

## THYROID INDUCED OXIDATIVE STRESS: IMPLICATION IN THERAPY

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**Abstract:** *Thyroid hormone is essential for growth, development and regulation of energy metabolism. Hyperthyroidism increases basal metabolic rate due to higher consumption of oxygen leading to enhanced Reactive oxygen Species ( ROS) generation. Oxidative modification of biomolecules and oxidative insult to mitochondria, due to enhanced ROS, has been implicated as a major causal factor in the thyroid hormone associated differential response of patients to therapy. Flow cytometric analysis of ROS levels in Peripheral blood mononuclear cell (PBMC) revealed two sub-groups of patients in hyperthyroids, one with a 3 fold increase in the oxidative stress levels as compared to euthyroids and the other where a 1.7 fold increase in the oxidative stress was noted. However, a general increase in macromolecular damage was observed in all hyperthyroid irrespective of the differences in oxidative state. The macromolecular damage and mitochondrial status was similar in hypothyroid and euthyroid subjects. These observations suggest that a critical analysis of differences in the levels of oxidative stress, experienced by patients might contribute in the future design of therapies for effective clinical management of thyroid dysfunction*

**Key words:** Hyperthyroid, Oxidative stress

### INTRODUCTION

Hypersecretion of thyroid hormone has been shown to increase the production of reactive oxygen species (ROS). Our earlier work [1] has demonstrated a three fold increase in reactive oxygen species generation in peripheral blood mononuclear cells of hyperthyroid patients as compared to euthyroids. Increased production of ROS in hyperthyroid is suggested to be due to enhancement in the oxidative phosphorylation (OXPHOS) as shown by thyroidectomy in the rat model [2] On the other hand, in hypothyroid rats administration of thyroid hormone increases oxygen consumption and oxidative phosphorylation, measured in isolated liver mitochondria. Thyroid hormone up- regulates the expression of selected nuclear genes that encode OXPHOS complex subunits [3]. Mitochondria from

thyroxine treated rats incubated with succinate as substrate shows an increase in the respiration rate and membrane potential suggesting thyroxine induced upregulation of mitochondrial function [4]. In addition, T<sub>3</sub> is considered as a major regulator of mitochondrial biogenesis [5] suggesting that the hormone acts on both nuclear and mitochondrial genes expression.

Mitochondrial H<sup>+</sup> leak can decrease superoxide generation while mitochondrially-generated superoxide can increase H<sup>+</sup> leak. A feed back loop exists between ROS and H<sup>+</sup> leak [6]. A proton leak across the inner membrane is suggested to be involved in the generation of ROS. The proton leak represents about 20% of the multifactorial control of mitochondrial respiration and convergent data show that it is increased by thyroid hormone by several mechanisms [7]. Further, thyroid hormone has been

shown to alter the phospholipid composition [8-9] leading to increased permeability to protons. More recently, a substantial correlation between UCP3 mRNA levels [10] and thyroid state has been demonstrated, suggesting the role of UCPs expression in  $T_3$  regulation of the proton leak. Another well established influence of thyroid hormone concerns the stimulation of mitochondriogenesis, considered as a long term influence. Mitochondriogenesis is the result of numerous events leading to membrane phospholipid synthesis and assembly, DNA replication and stimulation of the expression of the mitochondrial genome and of nuclear genes encoding mitochondrial proteins.

Thyroid hormone induced stimulation of cardiolipin synthase activity has been shown to be due to a rise in the mitochondrial phosphatidylglycerol [11], thereby increasing the amount of cardiolipin. As cardiolipin stimulates several mitochondrial carriers and enzyme activities [12-13] this event would contribute to the delayed hormone influence on mitochondrial respiration. However, altered mitochondrial respiration in thyroid patients is expected to vary depending upon the extent and duration of exposure of thyroid hormone which may in turn affect the response of patients to drugs used in the management of thyroid disorders despite having similar biochemical levels of  $fT_3$  and TSH in serum. Therefore, it has been hypothesized that contribution from non genomic factors that include alterations in metabolic state due to thyroid hormone secretion and other environmental factors may account for differential response of patients to the management of disease. Present studies have been carried out to investigate the impact of higher levels of circulating thyroid hormone in patients with Grave's disease, on oxidative damage and its correlation with the clinical symptoms in these patients that could form the basis of designing combined therapies using antioxidant.

## MATERIALS AND METHODS

**Selection of subjects:** A total of 114 non-smoking patients (within 40 years of age) visiting thyroid clinic at the Institute of Nuclear Medicine & Allied Sciences, Delhi, were included in the study. Complete clinical profile of the patients was recorded and patients with any other illness or under any medication were excluded. The subjects were grouped as euthyroid (n=38), hypothyroid (n=38) and hyperthyroid (n=38) based on thyroid function tests

carried out by measuring  $fT_4$  and TSH by using electrochemiluminescence method (based on the use of ruthenium(II) –tris (bipyridyl)  $[Ru(bpy)_3^{2+}]$  complex and tripropylamine}.

