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Evaluation of reactive oxygen species in term low birth babies

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Abstract

Background: Oxidative stress has been implicated as one of the major cause in the pathophysiology of decreased or impaired foetal weight in normal term low birth weight babies. Either increased generation of reactive oxygen species (ROS) or decreased antioxidant levels can be measured to substantiate this finding in view of the increased incidence of term babies with low birth weight.

Aim: The aim of this study is to evaluate the role of reactive oxygen species by measuring the serum malondialdehyde levels in term low weight babies with that of controls with normal weight.

Materials and methods: The study group consisted of 72 term low weight babies weighing < 2500 gms of both sexes and 70 controls with >2500 gms of both sexes were included in the study. In the study group babies with birth weight of <2000 g was grouped as group 1a and with weight of >2000 – 2500g as group 1b. Serum malondialdehyde (MDA) an end product of lipid peroxidation (a measure of free radical damage) were measured using calorimetric thiobarbituric acid method in both study group and control group and were compared with their birth weight and ponderal index. Data was collected and analysed by suitable statistical methods

Results: The mean MDA levels estimated in the study group was significantly higher (MDA levels – 28.5250 ±3.0 nmol/L) than in the control group (19.3693±1.60). As the birth weight increased the MDA levels decreased (P<0.01). The mean MDA levels in group 1a (20 among 72) was 32.205 ±2.49 nmol/ml and group 1b (52 among 72) was 27.1096±1.72 with p value of 0.01 which was statistically significant

Conclusion: Our results indicate that in the term low birth weight babies the MDA levels were significantly increased which concludes that ROS species plays a major role in etiopathogenesis of low birth weight babies.

Keywords: Malondialdehyde (MDA) levels and foetal growth.

1. Introduction

Foetal growth has been divided into three conservative cell growth phases [1]. The initial phase of hyperplasia that occurs during the first 16 hrs and is characterised by rapid increased in cell number. The second phase which extends up to 32 hours includes both cellular hyperplasia and hypertrophy. The corresponding growth rates during these three growth phases are from 5 g/day at 15 weeks, 10 – 15 g/day at 34 weeks [1]. In early foetal life the major determinant of growth is the foetal genome, but late in pregnancy, environmental, nutritional and hormonal influences become increasingly important.

` The influence of dietary factors i.e. Vit. E requirements (antioxidant) for the low birth weight infant varies with other factors such as iron and poly unsaturated fatty acids. This interaction is caused by the influence of iron on the oxidative breakdown of lipids (lipid peroxidation). Thus, the need for fortification of infant formulas with Vit. E if the formulas are iron fortified or has high content of PUFA [2].

During perinatal hypoxia, reactive oxygen species cause lipid peroxidation of cell membranes, yielding oxidation products that constitute thiobarbituric acid reacting substances (TBARS). Urine sample collected from term and near term infants on the 1st day of life shows elevated urinary TBARS substance in term and near term babies was of postnatal hypoxia (as determined by Apgar score). The lipid peroxidation end product, malondialdehyde (MDA) is also found to be elevated in plasma from low birth and very low birth weight babies [3].

In 78% of low birth weight, Glutathione peroxidases were low in the premature infants than in the full term infants. This was due to the low concentration of selenium known to be present in the southern New Zealand [4].

The concentration of circulating antioxidants may be important in the etiology of disease in low birth weight and pre-mature infants. Blood samples taken from them within 2 hours of birth showed a strong correlation with Plasma ascorbic acid and ceruloplasmin to prevent lipid peroxidation *in vitro* [5].

The role of free oxygen radicals in the pathogenesis of low birth weight infants was implied. Significant correlation was found between Elevated lipid peroxidation end and low birth weight babies [6].

Though elevation of ROS has been implicated to play a major role in the low birth weight babies, likes wise it has a role in development of macrosomic babies. Malondialdehyde has been identified as a product of lipid peroxidation and served as an index of determination of extent of lipid peroxidation.

Hence the aim of the study is to determine the relation between reactive oxygen species and term low birth Indian babies.

2. Materials and Methods

This study was done on the new born term & low birth babies in Neonatology Department at Raja Sir Ramaswamy Mudaliar Lying in Hospital, attached to Stanley Medical College for the period March 2003 to August 2003.

