

Original Research Article**Ischaemia modified albumin and Malondialdehyde level in subjects suffering from hypothyroidism.****Abstract:**

Thyroid hormone deficiency (hypothyroidism) leads to many serious conditions in body. Oxidative stress (OS), a state of excess free radicals and reactive metabolites formation which ultimately leads to an imbalance between the production of oxidants and their elimination by antioxidative systems in the body. Researchers all around the globe are in search of the link between this oxidative stress by estimating its different markers and hypothyroidism. In this present study oxidative stress is evaluated in hypothyroidism by estimating serum ischaemia modified albumin (IMA) and malondialdehyde (MDA).

Settings and Design:

56 patients attending different Out-Patient Department (OPD) who have fulfilled inclusion criteria were included as cases and 43 apparently healthy persons were selected as control. Serum fT_4 and TSH level measured by immunoassay and serum IMA and MDA level measured by well standardised validated methods.

Results:

By undertaking independent sample 't' test it is found that mean and standard deviation (SD) of IMA ($t=4.149$, $p<0.001$) and MDA ($t=19.171$, $p<0.001$) are significantly increased in case group than the control group. In case group it was found that serum IMA & MDA are significantly correlated with fT_4 $\{r= -0.835$ (IMA) & -0.765 (MDA)} & TSH $\{(r= +0.859$ (IMA) & $+0.672$ (MDA)}.

Keywords:

Hypothyroidism, ischaemia modified albumin, malondialdehyde, oxidative stress, free radicle.

27 **Introduction:**

28 The thyroid gland produces two related hormones, thyroxine (T4) and triiodothyronine (T3).

29 Acting through nuclear receptors, T3 and T4 play a critical role in cell differentiation during

30 development and help to maintain thermogenic and metabolic homeostasis in the adult.

31 Thyroid hormone deficiency (hypothyroidism) leads to many serious conditions in body like

32 growth failure, atherosclerosis, pleural and pericardial effusion, perceptive deafness,

33 infertility etc.

34 Oxidative stress (OS) represents an imbalance between the production of oxidants and their

35 elimination by antioxidative systems in the body. Many studies have linked OS to thyroid

36 dysfunction by showing its association with abnormally regulated oxidative or antioxidative

37 molecules (2, 3). Although, the high reactivity and short half life of reactive oxygen and poly

38 unsaturated fatty acid (PUFA) or nitrogen species make difficult their direct determination

39 (3), several methods have been tried. We propose an approach for the determination of

40 oxidative stress in human serum by measuring ischaemia modified albumin (IMA) and

41 malondialdehyde (MDA) which is a familiar member of thiobarbituric acid reacting

42 substances (TBARS).

43 Ischemia Modified Albumin (IMA) has been developed and found to be very useful for the

44 detection of acute myocardial ischemia and the study of oxidative stress in different disorder.

45 Initially the test was named as Albumin Cobalt Binding (ACB) assay since it is based on the

46 reduced binding affinity of the human serum albumin for metal ions (Cobalt) in patients

47 suffering from oxidative stress. The reduction in the binding affinity of albumin has been

48 attributed to the free radical damage to the N-terminal of albumin molecule in patients (4-7).

49 In 2012 Ma et al. reported that IMA levels are increased in patients with thyroid dysfunction,

50 particularly in overt hypothyroidism (6). Contradicting to this Ersoy et al. (2013) stated that

51 Serum IMA levels did not differ among patients with overt or subclinical hypothyroidism (5).

52 Malondialdehyde (MDA), a secondary product of lipid peroxidation (LPO) and is used as an
53 index of tissue damage (11, 12), as it is the major reactive aldehyde resulting from
54 peroxidation of biological membrane polyunsaturated fatty acids (PUFA). The peroxidation of
55 poly unsaturated fatty acids of the biological membranes leads to conjugated dienes (CD)
56 formation, followed by the cleavage of the fatty acid chains and subsequent release of the
57 reactive aldehyde products, i.e.; malondialdehyde, 4-hydroxy 2,3-transnonenal, 4-hydroxy-
58 2,3-transhexanal etc. often referred to as thiobarbituric acid reacting substances (TBARS) (9,
59 10). Malondialdehyde is only formed by fatty acids with three or more double bonds and is
60 relatively more stable than others (13).

61 **Aims and objectives:**

- 62 1. To find out if there is any correlation between fT4 and TSH with IMA and MDA in
63 our specified groups.
- 64 2. To find out if there is any difference of the means of IMA and MDA between our case
65 and control group.