### Isolation of peripheral blood mononuclear cells:

Blood from subjects was collected in sterile vacutainer coated with EDTA as anticoagulant (Beckton Dickinson, New Jersey, USA). Two ml of Histopaque (containing polysucrose and sodium diatrizoate) was layered with 2 ml of whole blood and centrifuged at 400xg for 10 min. After centrifugation, the interface containing mononuclear cells at the interface between the histopaque and the plasma. The interface was collected and washed thrice in phosphate buffered saline (PBS). Isolated peripheral blood mononuclear cells (PBMC) was resuspended in PBS for flow cytometric analysis.

### Measurement of reactive oxygen species:

Generation of reactive oxygen species was measured by using a dye 2', 7' dichlorofluorescein diacetate (DCFDA) [14-16]. The isolated PBMC were washed with PBS, resuspended to a concentration of  $1 \times 10^6$  cells/ml. and incubated with 10mg/ml of freshly prepared DCFDA for 30 minutes in dark at 37 °C. Cells were then washed, resuspended in PBS and kept on ice for an immediate flow cytometric analysis. Green fluorescence due to intracellularly trapped DCF was collected on FL1 Channel on a log scale on a FACS caliber using CELL Quest software provided by Beckton Dickinson, USA. A minimum of 10,000 cells per sample were analyzed.

### Mitochondrial membrane potential:

Mitochondrial membrane potential was analysed using the potentiometric dye Rhodamine 123 as described by [15] The PBMC ( $1 \times 10^6$  cells/ml) was resuspended in PBS and incubated with Rhodamine 123 (dissolved in ethanol) for 30 min in dark at 37 °C and flow cytometric analysis was carried out as described earlier.

**Mitochondrial mass:** The fluorescent dye 10-n-Nonyl-Acridine Orange (NAO) which binds specifically to cardiolipin at the inner mitochondrial membrane independently of membrane potential was used to monitor the mitochondrial mass [17]. To the PBMC ( $1 \times 10^6$  cells/ml) suspended in PBS, 10  $\mu$ M NAO was added and incubated for 10 min at 25°C in the dark and then transferred immediately to tubes on ice for flow- cytometric analysis.

**Assessment of mitochondrial status:** The mitochondrial status of the mononuclear cells was analyzed by colorimetric assay involving the conversion to formazan that occurs in active mitochondria in living cells. MTT was dissolved in PBS at a concentration of 5 mg/ml and filtered through 0.22 $\mu$  filter to remove small amounts of insoluble MTT. The equal number of mononuclear cells was seeded in each well. The cells were incubated with 20 ml MTT and after 2 hour incubation, the cells were lysed with 150ml DMSO and the absorbance was read at 570 with 630 as reference wave length [18].

**Lipid peroxidation:** Lipid peroxidation was measured by using TBA method as described earlier [19]. To isolated mononuclear cells suspension, 500 $\mu$ l each of TBA, potassium chloride, trichloroacetic acid and tris-HCl (52mM TBA, 0.15M KCl, 30% TCA, 10 mM Tris-HCl) was added and heated to 80 °C for 20 Min. After cooling, the precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of supernatant was taken at 532 nm.

**Protein oxidation:** Protein oxidation leads to the formation of carbonyl groups. 2, 4 dinitrophenylhydrazine (DNPH) is converted into 2, 4 dinitrophenylhydrazone by reaction with these carbonyl groups. 20  $\mu$ l of serum was precipitated with 10% trichloroacetic acid on ice. The precipitates were incubated with 10 mM DNPH in 2N HCl for 1 hr at room temperature with vortexing every 10-15 min intervals and precipitated again with 10% trichloroacetic acid. The resulting precipitates were washed three times with an ethanol-ethyl acetate mixture (1:1) (v/v) to remove excess DNPH. The final protein pellet was dissolved in 8 M urea and the absorbance at 370 nm was measured [20].

**DNA damage by comet assay:** Assessment of DNA damage was carried out by the neutral comet assay, which predominantly detects DNA strand breaks in individual cells [21-22]. Briefly, microscopic slides were precoated with 0.1% agarose by drying on a hot plate at 45 °C. About 10  $\mu$ l of whole blood was mixed with 500  $\mu$ l pre-warmed 0.75% ultra-low gelling agarose and layered on the precoated microscopic slides, and the slides were kept at 4°C to allow formation of agarose gel. The lysis was carried out by submerging the slides in lysis buffer (2.5% SDS, 1% sodium sarcosine, 25 mM EDTA, pH 9.5) for 15 min at 25-30° C. Slides were rinsed gently for 5 min in distilled water at 10 °C and

electrophoresis (90 mM Tris Base, 90 mM boric acid, 2.5 mM EDTA; pH8.3) was carried out at 2v/cm for 5 min. After electrophoresis the slides were briefly rinsed in distilled water, air dried at 45 °C on a hot plate and stored in a cool humid box. After rehydration of slides in distilled water for 5 min, slides were stained with propidium iodide (50 $\mu$ M in PBS) and observed under fluorescence microscope (Olympus BX60). Images were acquired by Grundig FA8 monochrome CCD camera (Grundig, Germany) using the Optimas image analysis software (Optimas USA: version 5.2). Analysis of DNA distribution in the comets was carried out using The Optimas Comet assay module (version 2.0).

