



## ALTERATION OF THYROID HORMONE PROFILE AS A BIOMARKER OF CARCINOGENESIS

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### ABSTRACT

Serum is a good source to measure thyroid hormone profile from different cancer patients. Blood samples were collected from patients suffering from different types of cancer. Serum were prepared from collected blood samples and stored at -20°C. Thyroid profile (T<sub>3</sub>, T<sub>4</sub> & TSH) of different test serum samples were measured by ELISA with the help of kits obtained from UBI-MAGIWEL. Thyroid hormones (T<sub>3</sub> & T<sub>4</sub>) and their inducer TSH are mainly involved in regulating BMR. Their levels are fluctuating during normal and pathogenesis conditions and can also be correlated with the development of carcinogenesis. The mean sample T<sub>3</sub> value is 247.27 ng/dL, which is significantly higher than the control T<sub>3</sub> value (103.1 ng/dL). The mean sample T<sub>4</sub> value is 13.52 µg/dL, which is significantly higher than the control T<sub>4</sub> value (10.13 µg/dL). The mean sample TSH value is 5.94 µIU/mL, which is significantly higher than the control TSH value (2.21 µIU/mL). Thyroid hormone profiles are remarkably high than prescribed normal range in cancer patients as compared to control (normal healthy person) and its measurement might support the clinical estimation of cancer risk (biomarker).

**KEY WORDS:** Serum, Thyroid hormone profile, ELISA, Cancer.

### INTRODUCTION

In carcinogenesis cells get abnormally proliferated and leads to several changes which may include hormonal changes specially thyroid hormone profile which is involved in controlling BMR of the organism. High metabolic activities in cancer cells may be due to high thyroid hormone profile or changes in its receptor level or position which is a prerequisite for cancer initiation and development.

Down-regulation of GH signaling can block carcinogenesis in prostate cancer. As carcinogenesis progressed, prostate GHR mRNA and protein expression increased significantly (Wang *et al.*, 2000).

Elevated serum thyrotropin (thyroid-stimulating hormone [TSH]) concentrations are associated with an increased risk of differentiated thyroid cancers in patients with nodular goiter. There is a positive trend in correlation between nodule size and TSH levels in patients with PTMC (p = 0.066). TSH is not elevated in subjects with (PTMCs) papillary thyroid microcarcinoma, indicating that it is not likely involved in the de novo oncogenesis of these small cancers. However, TSH might rather play a role in the progression of preexisting PTMCs (Gerschpacher *et al.*, 2010).

In the absence of a wild-type allele, the mutation of one TR-β allele is sufficient for the mutant mice to spontaneously develop follicular thyroid carcinoma. These results provide, for the first time, in vivo evidence to suggest that the TR-β gene could function as a tumor suppressor gene (Kato *et al.*, 2004).

Non-thyroid illness can cause changes in thyroid function that have been described as low triiodothyronine (T<sub>3</sub>) and

low thyroxine (T<sub>4</sub>) states. Reduced peripheral conversion of T<sub>4</sub> to T<sub>3</sub> explains the low serum T<sub>3</sub> concentration. The explanation for the low serum T<sub>4</sub> level is multifactorial; whether free-thyroxine (FT<sub>4</sub>) level is normal or reduced remains controversial. Cytokines such as tumor necrosis factor α, which are produced by the immune system during severe illness, may inhibit thyroid function directly and be responsible for the changes in pituitary-thyroid function (Wong and Hershman, 1996).

An association is observed between autoimmune thyroid disease and cancer. Thyroid assessment is strongly recommended in patients with idiopathic inflammatory myopathy and clinical relapse (Selva-O' Callaghan and Rodondo-Benito, 2007).

The inhibition of release of gastrointestinal trophic hormones by analogue of somatostatin (SMS 201-995) does not reduce the number of tumors in the intestine of azoxymethane-treated rats. (Savage *et al.*, 1999).

The involvement of thyroid hormones in the development and differentiation of normal breast tissue has been established. Women with breast cancer have higher levels of anti-thyroid peroxidase antibodies. The protein expression pattern of thyroid hormone receptors in different human breast pathologies is determined and their possible relationship with cellular proliferation is evaluated. The substantial changes in the expression profile of thyroid hormone receptors are revealed suggesting a possible deregulation that could trigger breast cancer development (Zomora and Conde, 1986).