The study group consisted of 72 terms low birth weight babies less than 2500 gms of both sexes and control group were 70 babies of normal birth weight about 2500 gms of both sexes [Table1a & b and Figure I, II & III]. The study groups were further divided into two groups A & Group B. Group A – babies weighing less than 2000 gms and Group B – babies weighing more than 2000 gms to 2500 gms. All the babies were examined according to standard protocols. They were subjected to a detailed general examination to rule out major congenital anomalies. Apgar scoring was done to rule out asphyxia.

All term babies weighing <2500 gms with Apgar score more than 8 were included in the study. Maternal illness during pregnancy like DM, PIH, Jaundice, Anaemia were excluded. Congenital anomalies in the newborn and all sick babies suffering from infection, sepsis were excluded.

The blood sample was collected from both the groups and subjected to centrifugation at 3000 revolutions/min for 10-15 minutes. The serum was separated and analysis was done.

Malondialdehyde the end product of lipid peroxidation was estimated [7] in serum by calorimetric thiobarbituric acid method. Under the acid and heating condition of the reactions, the lipid peroxides break down to form a stable compound malondialdehyde (MDA) which complexes with Thiobarbituric acid (TBA). The resulting MDA – TBA chromogen can be measured calorimetrically at 540 nm.

Tetramethoxy preparation (Malondialdehyde – TCL, Tokyo Kasai, Japan) was used as a standard and double distilled water as control. The method consisted of treating 0.5 ml of serum with 3.6 ml of 10% TCA followed by addition of 1.5 ml of 0.67% TBA (Thiobarbituric acid) and to that solution 1 ml of distilled water was added.

The reaction mixture was heated in a boiling water bath for 10 minutes and then centrifuged at 5000 rpm for 10 – 15 minutes until a clear pale pink supernatant was collected. Colour intensity was measured at 540 nm in calorimeter. The optical density value was noted down.

The same methods were adopted in control group. From the optical density values using the formula, the amount of MDA present was calculated and results were expressed as nmol/ml.

2.1 Statistics

All statistical analyses were done using SPSS v16.0 software. Results were expressed as mean & SD. Independent unpaired student 't' test was used to compare mean value between groups. A P-value less than 0.05 was considered significant.

3. Results

The number of female babies recruited in both groups was more than that of male babies. There was no significant difference in the distribution of babies based on sex between the two groups. The gestational age was comparable in both the groups, Mean gestational age of the study group was 39.47 and that of the control group was 39.53. There is no statistical significance in the mean gestational age by dates in the two groups.

The mean birth weight, length and ponderal index of study group was found to be significantly less than that of control group. The mean birth weight in the study group was 2164 gms and that in the control group was 2893.57 gms. The mean length of the babies in the study group was 49.8 cms and that in the control group was 51 cms. Likewise, the ponderal index calculated for the study and the control group was 1.74 and 2.21 suggesting and confirming the fact that ponderal index of <2 signifies low birth weight and >2 as term normal birth weight baby. Similar relationship was observed between subgroups Ia & IIb of study group. [Table 2 & 3]

The MDA levels in the normal term babies and term low birth weight babies were compared. The values of MDA in term low birth weight babies ranged from a minimum of 26 nmol/ml to a maximum of 40 nmol/ml. The MDA levels in the control group ranged from minimum of

17.37 nmol/ml to a maximum of 23.45 nmol/ml. The mean MDA level in the study group was 28.53 ±3.01 nmol/ml and in the control group as 19.37 ±1.69 nmol/ml. The 'p' value is less than 0.001 and it was highly significant. There was a difference of about 9 nmol/ml between the two groups which is statistically highly significant. Within the study group male babies show a significant increase in MDA levels than female babies. (Table 4 & 5 and figure IV & V)

Similarly the mean MDA levels estimated in the study group Ia (32.2) & Ib (27.11) were significantly higher in Group Ia than in Group Ib. As the birth weight increased the MDA level decreased. [Table 6]

Table 1a: Distribution of low birth weight babies (<2500 gm)

Birth weight (gm)	No	%
1400 – 1699	4	6.00
1700 – 1999	16	22.00
2000 – 2299	33	46.00
2300 – 2499	19	26.00
Total	**72**	