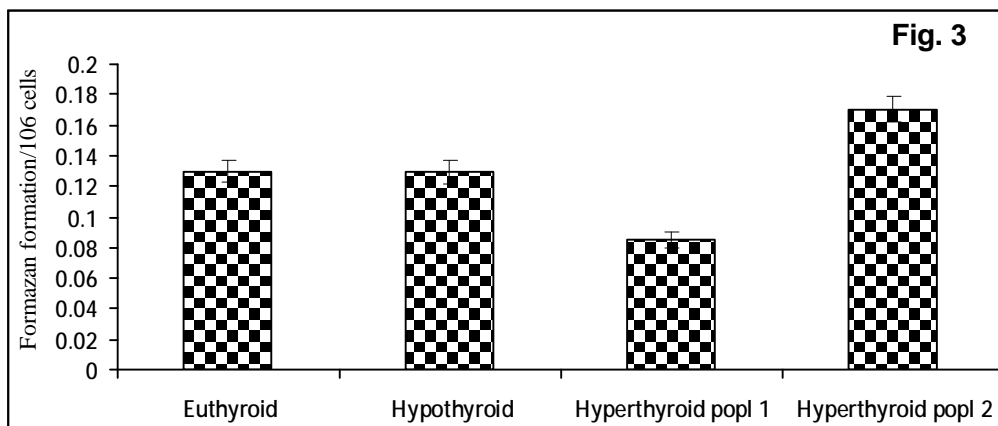
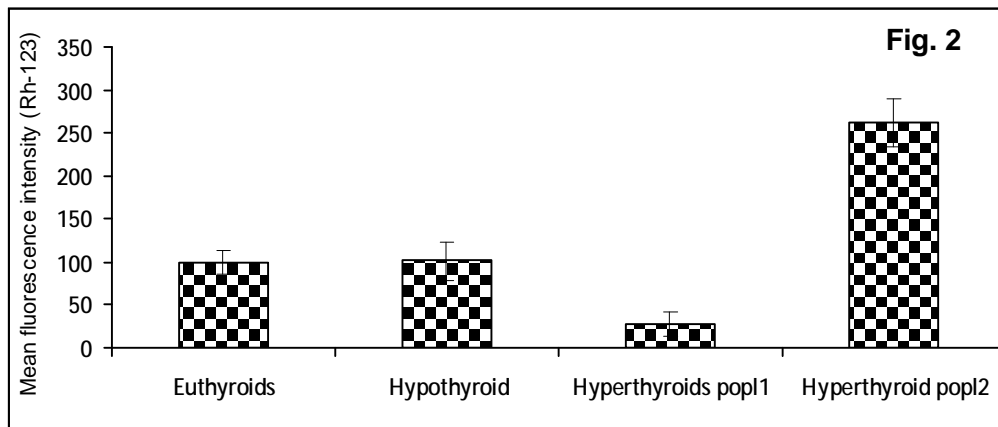
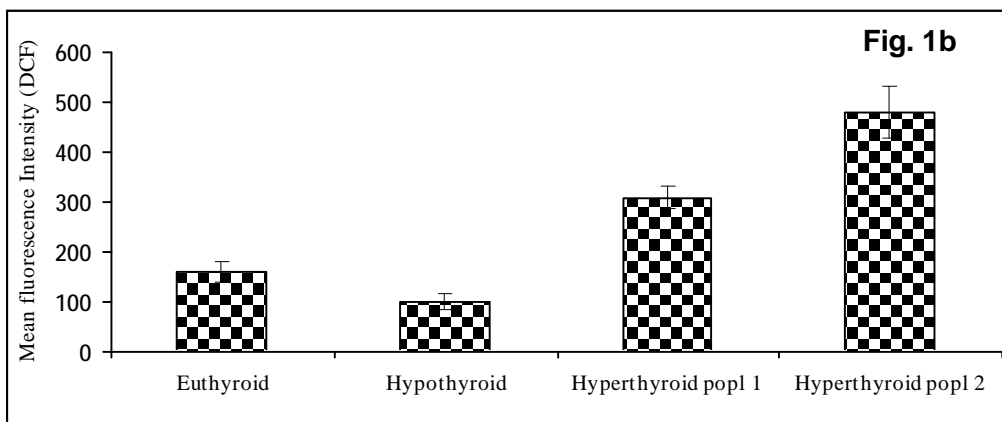
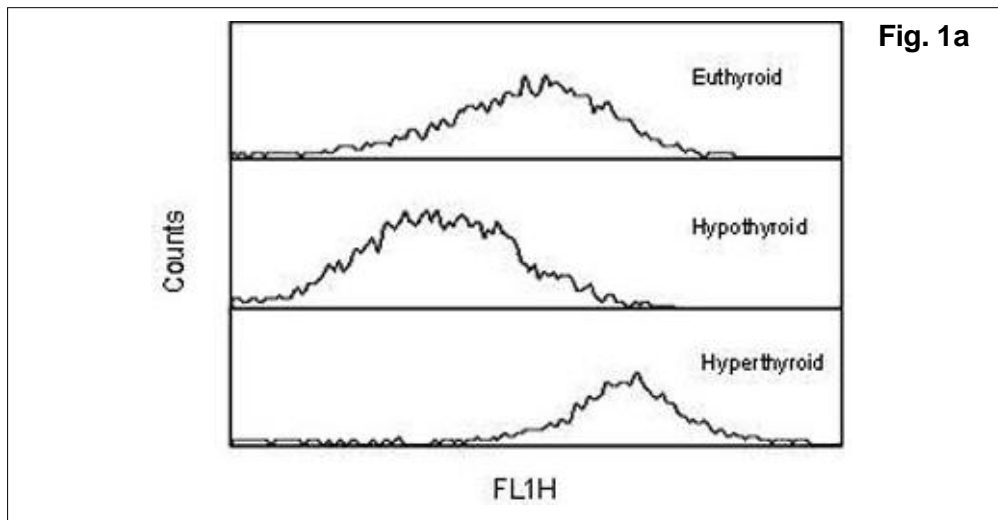
**Statistical Analysis:** Statistical analysis was carried out using the SPSS package. The data obtained are expressed as mean values  $\pm$  SD. One way ANOVA was used to determine whether the differences observed between the means were significant, with  $p < 0.05$  taken as the level of significance.