The malignancy of thyroid oncocytic tumors, or oncocytomas, is higher than that of follicular tumors. The role of thyroid-specific genes in oncocytic tumors and

papillary carcinomas is investigated. TSHR, TTF-1 and TRbeta1 levels are significantly lower in oncocyctic tumors than in papillary carcinomas, as a result of specific biological changes in oncocyctic tumors (Prunier *et al.*, 2004).

Kojic acid (KA) interrupts thyroid function, primarily by inhibiting iodine intake, consequently causing a decrease in serum T<sub>3</sub> and T<sub>4</sub>. Increased TSH from the pituitary gland in turn stimulates thyroid hyperplasia. (Fujimoto and Hiroshi, 2004).

Application of PTU before and during MNU-induced mammary gland carcinogenesis yielded in a marked decrease of the number and volume of tumors per animal, however, there was no effect of hypothyroid state in thyroidectomized rats as well as hyperthyroid state concerning the number and volume of tumors. Mammary tumors of in euthyroid group of MNU animals showed that there is no tumor, in which all of subtypes of retinoid and rexinoid receptors are expressed. A different pattern of expression of retinoid or rexinoid receptors has been found either in MNU-induced mammary carcinomas in both hypothyroid and hyperthyroid rat ( Macejova *et al.*, 2005). The suppression of testicular androgen inhibits bladder carcinogenesis and investigated that which phase of bladder carcinogenesis is inhibited by the hormonal change of the hypothalamus-pituitary-testicular axis induced by the depot form of the LH-RH agonist (Matsuki *et al.*, 2002).

cyclin D1, pituitary tumor transforming gene-1, cathepsin D and transforming growth factor alpha genes are over-expressed in human thyroid cancers. The signaling pathways mediated by thyrotropin peptide growth factors, transforming growth factor-beta, tumor necrosis factor-alpha and nuclear factor-kappa B are activated, whereas pathways mediated by peroxisome proliferation activated receptor gamma are repressed. Complex alterations of multiple signaling pathways contribute to thyroid carcinogenesis (Ying *et al.*, 2003).

A high prevalence of endocrine and metabolic disorders in young adult survivors of childhood acute lymphoblastic leukaemia (ALL) or non-Hodgkin lymphoma (NHL) is observed and treatment with bone marrow transplantation 1. after total body irradiation (BMT/TBI) is the most detrimental and many will develop GHD, hypothyroidism, 2. hypogonadism, insulin resistance and dyslipidaemia (Steffens *et al.*, 2008).

Triiodothyronine regulates proliferation and acting as stimulator or inhibitor. E2F4 and E2F5 in complexes with pocket proteins p107 or p130 stop cells in G1, repressing 4. transcription of genes important for cell cycle progression. p107 and p130 inhibits activity of cyclin/cdk2 complexes. Expression of all those proteins could be regulated by 5. triiodothyronine. T3 inhibits proliferation of HK2 lines and stimulates it in Caki lines. Those differences are result

of disturbed expression of TR, causing improper regulation of E2F4, E2F5, p107 and p130 in cancer cell (Poplawski *et al.*, 2006).

The effect of molecular iodine (I<sub>2</sub>), potassium iodide (KI) and a subclinical concentration of thyroxine (T<sub>4</sub>) on the induction and promotion of mammary cancer induced by N-methyl-N-nitrosourea is analysed and concluded that continuous I<sub>2</sub> treatment has a potent antineoplastic effect on the progression of mammary cancer and its effect may be related to a decrease in the oxidative cell environment (Solis *et al.*, 2006).

Present study is needed to establish a correlation between thyroid hormone profile level and incidence of carcinogenesis so that this can be used as a biomarker of cancer risks, early detection and its assessment to progression into different stages.