66 **Materials and methods:**

67

68 Patients attending different Out-Patient Department (OPD) of R. G. Kar Medical College &
69 Hospital, Kolkata, West Bengal who had serum TSH value > 6.5 mIU /L were included in
70 this study. 56 patients were well thought-out as cases. They had attained the age of 18 years
71 and could give informed consent after understanding the objectives of the study. A group of
72 43 age and sex matched healthy individuals with no history of thyroid disorder served as
73 controls. Control subjects were selected from persons accompanying the patients in OPD who
74 were free from any detectable disorders including hypothyroidism or conditions which may
75 interfere with our study parameter. Patients who are suffering from diabetes mellitus,

76 neoplasm, neurological diseases were excluded from study. All of the selected patients are
77 free from intake of alcohol or nicotine by any means in any form. As participants attended the
78 hospital OPD from a large rural base, they had approximately similar ethnicity,
79 socioeconomic status and dietary habits.

80 Fasting blood was collected by venipuncture for the determination of different biochemical
81 parameters. The blood samples were subjected to centrifugation at 3,000 rpm for 10 min for
82 separation of serum. Serum thus obtained was analysed for biochemical parameters Serum
83 fT_4 and TSH level were measured by ELISA with kit provided by Accubind-Monobind with
84 help of TECAN ELISA reader and washer. Within assay precision of fT_4 , TSH was
85 determined by analysis control materials.

86 IMA estimation (30-33) is based on the premise that hypothyroidism due to oxidative stress
87 causes changes in human serum albumin (HSA) that are demonstrated by reduced exogenous
88 cobalt binding. The concentration of ischemia modified serum albumin can be determined by
89 addition of a known amount of cobalt (II) to a serum specimen and measurement of the
90 unbound cobalt (II) by colorimetric assay using dithiothreitol (DTT). An inverse relationship
91 thus exists between the level of albumin bound cobalt and the intensity of the colour
92 formation. Preparations for the Co (II) albumin binding protocol involved the addition of 200
93 μ l of patient serum to 50 μ l of a solution of 1gm/l cobalt chloride, followed by vigorous
94 mixing and 10-min incubation. Dithiothreitol (50 μ l of a 1.5 g/l solution) was then added and
95 mixed. After 2 min. incubation, 1.0 ml of a 9.0 g/l solution of NaCl was added. The
96 absorbance of the assay mixture was read at 470 nm using a Spectrophotometer. The blank
97 was prepared similarly with the exclusion of DTT. IMA assay was standardized in the
98 Department of Biochemistry R. G. Kar Medical College and a standard curve was prepared in
99 the range 6.0-60.0 μ g $CoCl_2$ /ml. One IMA unit was defined as “ μ g of free Co (II) in the

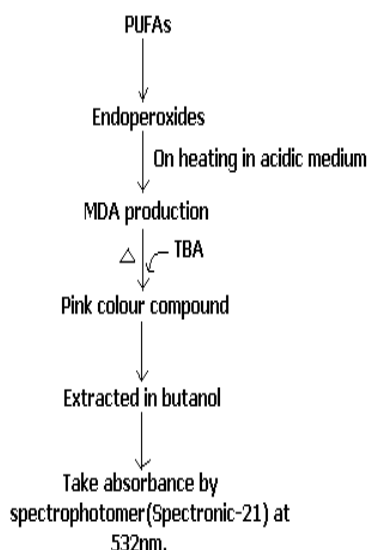
100 reaction mixture per ml of serum sample". The assay was found to maintain linearity within
101 this range. The calculated factor from this standard curve is 411.8.

102 So,

103 The IMA = $\frac{411.8 * (\text{Absorbance of unknown} - \text{Absorbance of blank}) * 5 \mu\text{g/ml}}{1}$.

104 For the convenience in the present study we expressed our values as absorbance units
105 (ABSU).

106 MDA estimation (34-36) is based on that polyunsaturated fatty acids react with molecular
107 oxygen to undergo destructive free radical mediated auto-oxidation. During this process
108 numerous peroxides and aldehyde compounds are formed. These non-volatile compounds
109 decompose to form MDA and other substances during acid heating in laboratory (Prayer et
110 al., 1976). These substances react with thiobarbituric acid in an acidic medium to form a pink
111 colour compound. Then the product is readily extractable into organic solvents such as
112 butanol and absorbance was taken at 532nm using dual beam spectrophotometer.



113

114 Estimation of MDA in samples:

115 To 0.5 ml of freshly prepared serum 2.5 ml of trichloroacetic acid (20mg/dl) was added and
 116 the tube was allowed to stand for 10 minutes at room temperature. Then 2.5 ml. of sulphuric
 117 acid {0.05(M)} was added and stirred thoroughly. 3.5 ml. of TBA reagent (200mg of TBA
 118 was mixed with 100ml of 2(M) sodium sulphate solution) was added to this. The coupling of
 119 lipid peroxide with TBA was carried out by heating in boiling water bath for 30 minutes. It
 120 was then cooled in water. 4.0 ml of n-butanol was added and chromogen was extracted to
 121 organic phase by vigorous shaking and vortexing followed by centrifugation at 3000 rpm for
 122 10 minutes.