## RESULTS

**Hormonal levels:** The  $fT_4$  (pmol/l) levels of euthyroids, hypothyroids and hyperthyroids were  $16.6 \pm 2$ ;  $9.4 \pm 5.6$  and  $72.4 \pm 20.2$  respectively. The TSH ( $\mu$ U/ml) levels were  $2 \pm 0.8$  in euthyroids,  $72.9 \pm 55.1$  in hypothyroids and  $0.02 \pm 0.015$  in hyperthyroids.

**ROS levels in PBMC of thyroid patients:** Our earlier studies with limited number of subjects had shown that the ROS levels in the PBMC of hyperthyroid patients are significantly higher than in euthyroid and hypothyroids [1]. Interestingly, a critical analysis from a larger population of hyperthyroid subjects revealed that there are two subpopulations of hyperthyroid patients experiencing different levels of oxidative stress as indicated by the mean fluorescence intensity (MFI) values of DCF fluorescence (Fig.1b). Nearly 75% of hyperthyroid patients showed a 1.7 fold increase in the level of oxidative stress (MFI value of  $300 \pm 20$ ) while 25% patients had a very significantly higher level (3 fold) of oxidative stress (MFI value of  $470 \pm 50$ ) as compared to the levels in hypothyroids (MFI value of  $103 \pm 21$ ) and euthyroids (MFI value of  $145 \pm 40$ ).

**Effect of thyroid hormone on mitochondria:** Since, mitochondria are the major intracellular source and primary site for the generation and action of ROS



, the mitochondrial membrane potential, mass and functional status were assessed to determine the effects of increased ROS generation on mitochondria observed in the hyperthyroid patients.

**Mitochondrial membrane potential:** Mitochondrial membrane potential (MMP) was measured with the help of potentiometric dye Rhodamine-123 that selectively accumulate in mitochondria whose membrane potential is maintained. Disruption in the membrane potential is indicated by a decrease in the Rhodamine- 123 uptake by the cells and hence a shift of the histogram to the left or decrease in the mean fluorescence intensity.

In 75% of the hyperthyroid patient, the mitochondrial membrane potential was significantly lower (MFI  $31.5 \pm 6.2$ ) as compared to hypothyroid (MFI  $88 \pm 6.7$ ) patients and euthyroids (MFI  $88 \pm 8$ ) subjects where the membrane potential was essentially similar (Fig.2). Interestingly in the remaining 25 % of hyperthyroid patients, a significant increase was observed in the mean fluorescence intensity ( $240 \pm 46$ ) suggesting an increase in the number of mitochondria as compared to the other two groups.

**Functional status of mitochondria:** MTT reduction by succinate dehydrogenase, a component of the complex II of the respiratory chain, was used as an indicator of mitochondrial function [23]. In the group of hyperthyroid patients (75%) where a decrease in membrane potential was observed, the reduction of MTT to formazan was nearly 30% lower in comparison to the euthyroids and hypothyroid patients, which is correlated well with the decrease in the mitochondrial membrane potential observed in this group. In remaining 25%, the reduction of formazan

was found to be significantly higher which further indicated towards the increase in number of functional mitochondria in these groups (Fig. 3).

**Mitochondrial mass:** Mitochondria contain cardiolipin, a phospholipid located exclusively in the inner membrane, particularly in the intermembrane contact sites. Mitochondrial mass was assessed using the fluorescent dye 10-N- nonyl acridine orange (NAO), which displays specificity for cardiolipin.

