

Original Research Article

Ischaemia modified albumin and Malondialdehyde level in subjects suffering from hypothyroidism.

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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 fT₄ 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 fT₄ {r= -0.835 (IMA) & -0.765(MDA)} & TSH {(r= +0.859(IMA) & +0.672(MDA))}.

Keywords:

30 Hypothyroidism, ischaemia modified albumin, malondialdehyde, oxidative stress, free
31 radicals.

32 **Introduction:**

33 The thyroid gland produces two related hormones, thyroxine (T4) and triiodothyronine (T3).
34 Acting through nuclear receptors, T3 and T4 play a critical role in cell differentiation during
35 development and help to maintain thermogenic and metabolic homeostasis in the adult.
36 Thyroid hormone deficiency (hypothyroidism) leads to many serious conditions in body like
37 growth failure, atherosclerosis, pleural and pericardial effusion, perceptive deafness,
38 infertility etc.

39 Oxidative stress (OS) represents an imbalance between the production of oxidants and their
40 elimination by antioxidative systems in the body. Many studies have linked OS to thyroid
41 dysfunction by showing its association with abnormally regulated oxidative or antioxidative
42 molecules (2, 3). Although, the high reactivity and short half life of reactive oxygen and poly
43 unsaturated fatty acid (PUFA) or nitrogen species make difficult their direct determination
44 (3), several methods have been tried. We propose an approach for the determination of
45 oxidative stress in human serum by measuring ischaemia modified albumin (IMA) and
46 malondialdehyde (MDA) which is a familiar member of thiobarbituric acid reacting
47 substances (TBARS).

48 Ischemia Modified Albumin (IMA) has been developed and found to be very useful for the
49 detection of acute myocardial ischemia and the study of oxidative stress in different disorder.
50 Initially the test was named as Albumin Cobalt Binding (ACB) assay since it is based on the
51 reduced binding affinity of the human serum albumin for metal ions (Cobalt) in patients
52 suffering from oxidative stress. The reduction in the binding affinity of albumin has been
53 attributed to the free radical damage to the N-terminal of albumin molecule in patients (4-7).
54 In 2012 Ma et al. reported that IMA levels are increased in patients with thyroid dysfunction,

55 particularly in overt hypothyroidism (6). Contradicting to this Ersoy et al. (2013) stated that
56 Serum IMA levels did not differ among patients with overt or subclinical hypothyroidism (5).
57 Malondialdehyde (MDA), a secondary product of lipid peroxidation (LPO) and is used as an
58 index of tissue damage (11, 12), as it is the major reactive aldehyde resulting from
59 peroxidation of biological membrane polyunsaturated fatty acids (PUFA). The peroxidation of
60 poly unsaturated fatty acids of the biological membranes leads to conjugated dienes (CD)
61 formation, followed by the cleavage of the fatty acid chains and subsequent release of the
62 reactive aldehyde products, i.e.; malondialdehyde, 4-hydroxy 2,3-transnonenal, 4-hydroxy-
63 2,3-transhexanal etc. often referred to as thiobarbituric acid reacting substances (TBARS) (9,
64 10). Malondialdehyde is only formed by fatty acids with three or more double bonds and is
65 relatively more stable than others (13).

66 **Aims and objectives:**

- 67 1. To find out if there is any correlation between fT4 and TSH with IMA and MDA in
68 our specified groups.
- 69 2. To find out if there is any difference of the means of IMA and MDA between our case
70 and control group.

71 **Materials and methods:**

72

73 Patients attending different Out-Patient Department (OPD) of R. G. Kar Medical College &
74 Hospital, Kolkata, West Bengal who had serum TSH value > 6.5 mIU /L were included in
75 this study. 56 patients were well thought-out as cases. They had attained the age of 18 years
76 and could give informed consent after understanding the objectives of the study. A group of
77 43 age and sex matched healthy individuals with no history of thyroid disorder served as
78 controls. Control subjects were selected from persons accompanying the patients in OPD who

79 were free from any detectable disorders including hypothyroidism or conditions which may
80 interfere with our study parameter. Patients who are suffering from diabetes mellitus,
81 neoplasm, neurological diseases were excluded from study. All of the selected patients are
82 free from intake of alcohol or nicotine by any means in any form. As participants attended the
83 hospital OPD from a large rural base, they had approximately similar ethnicity,
84 socioeconomic status and dietary habits. Institutional ethics committee permission was taken
85 prior to start of the work. Informed consent was taken from all participants before inclusion
86 in the study in their convenient language as applicable

87 Fasting blood was collected by venipuncture for the determination of different biochemical
88 parameters. The blood samples were subjected to centrifugation at 3,000 rpm for 10 min for
89 separation of serum. Serum thus obtained was analysed for biochemical parameters Serum
90 fT₄ and TSH level were measured by ELISA with kit provided by Accubind-Monobind with
91 help of TECAN ELISA(Austria based company) reader and washer. Within assay precision
92 of fT₄, TSH was determined by analysis control materials.

