

## Thyroid and its Regulatory Hormonal Homeostasis in Current and the Former Smokers

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**Abstract.-** Effect of cigarette smoking on serum thyroid hormones, *i.e.*, thyroxine ( $T_4$ ) and tri-iodothyronine ( $T_3$ ) and their regulatory hormone, *i.e.*, thyrotropin (TSH) was evaluated in male current smokers ( $n=80$ ), ex-smokers ( $n=50$ ) and never smokers ( $n=50$ ), ages ranging 30-60 years. Current smokers were categorized as group I ( $n=40$ ) including short duration (1-5 years) and group II ( $n=40$ ) including long duration (6-30 years) smokers. Body mass index (BMI), blood pressure (BP) and heart rate (HR) were also recorded. Enzyme-linked-Immunesorbent Assay was used to estimate levels of  $T_4$ ,  $T_3$  and TSH.  $T_4$  varied non-significantly in comparable groups.  $T_3$  and TSH were found to be significantly elevated in current compared to never smokers. Elevation in TSH was more intensified in group II compared to group I. Ex-smokers indicated a significant rise in  $T_3$  when compared with never smokers. BMI exhibited a significant decline in smokers than never smokers but a highly significant elevation in former compared to current and never smokers. BP decreased significantly in current compared to never smokers. The trend persisted, although less intensified, in former than never smokers. HR, in current and ex-smokers, was significantly elevated as compared to never smokers. The study indicated significant variations in thyroid and their regulatory hormonal pattern in smokers and ex-smokers. BP, BMI and HR also presented significant alterations from the normal pattern. The results, specifically in ex-smokers, have suggested that smoking has long term effects on serum  $T_3$ , BP and HR, whereas, BMI has shown inverse relationship between current and the former smokers.

**Keywords:** Thyroxine, tri-iodothyronine, thyrotropin, smoking.

### INTRODUCTION

Smoking has a significant impact on thyroid function. Thiocyanate, a major component of smoke, derived from hydrogen cyanide leads to increased excretion of iodine, inhibits iodide uptake by the thyroid, competes with iodide in the organification process (Ermans *et al.*, 1980) and inhibits thyroid hormone synthesis (Fukayama *et al.*, 1992).

The noxious effect of smoking on the thyroid gland seems to become apparent when thyroid function is slightly compromised, while in euthyroid patients, the pool of circulating thyroid hormones is adequate to compensate for the smoking-induced defect of thyroid hormone action (Muller *et al.*, 1995). Thus, in normal adults, smoking has no effect on thyroid function or a weak pro-thyroid effect, causing small, thyrotrophin-independent increase in thyroid function, most often small increase in serum triiodothyronine concentrations (Utiger, 1995).

Epidemiological studies have reported a small inverse association of thyroid cancer with cigarette consumption (Kreiger and Parkes, 2000; Rossing *et al.*, 2000). Cigarette smoking has been found to be associated with a moderately reduced risk of thyroid cancer. This relationship is more pronounced in current smokers than former smokers. There are significant trends of reduced risk with greater duration and frequency of smoking (Mack *et al.*, 2003). The smoking-related reduction in TSH secretion and the lower body weight among smokers compared to nonsmokers are proposed explanations, as increased body weight is associated with a slightly increased thyroid cancer risk (Henderson *et al.*, 1982; Mack *et al.*, 2003; Williams, 1990).

Both decreased and increased thyroid functions have been described in smokers (Bartalena *et al.*, 1995; Bertelsen and Hegedus, 1994; Ericsson and Lindgarde, 1991; Knudsen *et al.*, 2002). Higher thyroxine and lower thyroid stimulating hormone levels have been reported in smokers (Utiger, 1998). Moreover, heavy smokers had a smaller increase in thyroxine levels than did light smokers, when compared to non-smokers. Cigarette smoking is, therefore, associated with a significant increase in the  $T_4$  level and a decrease in the TSH level. The

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effect of smoking on thyroid hormone levels is related to increased levels of thyroxine-binding globulin (Fisher *et al.*, 1997).

It has further been reported by several workers that current smokers have higher T<sub>4</sub> levels and lower TSH levels than never smokers and former smokers (Eden *et al.*, 1984; Ericsson and Lindgarde, 1991; Karakaya *et al.*, 1987). Certain workers, on the other hand, have reported similar or lower thyroid hormone levels in smokers as compared to non-smokers (Sepkouic *et al.*, 1984).

Cigarette smoking causes acute blood pressure (BP) elevation, although some studies have reported similar or lower BPs in smokers compared with nonsmokers (Green *et al.*, 1986; Primatesta *et al.*, 2001). Ex-smokers tended to have BPs similar to non-smokers (Savdie *et al.*, 1984; Seltzer, 1974).

Cigarette smoking increased heart rate and blood pressure in chronic smokers compared to non-smokers (Carter *et al.*, 2006). Moreover, the lowered body weight, in smokers, compared to non smokers, was found to be subsequently increased following smoking cessation (Puddey *et al.*, 1985).