The MFI values derived from NAO fluorescence was nearly identical in euthyroids and in hypothyroids, while it was three to four times higher in sub population of hyperthyroid experiencing higher degree of oxidative stress suggestive of ROS activated mitochondrial biogenesis in these patients (Fig. 4).

**Effect of thyroid hormone on the macromolecular damage:** Biomolecules such as lipids, proteins and DNA are well known targets of ROS resulting in a variety of damages that is primarily responsible for the pathophysiology of many diseases. Since, a significant increase in ROS generation that was observed in the PBMC from hyperthyroid patients, the induction of macromolecular damage was investigated.

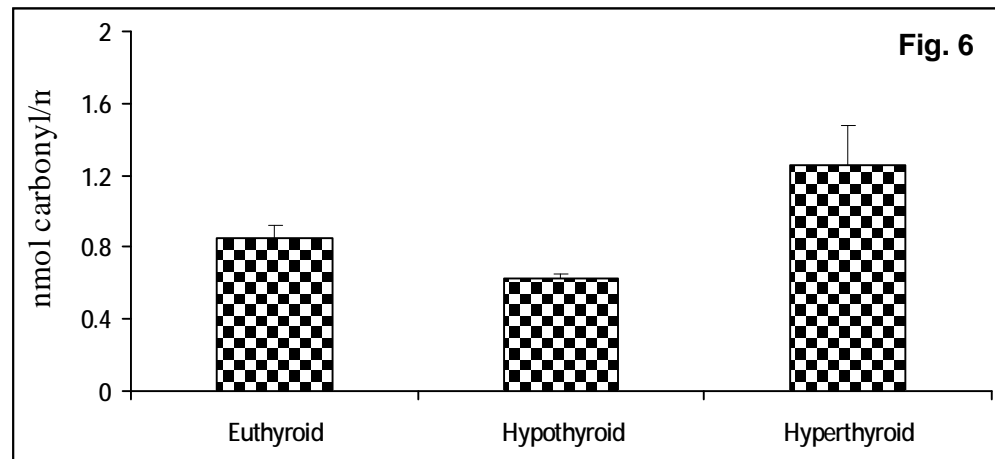
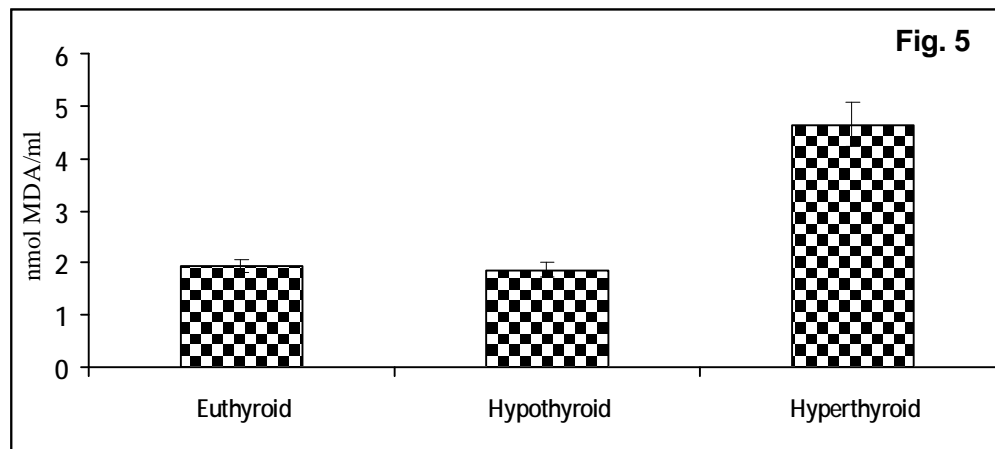
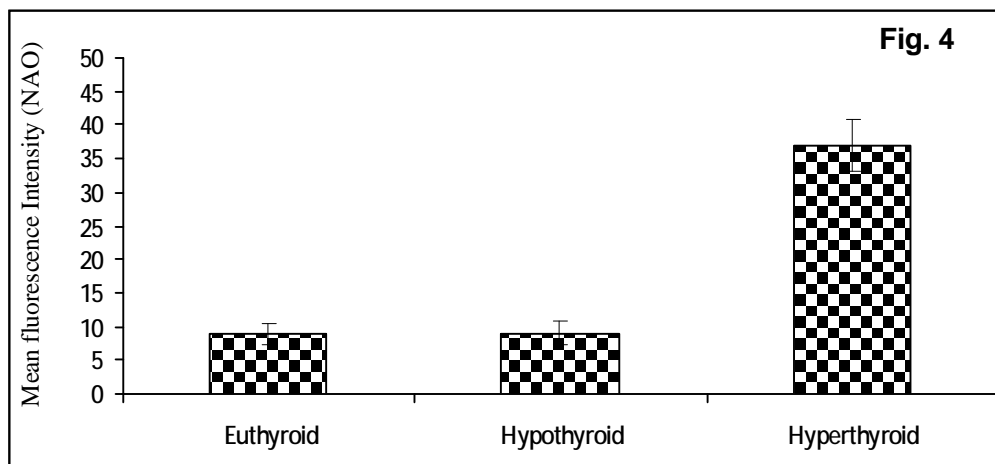
**Lipid peroxidation:** ROS damages lipids mainly by causing its oxidation. Malondialdehyde (MDA) formed was used as a measure of lipid peroxidation and was measured in erythrocytes. The MDA levels (nmol/ml) in hyperthyroid group of patient's ( $4.95 \pm 1.95$ ) were significantly higher ( $p < 0.005$ ) than the euthyroid ( $1.8 \pm 0.8$ ) and hypothyroid group ( $1.5 \pm 0.3$ ) suggesting possible alterations in the membrane of various organelle and plasma membrane (Fig. 5.)