Table 1b: Distribution of normal birth weight babies (≥2500 gm)

Birth weight (gm)	No	%
2500 – 2799	23	47
2800 – 3099	25	36
3100 – 3399	7	10
3400 – 3699	4	6
>3700	1	1
Total	**70**	

Table 2: Base-line statistical parameters of study and control group

Features	Study group (n = 72)		Control group (n = 70)		t value	p value
	Mean	S.D.	Mean	S.D.		
Birth weight (gm)	2164.72	232.53	2893.57	247.15	18.10	<0.001
Length (cm)	49.8167	1.8251	50.94	0.16	-4.19	<0.001
Ponderal index	1.7440	0.1464	2.213	0.1911	16.45	<0.001
Gestational age (weeks)						
By dates	39.47	0.99	39.53	0.85	-0.36	>0.05
By Ultrasound	>39 wk	-	>39 wk	-	-	-
Apgar						
1 minute	7.0	0.69	7.40	0.73	-3.351	<0.001
5 minutes	8.11	0.49	8.46	0.06	-3.342	<0.001

Table 3: Base-line statistical parameters of low birth weight babies (study group) subdivided as Group Ia and Group Ib

Features	<2000 gms Group Ia (n = 20)		>2000 – 2500 gms Group Ib (n = 52)		t value	P value
	Mean	SD	Mean	SD		
Birth weight (gm)	1850.00	175.47	2285.77	100.10	13.24	<0.001
Length (cm)	48.7250	2.308	50.236	1.41	-3.37	<0.001
Ponderal index	1.6113	0.160	1.7	0.100	-5.7	<0.001
Gestational age (weeks)						
By Dates	39.65	1.04	39.4	0.98	0.94	>0.05
By Ultrasound	>39 wk	-	>39 wk	-	-	-
Apgar						
1 minute	7.00	0.64	7.04	0.71	-0.75	>0.05
5 minutes	8.00	0.56	8.15	0.46	-1.2	>0.05

Table 4: MDA levels in the study and control group

Group	Number	Mean MDA level (nmol/ml)
Study	72	28.5250±3.01
Control	70	19.3693±1.69
	t = 22.21	p < 0.01

Table 5: Showing the correlation between sex of babies and MDA level

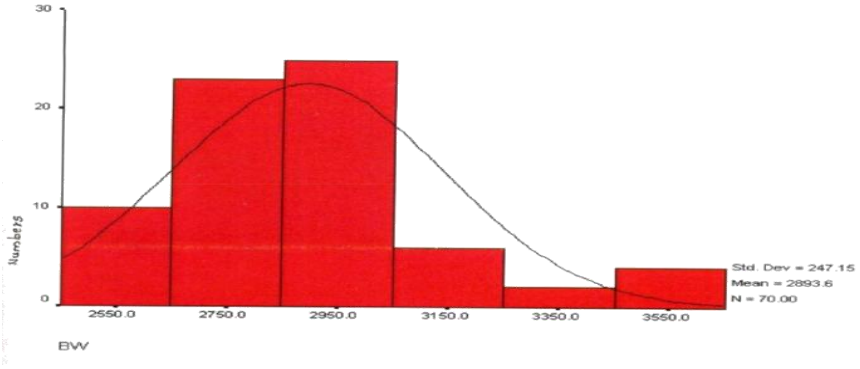
	2500 gm (LBW)		>2500 gm normal birth weight	
	Mean MDA	SD	Mean MDA	SD
Gender				
Male	29.56	2.3	19.14	1.58
Female	28.09.	3.18	19.44	1.74
	p = 1.90	p = 0.06	t = 0.61	p = 0.5

Table 6: MDA levels in the study subgroup Ia and Ib

Study subgroup	Number	Mean MDA level (nmol/ml)
A	20	32.205±2.49
B	52	27.1096±1.72
	t = 9.864	p < 0.01

The distribution of normal birth weight babies is illustrated in figure II.

Figure II: Distribution of normal BW



The distributions of all the babies recruited within study i.e. the low birth weight and normal birth weight babies scoring to their birth weight are illustrated in figure III. It follows "normal" distribution patterns.

