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PARAOXONASE-1 ACTIVITY, HYDROPEROXIDE LEVELS AND THEIR CORRELATION IN MALNOURISHED CHILDREN

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Abstract:

Background- PON1 (paraoxonase1) is an HDL-associated enzyme having antioxidant activity. PON1 is synthesized in the liver, and there is decreased activity of PON1 with increased lipid peroxidation. Malnutrition is state of oxidative stress due to overproduction of oxidants, decrease in antioxidant defences or a combination of these factors. As per our search, Studies related to childhood malnutrition and increased oxidant stress have been reported but there is scanty research on correlation of PON1 activity and hydroperoxide levels in Malnourished children

Objectives-Present study was carried out to find the effect of malnutrition on changes in PON1 activity, lipid profile and lipid hydroperoxide formation.

Material and methods: 30 Malnourished children (upto 5yrs) and age matched 30 controls were included. Serum arylesterase activity, lipid profile and lipid hydroperoxide were measured.

Results: Malnourished children had significantly lower PON1 arylesterase activity (105.33±14.69 V/S 175.23±22.64) lower total cholesterol (89.7±13.61V/S 126±20.19mg/dl), HDL-c (32.35±8.27 V/S 42.66±4.06 mg/dl) and significantly higher TG(180 ±28.03V/S 125±16.63mg/dl) level and hydroperoxide levels. (6.405± 1.361 V/S 2.389± 0.723µM/L).

Conclusions: Malnutrition leads to increase in hydroperoxide with decrease in arylesterase activity also increase in triglyceride level explaining in parts the complications of malnutrition.

Keywords: PON1 arylesterase; hydroperoxide; Malnutrition.

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1. INTRODUCTION

Malnutrition is one of the major public health problem in developing countries. It has devastating effects on children and very common In India[1]. The World Health Organisation (WHO) report states that in 2020, 149 million children under 5 were estimated to be stunted, 45 million were estimated to be wasted and 38.9 million were overweight [2]. Malnutrition can be due to insufficient calorie intake causing undernutrition or, insufficient intake of one or more of the essential nutrients, specially proteins causing deficiency which are responsible for marasmus and kwashiorkor, respectively based on the severity and features at the presentation [3].

Paraoxonase 1(PON1) is a calcium-dependent glycoprotein consisting of 354 amino acid residues with molecular weights of 43–47 kDa. PON1 is synthesized in the liver.[4] Paraoxonase1 (PON1) hydrolyzes organophosphate compounds and fatty acid lactones.[5,6]It is associated with the high-density lipoprotein, having an antioxidant property with arylesterase and lactonase activities [7,8]. it retard the oxidation of low density lipoprotein-cholesterol (LDL-C) by preventing the generation of lipid peroxides.[9] PON1 also found to hydrolyze hydrogen peroxide (H_2O_2),a major reactive oxygen species produced under oxidative stress which is produced in Marasmic children as it is state of oxidative stress. Thus, PON1 preserves anti-atherogenic functions of HDL and reduces oxidation of LDL [10].

As per our search, Studies related to childhood malnutrition and increased oxidant stress have been reported [11–13] but there is scanty research on correlation of PON1 activity and hydroperoxide levels In Malnourished Children.[14]

The aim of this study was to evaluate the anti-oxidant enzyme PON1 activity with oxidant stress markers including total hydroperoxide (TPX) level, triglyceride levels in order to assess oxidant/antioxidant status. The objective was also to investigate the possible correlations of oxidant/antioxidants and total hydroperoxides.

2. MATERIALS AND METHODS-

This was a hospital based case control study. study includes 30 malnourished children including twenty three males and seven females, in paediatric inpatient department of our Govt. Medical College were enrolled in our study with ages up to 5 yrs and 30 age matched healthy children including 20 males and 10 females, who had come to hospital for routine check-up or elective surgery, and had no clinical signs of any illness including malnutrition. Exclusion criteria were children with genetic and endocrine disorders causing stunting. Anthropometric indices of all children that are height, weight, head circumference were taken for all the children. Children were classified as malnourished depending as per WHO criteria .Children with weight for age < 50th percentile were taken as malnourished. No children had clinical findings of oedema. Weight and height of control group were between 15th and 50th percentiles according to WHO growth chart.