## MATERIALS AND METHODS

Collection of blood samples: The blood samples were collected from patients suffering from different types of cancer and named according to the type of cancer like G.B.= Gall bladder cancer samples, B.C.= Breast cancer samples, M.M.= Medistinal malignancy samples, R.C.= Rectum cancer samples, O.C.= Oesophagus cancer samples, E.S.= Ewing sarcoma samples, C.C.= Colon cancer samples, Ov.C.= Ovary cancer samples, M.C.= Myeloblast cancer samples, U.C.= Urinal cancer samples, E.C.= Endotracheum cancer samples, CML= Chronic myeloid leukemia samples, AML= Acute myeloid leukemia samples and patient number was given.

Preparation of serum from collected blood: 3 mL of collected blood sample was incubated for one hour at room temperature and the unclotted portion of the blood was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected into an eppendroff or microfuge tube and stored at -20<sup>0C</sup>. This supernatant (serum) was used for experiments. Control serum was prepared from the bold of normal healthy person.

Measurement of thyroid hormone profile of serum samples: Thyroid hormone profile of different test serum samples were measured by ELISA (Wim Gaastra, 1984).

Desired no. of coated wells were taken in the holder and 50 µL of standards, controls or serum samples were added.

100 µL of enzyme conjugate was added to each well and incubated at room temperature for 60 minutes in dark.

3. Incubation mixture was removed and the wells were rinsed 5 times with washing buffer solution (300 µL / well / rinse).

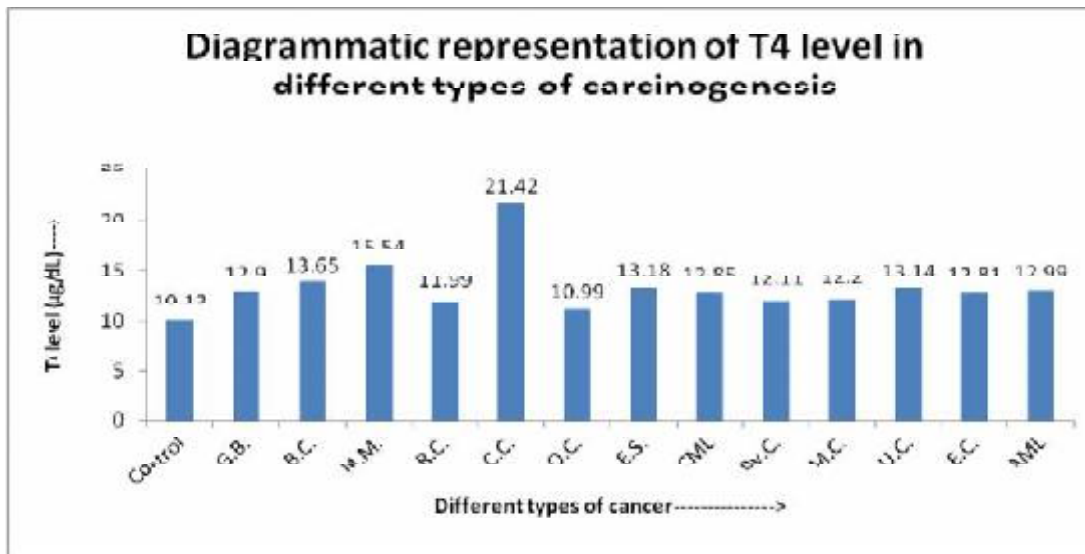
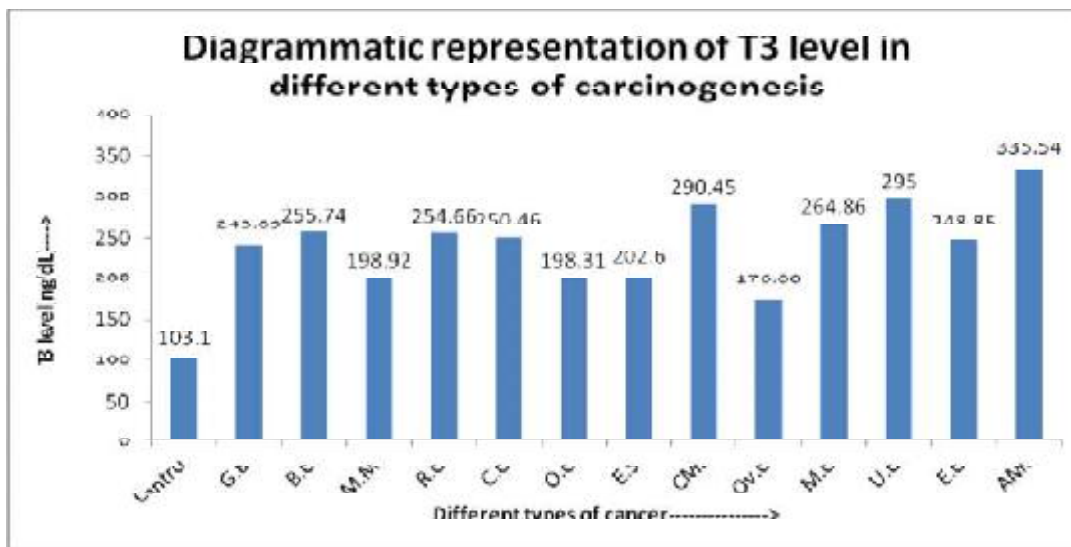
4. 100 µL of TMB solution was added to each well including blank and incubated at room temperature for 30 minutes in dark.