Figure III: Distribution of birth weight of all babies

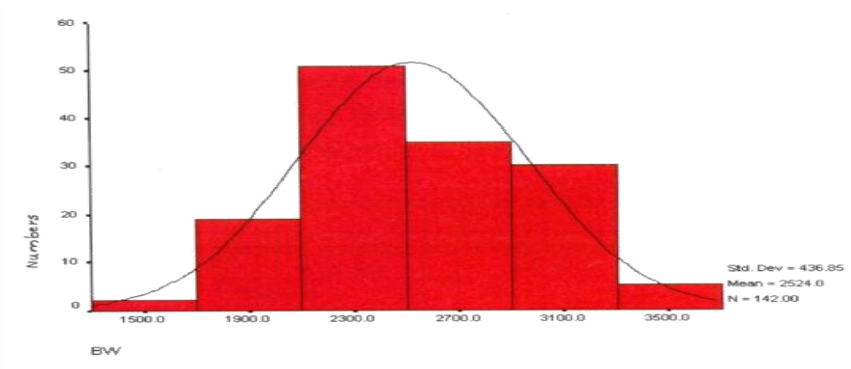


Figure IV: Correlation between MDA level & BW

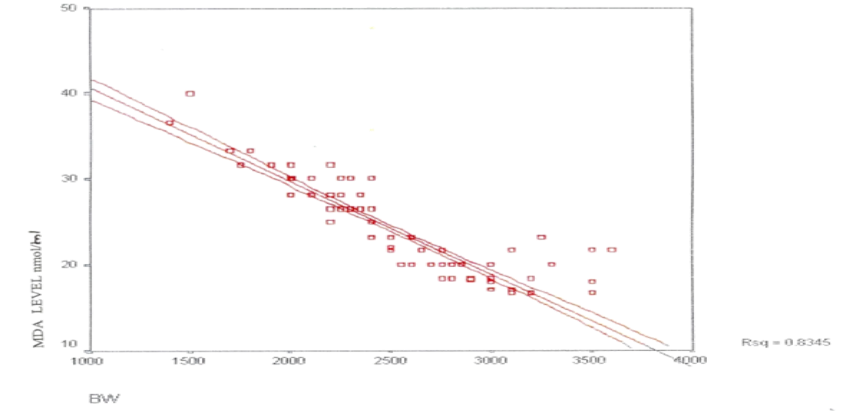
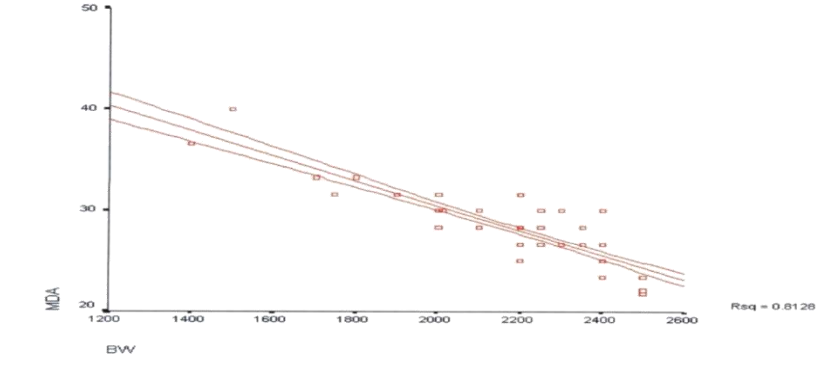


Figure V: Correlation between APGAR Score and MDA Level in Low Birth weight babies



4. Discussion

In the present study, the levels of lipid peroxidation end product i.e. MDA is compared between control group which comprises of normal term babies and study group which refers to term low birth weight babies.

The MDA levels in the normal term babies and term low birth weight babies were compared. There was a difference of about 9 nmol/ml between the two groups which is statistically highly significant. This finding confirms that the lipid peroxidation end product (MDA) level increases as birth weight decreases [3,6].

This further supports the finding that ROS may have a major role to play in the pathogenesis of low birth weight babies. This fact is in confirmation with the studies conducted [8].

In healthy individuals generation of reactive oxygen species should be in balance with antioxidant defenses. During the process of cellular differentiation and proliferation there is a rise in the intracellular level of H_2O_2.

