOD level in RBC was slightly but significantly more in non tribals, but not in plasma. Catalase level in RBCs also showed similar trend. Oxidative stress measured in terms of TBARs was slightly raised. Presently we cannot assert whether it was due to lower activity of SOD and Catalase in tribals or the results are just fortuitous. Surprisingly the Serum levels of Retinol and β- carotene were almost normal despite their poor intake. However, the Serum levels of Ascorbic acid and α-Tocopherols were usually and almost depleted in about 30% subjects. Presently Ascorbic acid in cytosol and α- tocopherols in lipid membrane are considered as important and potent in the hierarchy of dietary antioxidants. In spite of very poor intake of fat, the lipid

Table 4: Enzyme antioxidants, Plasma nutrient antioxidants and MDA levels in tribal normal and non tribal normal subjects

Parameters	1-9 years (n=21)		19-50 years (n=12)	10-18 years	51-60 years (n=53)		(n=15)		**Mean square**
	Tribal	Non Tribal	Tribal	Non Tribal	Tribal	Non Tribal	Tribal	Non Tribal	
SOD (EU/ml RBC)	6.5±1.4	7.5±1.6	6.9±2.4	7.3±2.2	6.9±2.1	8.6±2.9	5.8±1.2	6.6±2.7	73.40**
SOD (EU/ml Plasma)	3.2±1.3	2.7±0.8	2.9±1.4	3.2±0.7	3.6±1.6	3.4±0.9	2.1±0.9	4.5±1.3	2.37
CATALASE (µmol H2O2 consumed/ min/mg protein)	144±31	153±55	146±51	173±31	158±31	170±40	157±27	152±40	6248.56*
MDA(nmol/100ml plasma)	282±86 (n=19)	299±105	335±66 (n=10)	323±67	354±117 (n=45)	330±120 (n=39)	38±78	332±43	-----
MDA(nmol/mg urine creatinine)	8.6±1.7	8.4±1.7	8.7±1.5	12.7±5.9	10.9±2.8	8.9±3.4	11.5±2.6	9.5±2.9	25.39
Retinol (µg/dl)	18.5±3.5	20.1±4.3	20.6±6.1	22.7±6.2	21.5±5.9	25.4±6.4	21.9±5.9	26.2±4.7	500.73**
B-Carotene (µg/dl)	73.8±14.2	79.1±13.6	100.3±13.9	106±17.3	109.2±29.7	117±20.9	107±24.9	106±14.9	2255.52*
Tocopherol (mg/dl)	0.58±0.18	0.67±0.20	0.68±0.11	0.67±0.20	0.75±0.20	0.85±0.23	0.58±0.13	0.82±0.20	0.53**
Ascorbic acid (mg/dl)	0.56±0.20	0.62±0.17	0.64±0.26	0.70±0.37	0.73±0.24	0.88±0.29	0.43±0.10	0.91±0.21	1.37**

n= number of subjects

Table 4: Plasma Lipid Profile, Total Proteins and Hemoglobin levels in tribal normal and non tribal normal subjects

Parameters	**Age Groups (Years)**							
	1-9 years (n=21)	(n=14)	10-18 years (n=12)	19-50 years (n=13)	(n=53)	51-60 years (n=44)	(n=15)	(n=12)
Serum Cholesterol (mg/dl)	130±5.84	145.07±3.63	150.58±7.11	155.60±5.13 (n=10)	178.90±42.1	177.4±36.46	185.27±14.9	210.92±34.15
HDL-Cholesterol (mg/dl)	35.24±4.05	38.43±4.01	35.42±5.12	36.90±3.21 (n=10)	50.86±18	54.26±19.95	40.60±7.40	64.25±20.60
LDL- Cholesterol (mg/dl)	76.30±7.32	88.24±5.37	100.53±7.11	101.30±8.20 (n=10)	107.62±37.8	91.33±36.96	126.81±20.7	120.32±20.22
VLDL- Cholesterol (mg/dl)	18.46±4.25	18.40±3.02	14.63±2.53	17.40±2.90 (n=10)	20.58±7.57	31.95±14.83	17.85±3.65	26.35±12.38
Triglyceride (mg/dl)	92.29±21.62	92.29±15.05	73.17±12.66	87±14.48 (n=10)	102.90±37.8	159.74±74.1	89.27±18.29	131.75±61.64
Total Protein (gm/dl)	6.66±0.51	6.94±0.48	6.66±0.37	7.13±0.54	6.95±0.63	7.24±0.74	6.85±0.80	7.37±0.37
Hemoglobin (gm%)	9.93±1.56	10.84±1.26	10.29±1.48	11.04±0.97	12.51±0.72	12.77±0.88	12.30±0.46	12.81±1.08

n= number of subjects

profile was in accepted range and comparable to non tribals. Serum proteins were normal. Hemoglobin levels were slightly lower. All the observation taken together again gives inking of genetic adaptability.