Nutritional and health history of subjects were obtained. Anthropometric measurements including body weight, height, mid-arm circumference, head circumference and thorough physical examination were performed and recorded on admission. Recumbent length was measured with length board with a foot sliding board (Precision 1 mm) and weight was estimated with precision of 10 g. The values were compared with the median age-related World Health Organization (WHO) standard growth chart present in ward. Examinations of the children and anthropometric measurements were made by the same physician. The protocol for this study was approved by the Institutional Ethics Committee of the Medical College. Written informed consent was obtained from all the parents of children.

Blood Sample collection-

Samples were drawn by veinepuncture. With all aseptic precautions, blood samples were collected in vacutainer. Blood from plain bulb was centrifuged at 1000 rpm for 15 min for serum separation. Serum samples were analyzed immediately.

PON 1 ARE activity, TPX levels and baseline investigations that is total cholesterol, triglyceride, HDL cholesterol were then measured.

Measurement of Serum PON1 Arylesterase Activity-

PON1 arylesterase activities were measured according to the method previously described by Ekerson et [15] al. The assay mixture contained 4.0 mM/L phenylacetate, 1 mM/L CaCl2 dissolved in 20 mM/L Tris HCl buffer,pH 8.0 at 25 °C. Reaction was initiated by adding a 5-µL sample in a 3-mL assay mixture. The rate of phenol formation was recorded at 270 nm following 20 s lag time. One unit of arylesterase activity is equal to 1 mM of phenylacetate hydrolysed per min. The activity was expressed as unit per litre, based on the extinction coefficient of phenol of 1310 M^{-1} cm^{-1} at 270 nm, pH 8.0, and 25 °C. Blank samples containing water were used to correct non-enzymatic hydrolysis.[16] .The intra assay coefficient of variation (CVs) was 3.2%.

Total Hydroperoxide Concentration

Total hydroperoxide concentrations of serum samples were determined using the FOX2 method [17] The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250mM H_2SO_4 (10 mL) to give a final concentration of 250 µM ferrous ion in acid. This solution was then added to 90 mL of HPLC-grade methanol containing 79.2 mg butylated hydroxytoluene (BHT). Finally, 7.6 mg xylenol orange was added with stirring to make the final working reagent (250 µM ammonium ferrous sulphate, 100 µM xylenol orange, 25 mM H2SO4, and 4 mM BHT in 90% vol/vol methanol in a final volume of 100 mL).

Estimation of Lipid Parameters-

Lipid profile by a routine biochemical Kit methods .

Estimation of Serum Cholesterol was done by (Enzymatic) Dynamic extended stability CHOD – PAP method, End point with Lipid clearing agent.

Serum HDL cholesterol was estimated by precipitation method using Phosphotungstic Acid [18].

Serum triglycerides (TG) glycerol phosphate oxidase (GPO)-PAP Method (Method of Wako, modified by McGowan and Fossati)[19]

Statistical Analysis-

- Results were expressed as the mean ± standard deviation. The continuous variables were tested for normality using the Shapiro-Wilk's test. Statistical differences between the patients and control group were estimated using unpaired Student's t-test. The strength of association between two parameters is expressed by the Pearson's correlation coefficient. Stepwise forward regression was done to assess contribution from all parameters towards hydroperoxide formation in malnutrition.
- The results obtained are analyzed by Mystat-12 statistical software. The significance level assigned is < 0.05.

Ethical statement

- Ethical approval – all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee. Informed consent – written informed consent was obtained from all individual participants parents included in the study which had been approved by the Institutional Ethics Committee of our Government Medical College.

3. RESULTS

Sixty children up to age of 5 years were selected for the study (30 patients with malnutrition and 30 healthy controls were selected).

The anthropometric data and biochemical characteristics of the study subjects are summarized in Table1 and table 2. There is no significant difference for age and sex between cases and controls. Other parameters—weight ,height, head circumference,PON1 ARE activity and total cholesterol, HDLC were found to be decreased significantly in malnourished children than healthy controls. Whereas other parameters that are TG and total hydroperoxides were increased significantly in malnourished children than healthy controls. Significant negative

correlation was found between PON1 arylesterase and TPX (fig.1 Correlation in malnourished) and positive correlation between TG and TPX. Table 3 showing pearson's correlation of all parameters keeping total hydroperoxide formation as dependent variable.