## Explanation of figures:

**Fig. 1:** Intracellular ROS levels in PBMC of euthyroid, hypothyroid and hyperthyroid patients. A: Typical frequency distribution of DCF fluorescence (reflecting intracellular ROS levels) in PBMC of euthyroid. B: Average values of mean DCF fluorescence intensity (ROS levels) in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients. Data are mean values of measurements made in 38 nos of subjects in each group. The difference in the means between euthyroid and hyperthyroid and between hypothyroid and hyperthyroid is significant ( $p < 0.001$ ).

**Fig. 2:** Relative mitochondrial membrane potential (MFI of Rh123 fluorescence) measured using the potentiometric dye Rh123 in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients. Data are mean values of measurements made in 38 nos of subjects in each group.

**Fig. 3:** MTT reduction leading to formazan formation in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients. The OD of formazan formed is presented. Data are mean values of measurements made in 38 nos of subjects in each group.



### Explanation of figures:

**Fig. 4:** Levels of unoxidized mitochondrial cardiolipin in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients measured by MDA formation. Data are mean values of measurements made in 38 nos of subjects in each group.

**Fig. 5:** Lipid peroxidation levels in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients measured by MDA formation. Data are mean values of measurements made in 38 nos of subjects in each group. The difference in the means between euthyroid and hyperthyroid and between hypothyroid and hyperthyroid is significant ( $p < 0.001$ ).

**Fig. 6:** levels of oxidized proteins in PBMC of euthyroid, hypothyroid and two sub-populations of hyperthyroid patients measured by carbonyl formation. Data are mean values of measurements made in 38 nos of subjects in each group. The difference in the means between euthyroid and hyperthyroid and between hypothyroid and hyperthyroid is significant ( $p < 0.005$ ).



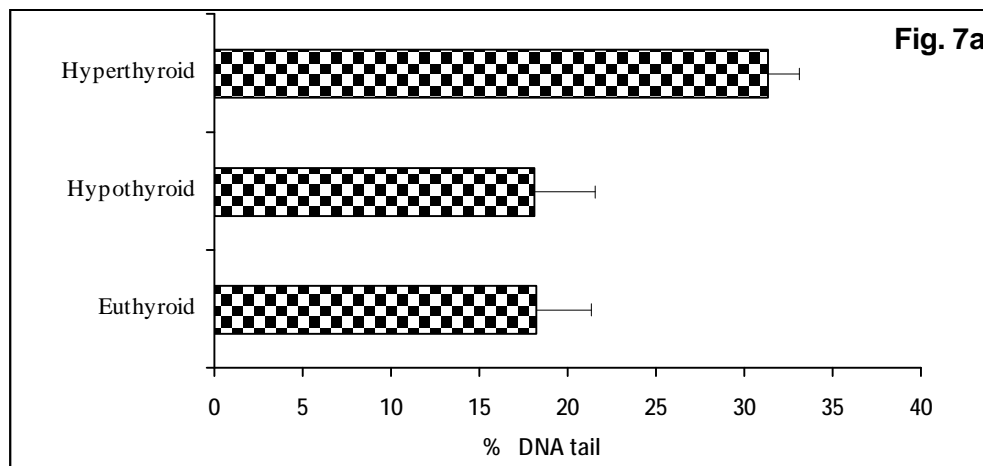
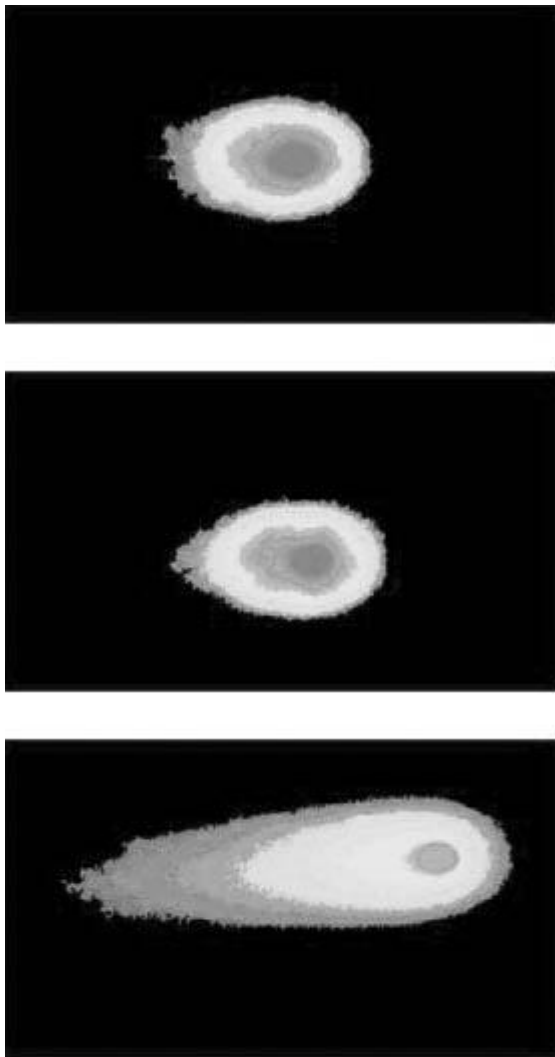