123 The supernatant organic phase was pipetted to a clean test tube. Its absorbance was
 124 determined at 532nm wave length by spectrophotometer. N-Butanol was used as blank to
 125 assure zero reading. The optical density was noted and level of MDA was calculated from
 126 standard curve in the range of 5-15 nmol/ml. The calculated factor from the standard curve is
 127 = 60.

128 Hence,

129 Concentration of MDA of a unknown solution is = Absorbance of unknown*60*2 nmol/ml

130

131 **Results:**

132 Mean & SD of serum fT4 (ng/dl), TSH (mIU /ml) , IMA (μ g/ml)and MDA (nmol/ml) in
 133 control vs. case group respectively are 1.63 ± 0.15 vs. $0.93 \pm .58$, 5 ± 0.87 vs. 18.3 ± 8.1 ,
 134 0.42 ± 0.05 vs. 0.51 ± 0.14 & 4.62 ± 1.1 vs. 9.27 ± 1.27 (vide table 1). In control group Pearson's
 135 bivariate correlation shows that serum IMA & MDA though correlated with fT4 (-ve) & TSH
 136 (+ve) but the level is not statistically significant in controls. ('t' values & p values are fT4: -
 137 7.75, <0.001; TSH: 10.719, <0.001; IMA: 4.149, <0.001 & MDA: 19.171, <0.001). In the

case group it was found that serum IMA & MDA are significantly correlated with fT4 ($r = -0.835$ & -0.765) & TSH ($r = +0.859$ & $+0.672$) (vide table 2).

Discussion:

Oxidative stress represents an imbalance between the production of oxidants and their elimination by antioxidative systems in the body. In this study, we had determined serum IMA and malondialdehyde as oxidative stress marker taking into account the relationship between free radicals and thyroid diseases. In 2012 Ma et al. reported that IMA levels are increased in patients with thyroid dysfunction, particularly in overt hypothyroidism (6) than in euthyroid subjects ($p < 0.05$).

Erdamar H et al revealed an increased generation of reactive oxygen species and impairment of the antioxidant system in patients with hypothyroidism. Malondialdehyde, nitrite, vitamin E, and myeloperoxidase activity increased in patients with hypothyroidism. Some other studies also showed association of thyroid dysfunction with abnormally regulated oxidative or antioxidative molecules (2, 3). Contradicting to this Ersoy et al. in 2013 stated that Serum IMA levels did not differ among patients with overt or subclinical hypothyroidism (5).

In this study 56 cases and 43 age and sex matched controls had been recruited. After analyzing different parameters and applying appropriate statistical analysis it was found that the mean and standard deviation (SD) of different studied parameters are significantly different in these two groups. After doing independent sample 't' test it was found that all the studied parameters has a statistically significant difference of mean of cases from that of controls group

In control group Pearson's bivariate correlation shows that serum IMA & MDA though correlated with fT4 (-ve) & TSH (+ve) but the level is not statistically significant in controls.

162 In cases we found that serum IMA & MDA are significantly correlated with fT4 ($r = -0.835$ &
163 -0.765) & TSH ($r = +0.859$ & $+0.672$).

164 Hence, from this study it can be concluded that in hypothyroidism there is oxidative stress
165 which is reflected by the existence of significant level of free radical mediated generation of
166 serum MDA and IMA.

167 Results reveal an increased generation of reactive oxygen species and impairment of the
168 antioxidant system in patients with hypothyroidism. Malondialdehyde and IMA
169 concentrations increased in patients with hypothyroidism and also correlated with the severity
170 of the disease as reflected by the levels of fT4 and TSH. These findings indicate that thyroid
171 hormones have a strong impact on oxidative stress and the antioxidant system.

172 Tables:

173 Table 1: Mean and standard deviation and their comparison by independent t test.

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VARIABLES	GROUPS	Number of subjects	Mean	Std. Deviation	t	Sig. (2-tailed)
fT4 (ng/dl)	CASES	56	.9250	4.149	-7.750	<0.001
	CONTROLS	43	1.6312	19.171		
TSH (m IU/ml)	CASES	56	18.3116	8.09758	10.719	<0.001
	CONTROLS	43	5.0002	.87419		
IMA (µg/ml)	CASES	56	.5148	.14350	4.149	<0.001
	CONTROLS	43	.4202	.04718		
MDA (nmol/ml)	CASES	56	9.2732	1.27102	19.171	<0.001
	CONTROLS	43	4.6172	1.09447		

175

176 Table 2 : Pearson's bivariate correlation of fT4 & TSH with different study parameters of
177 controls and cases are as follows:

178

Thyroid function tests		Control IMA	Control MDA	Case IMA	Case MDA
ft4	Pearson Correlation	-.269**	-.225**	-.835**	-.765**
	Sig. (2-tailed)	>0.05	>0.05	<0.001	<0.001
	N	43	43	56	56
TSH	Pearson Correlation	.296**	.241**	.859**	.672**
	Sig. (2-tailed)	>0.05	>0.05	<0.001	<0.001
	N	43	43	56	56

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