The present study is carried out to investigate the effect of smoking and smoking cessation on thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) from the thyroid gland and their regulatory, thyroid-stimulating hormone (TSH), from pituitary gland in our local population. The study was further extended to evaluate the body mass index, blood pressure and heart rate in order to access smoking related variations in these parameters.

## MATERIALS AND METHODS

### *Subjects*

A group of male current smokers (n=80), former smokers (n=50) and the control never smokers (n=50), with ages ranging 30-60 years, from the local population, agreed to participate in the study after receiving a detailed explanation of its nature and purpose. Participants were selected on the basis of their sex, age, duration of smoking and number of cigarettes consumed per day in case of current smokers, duration of smoking cessation in case of former or ex-smokers, socioeconomic status, and absence of any physical disease, etc. Attempts were made that the referents must closely resemble

the smoking groups except that they were non smokers. The exclusion criteria for the study were passive smoking, drinking and existing physical diseases like diabetes, cancer, cardiovascular disorders, hepatitis and autoimmune disease, etc. The study was approved by the Regional Ethics Committee of Lahore district. A proforma, based on the criteria of the study, was designed and initially a large number of subjects were interviewed. The volunteers fulfilling the criteria and agreed to participate potentially were selected finally.

Current smokers were further categorized in two groups, of 40 subjects each, depending upon the duration of smoking. Former smokers were placed in a separate group.

Group I: Short duration (1-5 years) smokers

Group II: Long duration (6-30 years) smokers

Group III: Former smokers or ex-smokers

Moreover, group I and II were chain smokers consuming 50-60 cigarettes/day and group III had quitted smoking for 15-20 years.

An informed consent was obtained from each of the participants before his recruitment for the study. The intend and purpose of investigation was explained to the volunteers in a reassuring manner and every effort was made to put the subjects at ease.

### *Sampling and procedures*

Sampling was done, at a temporary clinic, made for the purpose. Further, a uniform sampling schedule was observed for all of the participants. Body weight (kg) in each group and the control subjects was obtained by digital weighing machine (Tanita weight balance, model: 01701). Average of two readings was used. During weighing, the subjects were without shoes and were wearing very light cotton clothes due to summer. The weight of a sample suit was then subtracted from the body weight of each volunteer. The actual body weight (kg) was then interpreted and compared with reference to height (m), measured by a metric scale adjusted with the wall, through body mass index (kg/m<sup>2</sup>). Blood pressure (mmHg) was measured in day time between 08:00-09:00 a. m. Prior to blood pressure measurement, every subject was given a rest period of 10 minutes. For measurement, subject was seated with bared arm resting on a standard

table or other support so that the midpoint of the upper arm was at the level of the heart<sup>13</sup>). Mercurial sphygmomanometer (Kenzmedico Co. Ltd., Japan; model No. 600) was used to record the blood pressure in each group. Average of two measurements was used for the study. Heart rate (heart beats/min) in each group and control subjects was also determined in the day time between 08:00-09:00 a.m. with the help of stethoscope (Kenzmedico Co. Ltd., Japan; model No. 120). Auscultation was performed for one minute and an average of two consecutive heart rates was obtained.

Blood samples from the volunteers were obtained between 09:00-10:00 a. m. with the help of registered technician using good quality sterilized disposable syringes (Becton Dickinson Private Limited) and left at room temperature for 3-4 hrs. Serum separated from the clot was centrifuged at 3000 rpm for 15 minutes and stored at -20°C in the labeled plastic vials, till used for further analyses.

The levels of the hormones ( $T_3$ ,  $T_4$  and TSH) were analyzed by Enzyme linked immunosorbant assay (ELISA: Automated Microplate Reader ELx 800, Automated Strip Washer ELx 50, BioTek Instruments Inc., USA) using the standard ELISA test kits (Monobind, Inc., USA) for the respective hormones. The absorbance for each set of reference standards and the samples was given at 450 nm wavelength. Validity of assay with serum samples was within 10 minutes of adding the stop solution for  $T_3$  and  $T_4$  and 5 minutes for TSH. Limits of detection (LoD) were exclusion of serum samples demonstrating gross lipemia, gross hemolysis, and/or turbidity. The concentrations of  $T_3$  and  $T_4$  in ng/dl and TSH in  $\mu$ IU/ml were determined from the "Linear Regression Equation".

#### *Statistical analyses*

Data obtained for each parameter of the study was presented as mean $\pm$ SEM, analyzed statistically using Student t-test at  $P < 0.05$  (Minitab 13.0 for windows) and employed in comparing the variations among different groups of study, in relation to control subjects.

## **RESULTS**

Table I shows the average levels of  $T_3$  (ng/dl),  $T_4$  (ng/dl), TSH ( $\mu$ IU/ml), body mass index

(kg/m<sup>2</sup>), blood pressure (mmHg) and heart rate (beats/minute) in the following groups.

Controls: Never smokers

Group I: Short duration (1-5 years) smokers

Group II: Long duration (6-30 years) smokers

Group III: Former smokers or ex-smokers

#### *Thyroxine ( $T_4$ )*

No significant variations were observed in the level of  $T_4$  when control group was compared with group I and group II. Group III (Ex-smokers), however, indicated an 11% non significant decrease in  $T_4$  concentration as compared to controls.