Fig. 7a

**Fig. 7a:** Representative comets showing DNA damage studied by alkaline single cell gel electrophoresis in nucleated cells from the whole blood of euthyroids, hypothyroid and hyperthyroid patients (a)



**Fig. 7b:** Percent tail DNA in each euthyroids, hypothyroid and hyperthyroids subjects (n=38 each). The difference in the means between euthyroid, hyperthyroid, and between hypothyroid and hyperthyroid is significant ( $p < 0.005$ )

**DNA damage:** Since, DNA is one of the critical targets of ROS, DNA plays a central role in the information transfer between generations of somatic cells, and the assessment of DNA damage in PBMC was of particular importance in these three groups with different ROS status. Neutral version of the single gel electrophoresis assay (comet assay) which predominantly detects the double strand breaks. Representative comets from euthyroid, hypothyroid and hyperthyroid subjects are shown in fig 7a, b. The percentage of DNA in the comet tail of the hyperthyroid was found to be significantly higher ( $31 \pm 2.6$ ,  $p < 0.005$ ) as compared to the euthyroid ( $19 \pm 4$ ) and hypothyroids ( $19 \pm 4.2$ ) suggesting a higher level of damage in the cells from this group of subjects.

## DISCUSSION

Oxidative stress induced by a number of intracellular factors like metabolic disturbances and environmental factors has been shown to be responsible for many disease processes. The clinical manifestation results from functional disturbances arising on account of structural damage mainly in the form of damage to vital biomolecules like protein, DNA and lipids. Since the Oxidative stress manifests in several ways and oxidative damage to biomolecules vary under different conditions, insight gained in to the relationship between oxidative stress and clinical symptoms would go a long way in designing better therapies.

**Protein oxidation:** The protein carbonyls (nmol/ml) estimated in the serum samples of hyperthyroid patients ( $1.210 \pm 0.2$ ) were marginally higher than in euthyroids ( $0.8 \pm 0.08$ ) (Fig. 6). However, protein carbonyls estimated in the euthyroid was not found to be significantly different from the hypothyroids.

Significantly higher level of ROS observed in peripheral blood mononuclear cells of hyperthyroid compared to euthyroid or hypothyroids, lends support to the proposition that metabolic alteration in thyroid dysfunction especially in case of hyperthyroidism results in oxidative stress condition that may be manifested in the form of clinical symptoms experienced by the patients. The range of ROS levels was broader in hyperthyroids although the range of hormonal levels was narrow indicating that these two sets of patient population experience different levels of oxidative stress under similar hormonal conditions. Therefore, understanding the molecular and functional alterations caused by thyroid hormone deregulation will help in designing better therapeutic strategies among individuals for the management of the disease.

In the two sub-sets of hyperthyroid population experiencing different level of oxidative stress (Fig. 1), it is expected that mitochondrial status will be compromised, coupled with varying degree of symptomatic manifestations. From the clinical view point, it will be interesting to see whether these sub population of patients with high and low ROS levels would respond differently to anti-thyroid treatment. Mitochondria are a significant cellular source of ROS through mitochondrial respiration, which is carried out by sequentially-coordinated redox reactions and because the respiratory chain complexes are proton pumps, this property is inherently governed by the trans-membrane proton gradient and membrane potential. The decrease in the membrane potential observed in 75% of hyperthyroids may be due to the damage in the permeability transition pore complex (PTPC) causing the permeability transition pore to remain open for relatively longer period of time, resulting in a greater disruption of the proton gradient and consequently, a decrease in the membrane potential observed. The decrease in mitochondrial membrane potential with an increase in oxidant levels might also be associated with reduced mitochondrial function. Results of MTT assay clearly suggested a decrease in functional mitochondria in 75% of hyperthyroid patients, which might be the consequence of ROS induced damage to the mitochondria. Rottenberg and Shaolong have documented similar findings in the lymphocytes of aged mice where increase in the level of oxidants facilitated the opening of PTPC with a consequent decrease in membrane potential [24]. However, increase in membrane potential in rest 25% of the hyperthyroid population (Fig. 2) could be attributed