Stepwise forward regression analysis taking hydroperoxides formation as a dependent factor is shown in Table 4.Naglekerke's R2 when Stepwise forward regression is applied for only PON1 ARE activity is 0.632 and when TC was added it became 0.684 and when weight was added it became 0.711.This shows that even if by adding each parameters p value remain significant and Naglekerke's R2 goes on increasing so decreased PON1 ARE activity, TC, weight of children in combination predicts the formation of hydroperoxides in malnourished children.

4. DISCUSSION

This study supports finding previously reported that malnourished children have a state of oxidative stress and inflammation[14]. In malnourished children, Their is an imbalance in the oxidant/antioxidant system characterized by increased oxidative stress and decreased antioxidant activity. In This study malnourished children showed decreased Paraoxonase activity and increased total hydroperoxide levels. Mechanisms leading to oxidative stress in malnourished children is the subnormal intake of nutrients such as glucose, proteins and vitamins, leading eventually to accumulation of free radicals [11-A13], This is supported by significant reduction in PON activity and elevated TPX levels in malnourished children, malnutrition is an inflammatory condition leading to non-specific chronic activation of the immune system this is second mechanism for oxidative stress in malnourished children. Arora R et al showed in their study that there is imbalance between oxidant/antioxidant capacities in malnutrition and status of PON1 has been significantly and negatively correlated to status of MDA in kwashiorkor children ($p<0.05$) Which also supports to our study[20].

Lower activity of PON1 in malnourished children may be due to decrease hepatic synthesis or patients lower HDLc concentration since PON is located on HDL-C[21]. Also Infection, inflammation, immuno-compromised state leads to decrease in PON1 activity [22].

PON1 is associated with apolipoprotein (Apo) A1 in high-density lipoprotein (HDL) and is capable of preventing HDL and LDL oxidation by hydrolyzing lipid peroxides in the lipoprotein.[23] PON1 destroy biologically active peroxides so increase TPX levels explained by their lower PON1 ARE activity.[14] TPX is a marker of total oxidant status and include both lipid peroxides and hydroperoxides, high TPX levels of our malnourished children may result in lipid peroxidation leading to the cell injury. [24]

Low cholesterol value is manifestations of inadequate nutrition resulting from chronic inflammation.

In this study, We found significant negative correlation between TPX levels and all other parameters except TG showing positive correlation. Increase in triglyceride levels in parts showing the inflammatory state in malnutrition. When there is a severe lack of food, mobilization of fatty acids from adipose tissue. This fat mass catabolism may be partly responsible for the increased triglycerides levels in malnourished children, since triglycerides are released into circulation during catabolism of adipose tissue .[25]

To conclude, in our study decreased PON1 ARE Activity, TC, Weight of children in combination predicts the formation of hydroperoxides in malnourished children. Malnutrition is a state of oxidative stress, Extent of oxidative stress increases with the increase in TPX Formation. Antioxidant System Should be Strengthened by giving antioxidant rich diet to the malnourished children.

Conflicts Of Interest- No conflict of interest

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conception or the design of the manuscript, Dr Mayuri Palmate and author Dr,Mohit Rojekar to acquisition, analysis and interpretation of the data. All authors have participated to drafting the manuscript, author Dr.Sachin Bhavthankar revised it critically. All authors read and approved the final version of the manuscript. All authors contributed equally to the manuscript and approved the final version of the manuscript

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Tables

TABLE 1 : Anthropometric characteristics of Malnourished children and healthy control subjects (mean ± S.D.)

Parameters	Malnourished children (30) M=23 F=7	Control(30) M=20 F=10	P value (un-paired t-test)
Age (months)	21.8±12.34	17.03±11.46	P=0.505
Weight (kg)	7.05±3.38*	16.22±2.22	P=0.026
Height(cm)	74.34±11.36**	106.20±6.19	P=0.001
Head Circum. (cm)	43.05±3.21*	48.45±2.04	P=0.01

*p < 0.05 with respect to the control group
**p < 0.001 with respect to the control group

TABLE 2: Arylesterase activity and total hydroperoxide levels in Malnourished children and healthy controls (mean±S.D.)