Normally, if the existing antioxidant like superoxide dismutase, glutathione peroxidase and catalases are in balance with free radical formed, there is no cellular damage. Therefore, in disease processes there can be either a decrease in antioxidant level or an increase production of free radicals [9].

The placenta is said to be rich source of mitochondria during pregnancy. From early pregnancy the human placenta influences maternal homeostasis. When fully developed placenta consumes about 1% of BMR of pregnant women [10].

It is also observed that heavy work by the mother during the last trimester results in loss of energy stores i.e. there is decreased uteroplacental blood flow with resultant decrease in blood volume, oxygen supply and nutrient to the foetus. The loss of water and electrolytes in hot climate during hard work results in reduction of plasma volume with an increase in iron concentration [11]. Most of the intracellur free iron is in the ferric state (Fe^{3+}); it must first be reduced to the ferrous form to participate in the Fenton's reaction. This reduction step is catalysed by superoxide ion and thus iron and superoxide synergise to elicit maximal oxidative cell injury.

Fenton reaction ($Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH\bullet + OH$) could be one of the source of production of free radicals. Nitric oxide (NO) an important chemical mediator normally synthesised by a variety of cell types can act as a free radical or can be converted into highly reactive nitrite species.

Three reactions are particularly relevant to cell injury mediated by free radicals.

1. **Lipid peroxiation of membranes:** Double bonds in membrane polyunsaturated lipids are vulnerable to attack by oxygen derived free radicals. The lipid radical interactions yield peroxides, which are they unstable and reactive and an autocatalytic chain reaction ensues.
2. **DNA Fragmentation:** Free radicals react with thymine in nuclear and mitochondrial DNA and produce single strand streaks. Such DNA damage is implicated in both cell killing and malignant transformation.
3. **Cross linkage of proteins:** Free radicals promote sulphydryl mediated protein cross linkages, resulting in enhanced rate of degradation or loss of enzymatic reactions. Free radicals also cause polypeptide fragmentation.

Free radical generation is also normal part of the respiration and other cellular activities including microbial defence. Fortunately, free radicals are inherently unstable and generally decay spontaneously. Superoxide, for example, rapidly breaks down in the presence of water in to oxygen and hydrogenperoxide.

This H_2O_2 which is formed in the early stages of embryogenesis in higher concentration is found to inhibit the receptors (Tyrosine Kinases) for growth factors were including PGDF, EGF, FGF and IGFI [12] thereby causing a delay and arrest in the cell cycle leading on to growth restrictive foetus.

However, cells have also developed several enzymatic and non-enzymatic systems to inactivate free radicals.

1. The rate of spontaneous decay is significantly increased by the action of superoxide dismutase found in many cell types.
2. Glutathione peroxidase protects against injury by catalysisng free radical breakdown. The intracellur ratio of oxidised (GSSG) to reduced (GSH) glutathione is a reflection of oxidative state of the cell and an important aspect of cell's ability to catabolise free radicals.
3. Catalases help in degradation of H_2O_2.
4. Endogenous and exogenous antioxidants (E.g., Vit. E, A, and C and β carotene) may either block the formation of free radicals or scavenge them once they have formed.

Though in this study the MDA levels increase as the birth weights decrease we could not explain which the cause is and which is the effect. The subnormal growth and maturation of the foetus could be the cause for insufficient clearing of reactive oxygen species.

Though placenta is said to be one of the source of free radical formation, it is not possible because free radicals are highly unstable and by the time it circulates in the mother and get transferred to the foetus it would have initiated lipid peroxidation in the mother itself. So, the probable source of free radicals should be from the foetus.

Pregnant mothers are given only iron and calcium supplementation during the antenatal period but iron in excess amount is found to be a prooxidant.

As long as the incidence of iron deficiency anemia is more in the community, we cannot withhold from giving iron to the pregnant mother. But to prevent the damage induced by ROS, iron with antioxidants is indicated. Substitution with diets rich in antioxidants in the latter half of pregnancy will help in overcoming the problem of low birth weight babies.

5. Conclusion

This study clearly shows the free radical has a role in the etiopathogenesis of low birth weight babies. Low birth weight can be prevented by administration of antioxidants and medical education to the mothers. Levels of MDA can be measured in all low birth babies to confirm their exposer to oxidative stress.

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