to the stimulatory action of thyroxine on mitochondrial biogenesis [5,25] which not only increases the number of functional mitochondria per cell, but also induces structural (increase in cardiolipin content located exclusively in the inner membrane of the mitochondria) and functional changes in mitochondria designed to enhance their performance [13]. Measurement of unoxidized cardiolipin content using NAO reflecting the mitochondrial mass revealed that the values in hyperthyroid patients were very broadly distributed (higher SD values: Fig. 4) as compared to euthyroid and hypothyroid, although distinct population as seen in case of mitochondrial membrane potential (Rh-123; Fig. 2) was not evident. Increase in mitochondrial mass might be a compensatory mechanism in patients where mitochondrial function is compromised. In other group of patients increase in mitochondrial mass is consistent with the increased MMP and functional status. Studies in animal model [12,13] had suggested hypothyroidism to be associated with mitochondrial dysfunction and cardiolipin deficiency and this mitochondrial cardiolipin deficiency was correlated with the decrease of metabolite transport activity across the mitochondrial inner membrane. Interestingly, in this study mitochondrial dysfunction and cardiolipin deficiency was not observed in hypothyroids and moreover, no difference was observed in the mitochondrial mass between the euthyroids and hypothyroids. These results suggest that variation in ROS generation leading to alterations in mitochondrial structure and function elicited by thyroxine depends on either the length of its exposure or difference in life style and may have greater clinical importance.

In spite of large differences in the ROS level between the two groups of hyperthyroids, the extent of macromolecular damage form the basis of pathologic conditions were essentially similar in both the groups. The increase in MDA formation observed in the PBMC of hyperthyroids in comparison to the euthyroids and hypothyroids suggested that the hypermetabolic state in hyperthyroidism, results in enhanced lipid peroxidation. This result also correlated well with the increase in ROS generation observed in erythrocytes and PBMC of hyperthyroids (data not shown). Enhanced lipid peroxidation by hyperthyroids would lead to a multitude of structural and functional disturbances in mitochondrial structure, permeability and alterations in respiratory functions that may contribute to clinical status like muscular wasting, tachycardia, shortness of breath, less tidal



volume and oxidation of cholesterol leading to thickening of arteries etc.

Persistent production of large amounts of ROS may induce significant oxidation of proteins. Enhancement in protein oxidation, in addition to lipid peroxidation, by thyroid calorigenesis will lead to modification of various functional and structural proteins. Free-radical mediated lipid peroxidation and protein oxidation leading to protein degradation in skeletal muscle in animal model has been shown to be one of the prime mediators involved in the pathophysiology of hyperthyroidism and plays a pivotal role in subsequent tissue injury such as thyrotoxic myopathy and cardiomyopathy [26-28]. Lipid peroxidation and protein oxidation are suggested important mechanisms responsible for thyroid hormone-mediated tissue injury in Graves's disease [29].

Recent studies in rats have shown, breathlessness, which is a common complaint in patients with hyperthyroidism is resulted from oxidation of myofibrillar protein which may account for a loss of contractile regulation in diaphragm in hyperthyroidism [30]. Experimental and epidemiological evidences show that oxidation of LDL is markedly higher in hyperthyroidism than in hypothyroidism or control subjects and plays an important role in development of atherosclerosis [31], fluctuations in ROS may elicit regulation of protein function through reversible oxidation and/or nitrosation of protein sulfhydryls [32]. In addition, redox up regulation of gene expression as a secondary mechanism of ROS is also observed, through modulation of the activity of kinases, phosphatases, and redox-sensitive transcription factors [33-34]. However, the extent of these changes depends on the level of pro-oxidants, and the duration of exposure, which varies among different patients.

DNA damage can also result from the ROS generated directly through respiratory chain, as well as through the generation of secondary ROS such as lipid peroxidation products and in hyperthyroid patients oxidation of one macromolecule might augment the damage in other macromolecules through the generation of secondary / tertiary ROS production. Stimulation of both radical and non-radical species through metal catalyzed lipid peroxidation reactions has been shown to damage DNA [35]. Damage to the DNA elicits cellular responses by way of DNA repair, cell cycle perturbations and cell death through

multiple processes by inducing alterations in signal transduction pathways involved in all three responses [36-37]. Oxidative stress and DNA damage induce alterations in the expression of genes related to the antioxidants as well as many signaling pathways [37] that can cause alterations in cell function and therefore tissue damage. To what an extent the oxidative stress induced DNA damage and related genes changes contribute to the pathophysiological conditions of hyperthyroidism needs further investigation. However, it is pertinent to note that *in vitro* culture of PBMC from hyperthyroid patient undergo rapid apoptotic death as compared to PBMC from euthyroid and hypothyroid (data not shown).

Taken together, the results of the present studies clearly suggest that, the hypermetabolic state in hyperthyroidism is associated with tissue oxidative injury. This is associated with the changes in ROS levels in PBMC. The increased mitochondrial ROS generation appears to be side effect of the enhanced level of electron carriers, by which hyperthyroid tissues increase their metabolic capacity. This provides evidence that oxidative damage to the macromolecules can culminate in cell death and this aspect represents an important target of therapeutic intervention for the management of thyroid dysfunction.

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