Parameters	Malnourished children (30) M=23 F=7	Control(30) M=20 F=10	P value (un-paired t-test)
ARE Activity(IU/L)	105.33±14.69*	175.23±22.64	P=0.02
TC(mg/dl)	89.7±13.61*	126±20.19	P=0.03
TG(mg/dl)	180 ±28.03**	125±16.63	P= 0.006
HDL(mg/dl)	32.35±8.27**	42.66±4.06	P=0.0002
TPX(µM/L)	6.405± 1.361**	2.389± 0.723	P=0.001

*p < 0.05 with respect to the control group
**p < 0.001 with respect to the control group

TABLE 3: **Pearson's Correlation (r)**
Dependent variable TPX

Parameters	r value	p
ARYL. Activity	-0.796	<0.001
TC	-0.661	<0.001
TG	0.621	<0.001
HDL-c	-0.558	<0.001

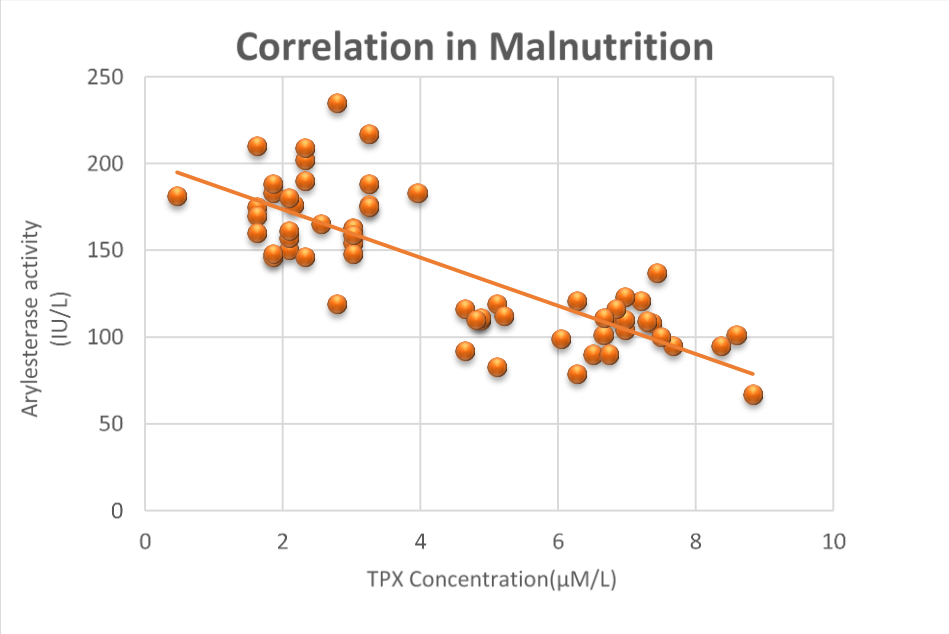
WEIGHT	-0.526	<0.001

ARE,arylesterase activity; HDL-C, high-density lipoprotein cholesterol;
TC, total cholesterol; TPX, total hydroperoxide.

TABLE 4: **Stepwise Forward Regression**

Model 1, (Naglekerke's R^2 =0.632 P<0.05)			
Parameter	Std. Coefficient	Standard Error	P value
ARE	-0.796	0.005	<0.01
MODEL 2(Naglekerke's R^2 =0.684 P<0.05)			
ARE	-0.626	0.005	<0.01
TC	-0.281	0.009	0.004
MODEL 3(Naglekerke's R^2 =0.711 P< 0.05)			
ARE	-0.563	0.006	<0.01
TC	-0.253	0.009	0.01
WEIGHT	-0.194	0.037	0.029

Figure No.1)



In fig 1 Total hydroperoxide has strong negative correlation with PON1 Arylesterase

activity. (r=0.796 p<0.001)

Abbreviations-

PON 1-paraoxonase1
ARE-arylesterase activity.
HDL-C- high-density lipoprotein cholesterol.
TC- total cholesterol
TPX- total hydroperoxide.