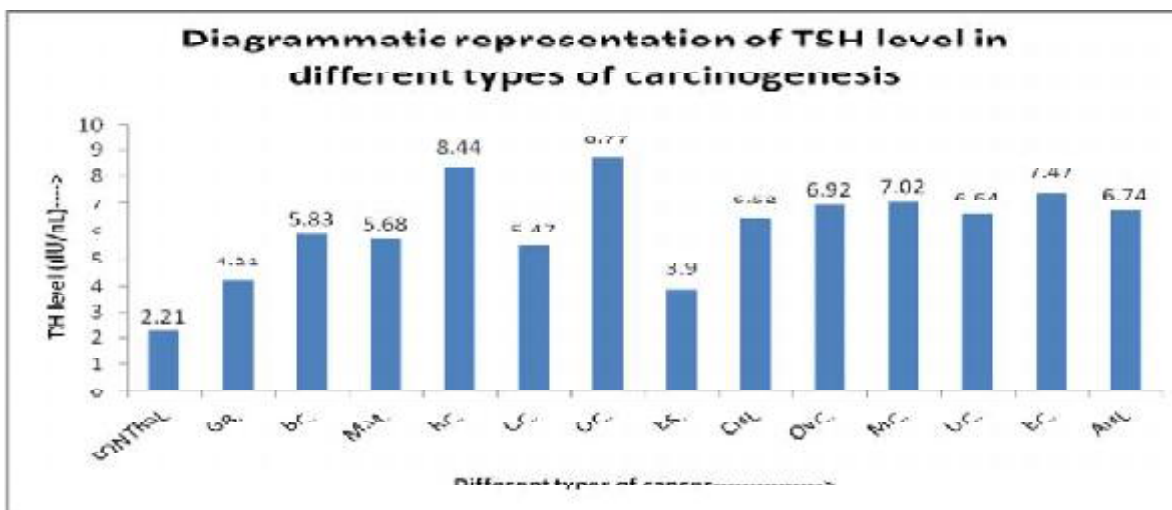
5. Reaction was stopped by adding 50 µL stop solution into each well and absorbance was measured at 450 nm with microwell (ELISA) reader within 10 minutes.

**RESULTS AND DISCUSSION**

The average values of thyroid hormone profiles measured from control and different test serum samples are tabulated as follows:

Serial Number	Test Sample	T3 (ng/dL)	T4 (µg/dL)	TSH (µIU/mL)
1	(Control) <sub>10</sub>	103.1	10.13	2.21
2	(G.B.) <sub>10</sub>	243.09	12.90	4.21
3	(B.C.) <sub>5</sub>	255.74	13.65	5.83
4	(M.M.) <sub>4</sub>	198.92	15.54	5.68
5	(R.C.) <sub>5</sub>	254.66	11.99	8.44
6	(C.C.) <sub>5</sub>	250.46	21.42	5.47
7	(O.C.) <sub>5</sub>	198.31	10.99	8.77
8	(E.S.) <sub>4</sub>	202.6	13.18	3.90
9	(CML) <sub>7</sub>	290.45	12.85	6.52
10	(Ov.C.) <sub>4</sub>	176.88	12.11	6.92
11	(M.C.) <sub>3</sub>	264.86	12.20	7.02
12	(U.C.) <sub>5</sub>	295.0	13.14	6.64
13	(E.C.) <sub>4</sub>	248.85	12.81	7.47
14	(AML) <sub>5</sub>	335.54	12.99	6.74