DISCUSSION

All the global health and nutritional agencies unequivocally recognize the crucial role of nutrition in growth, maintenance and tissue repair in human health, and that inadequate nutrition leads to: a) underweight b) stunting c) wasting d) recurrent infection and illness e) anemia and (f) lastly in extreme cases Kwashiorkor, Marasmus and increased mortality. Severe under nutrition has been reported among tribals from different parts of India (except North East).[16-22]The three commonly used methods for nutritional assessment are: a) diet b) anthropometery and c) biochemical indices.[23] Often clinical assessment is accompanied with either of these if a competent physician is associated with these projects. For the assessment of diet, dietary recall method was used to assess dense nutrients and vitamin A, C and beta carotene. In this study, clinical assessment was also conducted by a very competent and experienced senior physician. The data revealed extremely poor nutrition among tribals living in hills. The average calorie intake was 30-40 % less than that recommended by Indian Council of Medical Research.[15] Individual data revealed that, ≥ 90 individuals suffered from caloric deficit, thereby pointing out severe under nutrition. Almost similar observations have been made by others. Mandot *et al*, (2009), conducted a study on school going Garasia tribals (5-16 years) of Sirohi district of Rajasthan.[20] On the basis of their anthropometrical study, they noted high prevalence of stunting and wasting attributed to their low socio – economic status, poor dietary intake and lack of social awareness. Singh *et al* (2006) examined five clusters of population from Rajasthan in which one cluster consisted of tribals and scheduled caste.[19] Scheduled tribes (85%) and scheduled caste (88%) suffered more from under nutrition than other communities, the diet was generally but not always deficient in green leafy vegetables, fats, pulses and legumes and other vegetables. We also observed that the diet of tribals living in Arawali Hills was grossly deficient in calories and fat but surprisely either adequate or marginally poor in protein. The latter appeared to be due to relatively more intake of leafy vegetables available in hills and occasional non- vegetarian food from hunted animals and pet animals like goat and sheep etc. Admittedly their Food Basket was sparse, inadequate and usually imbalanced but it seemed to contain relatively more protein food than tribals from other places .[2,21]

Adak *et al* (2006) also, observed high prevalence of chronic energy deprivation in 90% tribals.[24] Zawar has distinctly two areas viz plane valley and Arawalli Hills surrounding them. The valley area has one of the richest zinc mines of India and has recently generated a lot of opportunity for employment in mines or zinc smelter. The tribals, who have migrated to valley are better placed, but they still appear to be negligent about their nutrition. Their nutrient intake was marginally better but still fell short of recommended intake.

In recent years there is a craze and fancy about quality and quantity of fat in the diet as it is the major determinant of atherosclerotic process.[25] Lopsided balance of fat intake adversely affects blood lipid profile primarily through HDL and LDL cholesterol levels. Tribals residing in hill areas cannot afford to buy fat for supplementing their fat intake. This is visible from their fat intake (Table). Earlier ICMR (1992) recommended 20 gm of visible fat intake and 20 gm of invisible fat intake in all age groups; it has now been reduced to almost 50%. However, ICMR still recommends higher intake of 25 gm in 1-3 year age group, it was just 16% of recommended intake among tribals. [26] It was 50-60% in 4 to 9 year age group and 90% in adolescents. It went down again to 60% in elderly people. Similar type of studies on Rajasthani tribals is not traceable in literature. The effect of this low fat on plasma lipid was examined. Both plasma HDL and LDL showed a more or less increasing trend with age and was within the normal limit. A systematic study on CVD in tribals is not available but a general impression is a lower incidence. This is, therefore, on expected lines. Lower fat intake may be one of the reasons, however further studies are needed in this direction. With the discovery of free radical involvement in 1956 by Herman in aging process and subsequent discovery of superoxide dismutase in human tissues in 1968 by Fridovich group,[27,28] a concept suddenly sprang up; that oxidative stress (OS) was "PANDORA BOX of EVILS" and that antioxidants were ordained as divine gate keepers for protection of " Cellular Homeostatic Box,[29,30] and that an imbalance between the two was seed of most of the diseases.[31,32] The antioxidants were initially presumed to be nutrient antioxidants and dietary antioxidants.[30,32] Presently both these concepts have undergone radical change. OS is now supposed be a consequence in most of human diseases, as there no evidence of torrential production of reactive species (ROS and RNS).[31] With regard to antioxidants nutrient antioxidants offer a very limited support eg. ascorbic acid and tocopherols or just a meager support (eg. vitamin A and beta carotene) (they have many other important metabolic activities) and other putative antioxidants (eg. lycopenes, polyphenoles and many others) may sometimes prove acrimonious rather than useful.[33] The intake of vitamin A and beta

carotene was low in tribals (Tables 1-3). So is also the case with ascorbic acid and alpha tocopherol (table 4). Their low levels must be traumatically affecting antioxidant and other physiological activates.

Presently endogenous antioxidants enzymes are supposed to be far more important to meet challenges of OS as they are inducible enzymes, thus flexible to meet the requirement.[30] The three quantitatively important groups of enzymes are SODs, and catalases. We have measured them in hemolysate of normal tribal and non – tribal subjects. Values are within normal range. No consistent differences are visible between tribals and non-tribals. However similar studies in other places are warranted. No comparable data are available.

Lastly we may mention that although we were unable to measure their alcohol consumption and smoking but oral questionnaire explicitly revealed that tribals in general were obsessively fond of alcohol (often binging) and smoking. They do so more often at the cost of their food and clothing and in all probability recourse to suppress their subconscious hunger. In due course of time they become addicted

In summary the tribals living in Arawali hills of Zawar (Rajasthan) are extremely poor and live in dire poverty and squalid conditions. Poverty and under nutrition are integrally related and interwoven with poor health problems[1]. Adding to these hazards is their pristine social structure archaic habits; incarcerated living style and closed circuit outdated and outmoded traditions and culture. They have been obstinately bracing to their centuries old customs. Studies on them often provide clues on human development on evolutionary scale because they are supposed to be in close proximity to the nascent human civilization as compared to modern one. The tribal who have migrated to planes of Zawar and have taken up employment in mines are economically better yet are negligent about their nutrition as observed by us in this study.

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