93 IMA estimation (30-33) is based on the premise that hypothyroidism due to oxidative stress
94 causes changes in human serum albumin (HSA) that are demonstrated by reduced exogenous
95 cobalt binding. The concentration of ischemia modified serum albumin can be determined by
96 addition of a known amount of cobalt (II) to a serum specimen and measurement of the
97 unbound cobalt (II) by colorimetric assay using dithiothreitol (DTT). An inverse relationship
98 thus exists between the level of albumin bound cobalt and the intensity of the colour
99 formation. Preparations for the Co (II) albumin binding protocol involved the addition of 200
100 µl of patient serum to 50 µl of a solution of 1gm/l cobalt chloride, followed by vigorous
101 mixing and 10-min incubation. Dithiothreitol (50 µl of a 1.5 g/l solution) was then added and
102 mixed. After 2 min. incubation, 1.0 ml of a 9.0 g/l solution of NaCl was added. The
103 absorbance of the assay mixture was read at 470 nm using a Spectrophotometer. The blank

104 was prepared similarly with the exclusion of DTT. IMA assay was standardized in the
105 Department of Biochemistry R. G. Kar Medical College and a standard curve was prepared in
106 the range 6.0-60.0 $\mu\text{g CoCl}_2/\text{ml}$. One IMA unit was defined as “ μg of free Co (II) in the
107 reaction mixture per ml of serum sample”. The assay was found to maintain linearity within
108 this range. The calculated factor from this standard curve is 411.8.

109 So,

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

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

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

120

121 Estimation of MDA in samples:

122 To 0.5 ml of freshly prepared serum 2.5 ml of trichloroacetic acid (20mg/dl) was added and
123 the tube was allowed to stand for 10 minutes at room temperature. Then 2.5 ml. of sulphuric
124 acid {0.05 M} was added and stirred thoroughly. Then 3.5 ml. of TBA reagent (200mg of
125 TBA was mixed with 100ml of 2 M sodium sulphate solution) was added to this. The

126 coupling of lipid peroxide with TBA was carried out by heating in boiling water bath for 30
127 minutes. It was then cooled in water. 4.0 ml of n-butanol was added and chromogen was
128 extracted to organic phase by vigorous shaking and vortexing followed by centrifugation at
129 3000 rpm for 10 minutes.

130 The supernatant organic phase was pipetted to a clean test tube. Its absorbance was
131 determined at 532nm wave length by spectrophotometer. N-Butanol was used as blank to
132 assure zero reading. The optical density was noted and level of MDA was calculated from
133 standard curve in the range of 5-15 nmol/ml. The calculated factor from the standard curve is
134 = 60.

135 Hence,

136 Concentration of MDA of a unknown solution is = Absorbance of unknown $\times \frac{60}{\text{Absorbance of standard}} \times 2 \text{ nmol/ml}$

137

138 **Results:**

139 Serum TSH ($18.3 \pm 8.1 \text{ mIU /ml}$) was significantly raised among case group in comparison to
140 control ($5 \pm 0.87 \text{ mIU /ml}$). IMA ($0.51 \pm 0.14 \text{ } \mu\text{g/ml}$) and MDA ($9.27 \pm 1.27 \text{ nmol/ml}$) also
141 significantly increased among case group in respect to control. Serum fT4 was significantly
142 decreased among case group (vide table 1). In control group Pearson's bivariate correlation
143 shows that serum IMA & MDA though correlated with fT4 (-ve) & TSH (+ve) but the level
144 is not statistically significant in controls. In the case group it was found that serum IMA &
145 MDA are significantly correlated with fT4 ($r = -0.835$ & -0.765) & TSH ($r = +0.859$ &
146 $+0.672$) (vide table 2).

147

148 **Discussion:**

Oxidative stress represents an imbalance between the production of oxidants and their elimination by antioxidative systems in the body. In this study, serum IMA and malondialdehyde had been determined 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.

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

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

Results revealed an increased generation of reactive oxygen species and impairment of the antioxidant system in patients with hypothyroidism.

Conclusion: Malondialdehyde and IMA concentrations increased in patients with hypothyroidism and also correlated with the severity of the disease as reflected by the levels of fT4 and TSH. These findings indicate that thyroid hormones have a strong impact on oxidative stress and the antioxidant system.

Tables:

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

Biochemical parameter	Case (mean±SD)	Control (mean±SD)	't' value	'p' value
fT4 (ng/dl)	0.93 ±0.58	1.6312 ± 0.15	-7.750	<0.001
TSH (m IU/ml)	18.31 ± 8.1	5.0 ± 0.87	10.71	<0.001
IMA (µg/ml)	0.51 ± 0.14	0.42± 0.047	4.14	<0.001
MDA (nmol/ml)	9.27± 1.27	4.6±1.09	19.17	<0.001

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

Thyroid function tests		Control IMA	Case IMA	Control MDA	Case MDA
fT4	Pearson Correlation	-.269**	-.835**	-.225**	-.765**
	Sig. (2-tailed)	>0.05	<0.001	>0.05	<0.001
TSH		.296**	.859**	.241**	.672**

	Sig. (2-tailed)	>0.05	<0.001	>0.05	<0.001
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189 IMA and MDA levels were significantly correlated with fT4 and TSH only among case group

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