Normal range of  $T_3$  is 60-200 ng/dL,  $T_4$  is 4-12  $\mu$ g/dL and TSH is 0.6-5.5  $\mu$ IU/mL. But in the above mentioned table, the mean sample  $T_3$  value is 247.27 ng/dL, mean sample  $T_4$  value is 13.52  $\mu$ g/dL and the mean sample TSH value is 5.94  $\mu$ IU/mL. The mean sample  $T_3$ ,  $T_4$  and TSH values are higher than their control (normal healthy person) values ( $T_3$  = 103.1 ng/dL,  $T_4$  = 10.13  $\mu$ g/dL and TSH = 2.21  $\mu$ IU/mL).

t-test is performed to test, the statistical significance of the obtained data.

The above data indicate that there is a significant increase in either thyroid hormones ( $T_3/T_4$ ) or both thyroid hormones ( $T_3$  &  $T_4$ ) or thyroid stimulating hormone or both. In some cases, where  $T_3/T_4$  level is low the TSH level is high so that it can cause overproduction of  $T_3$  &  $T_4$  from thyroid gland. There may be some correlation with thyroid hormones and developmental stages of carcinogenesis. In the earlier stages the thyroid hormones level remain low but as the stages of carcinogenesis progress, first the level of TSH increases and it will cause the increased level of  $T_3$  &  $T_4$ .

Thyroid hormones are basically involved in regulation of basal metabolic rate (BMR). As the level of thyroid hormone increases, the BMR will also increase a pre-requisite for abnormal proliferation of cell (carcinogenesis).

Thyroid hormones are lipophilic i.e. they can cross the plasma membrane of the target cell and bind to the intracellular thyroid hormone receptors (TRs). The TRs are acting as transcription factors, which regulate cell growth and differentiation. TRs causes the proliferation of cancer cells by breaking G1 arrest, enhances DNA synthesis and increases invasiveness of cancer cells. It also causes the activation of AKT and MAPK signaling pathways and enhancement of cyclin D1 levels. The alteration of THR beta1 functioning is an important change in human well differentiated thyroid carcinomas, affecting tumor cell growth and contributing to the malignant potential of cancer cells (Sedliarou *et al.*, 2007). The location (cytoplasmic or nuclear) of the thyroid receptors (TRs) varies in normal and carcinogenesis conditions, which reveal substantial changes in the expression profile of thyroid hormone receptors

suggesting a possible deregulation that could trigger carcinogenesis development (Saraiva *et al.*, 2005).

In this study thyroid hormone ( $T_3/T_4$ /TSH) levels in patients are significantly higher than control. Thyroid hormones are lipophilic and can cross the plasma membrane of the cell and bind to cytosolic thyroid hormone receptor, which is acting as a transcription factor and can regulate the expression of different genes involved in different signaling pathways responsible for cellular proliferation. Persons with elevated thyroid hormone profiles are susceptible to carcinogenesis and its measurement might support the clinical estimation of cancer risk (biomarker). So, elevation of the  $T_3/T_4$ /TSH level may indicate the beginning of the carcinogenesis and its level can be correlated with stages of oncogenesis.

If the level of thyroid hormone profile can be lowered, then it might be proved useful in suppressing oncogenesis development (suppressive therapy). Oral thyroid hormone helps keep TSH levels low while maintaining normal metabolism. But in the advanced stages cancer has a lower chance of returning if thyroid stimulating hormone (TSH) levels are low.

#### ACKNOWLEDGEMENTS

Dr. Mritunjay Kumar, Medical Superintendent, Dr. (Mrs.) Chandana Banerjee, Centre Manager and Mr. Raj Kumar Gupta, Laboratory In-charge, Curie-Abdur Razzaque Ansari Cancer Institute, Irba, Ranchi, Jharkhand are highly acknowledged for providing blood samples of different types of cancer patients.

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