#### *Triiodothyronine ( $T_3$ )*

The average  $T_3$  level was found to be highly significantly elevated by 14% each in group I and group II as compared to controls. A more pronounced and statistically highly significant elevation of 18% was observed in former compared to never smokers.

#### *Thyrotropin (TSH)*

The average TSH level in control group indicated a 17% significant rise in group I. Group II exhibited a more marked elevation of 57% as compared to controls. The pattern persisted, however, less intensified, with a non significant rise of 28% in TSH concentration, in former compared to never smokers.

#### *Body mass index*

BMI, in group I, indicated a 13% significant reduction as compared to control never smokers group. Group II indicated a comparatively more pronounced reduction of 22%, in BMI as compared to control group. Former smokers, however, presented a varying response, *i.e.*, a statistically highly significant increase of 16% when compared to control never smokers group.

#### *Blood pressure*

The average systolic blood pressure indicated an 11% highly significant decrease in group I as compared to control group. The average diastolic blood pressure in control group was also found to be highly significantly decreased by 14% in group I, as compared to control group.

**Table I.- Comparison of continuous variables in controls (never smokers), group I (smoking period 1-5 yrs), group II (smoking period 6-30 yrs), and group III (ex-smokers). Values are mean±SEM.**

Variables	Controls	Group I	Group II	Group III
Thyroxine (T <sub>4</sub> ), ng/dl	4.7±0.2	4.6±0.3	4.8±0.2	4.2±0.2
Triiodothyronine (T <sub>3</sub> ), ng/dl	150.8±5.3	172.8±4.2*	172.7±4.5*	177.8±6.4*
Thyrotropin (TSH), µIU/ml	2.4±0.3	2.8±0.1*	3.8±0.3*Δ	3.1±0.3
Body mass index, kg/m <sup>2</sup>	21.2±0.1	19.0±0.2*	16.6±0.2*	24.6±1.1*Δ□
Systolic blood pressure, mmHg	119.0±1.1	105.7±3.9*	105.0±5.2*	110.5±3.1*
Diastolic blood pressure, mmHg	81.2±0.7	69.5±4.1*	64.0±3.9*	73.5±2.3*
Heart rate, per minute	73.1±0.5	73.1±0.5	84.6±1.0*	85.3±0.8*

\*, Δ, □ Significance at  $P < 0.05$  when compared to control, group I and group II, respectively

The average systolic blood pressure exhibited a 12% significant decrease in group II compared to control never smokers group. The mean diastolic blood pressure in group II also indicated a 21% highly significant decrease as compared to control group.

Ex-smokers followed the same trend of elevation, however, with less intensity, indicating a 7% significant decrease in systolic blood pressure in group III, as compared to control group. The trend persisted but again less intensified indicating a 9% significant decrease in diastolic blood pressure in group III as compared to control group.

#### Heart rate

A highly significant elevation of 18% was observed in group I as compared to control group. A similar trend with 16% rise in heart rate, as compared to controls, was exhibited by group II. The pattern persisted in ex-smokers indicating a highly significant elevation of 17% as compared to never smokers.

### DISCUSSION

The present study addresses the human thyroidal and its regulatory hormonal, blood pressure, body mass index and heart rate responses to cigarette smoking.

In the present investigation, the level of T<sub>4</sub> did not vary significantly when smokers were compared with never smokers. Former smokers, however, indicated an 11% non significant rise as compared to controls. The T<sub>3</sub> and TSH were, however, found to be significantly elevated in

smokers compared to never smokers. Ex-smokers also indicated significant rise in T<sub>3</sub> and a non significant rise in TSH level when compared with control group.

It has earlier been observed that smokers have same or low thyroid hormone levels as compared to non-smokers (Sepkouic *et al.*, 1984). Significantly reduced serum thyroxine (T<sub>4</sub>) and non significantly elevated serum T<sub>3</sub> and TSH levels in smoking group when compared with control subjects have earlier been reported (Banerjee and Mathu, 1994). They further commented that thiocyanate, an important component of smoking, was found to be associated with lower levels of T<sub>4</sub> and higher levels of T<sub>3</sub> and TSH in smokers compared to controls (Banerjee *et al.*, 1997).

Moreover, heavy smokers exhibited lower T<sub>4</sub> levels than light smokers (Christensen *et al.*, 1984; Sepkouic *et al.*, 1984), whereas, an elevated T<sub>4</sub> and a reduced TSH level has been reported in heavy than light smokers and in current than former smokers and non smokers (Ericsson and Lindgarde, 1991; Fisher *et al.*, 1997; Karakaya *et al.*, 1987).

Body mass index, in present study, has exhibited a significant decrease in smoking group when compared with controls, however, a statistically highly significant elevation in BMI was observed in former compared to never smokers. Lower body weight among smokers compared to non-smokers has also been reported (Mack *et al.*, 2003). Smoking cessation, however, resulted in subsequent increase in body weight (Puddey *et al.*, 1985). Moreover, reduced blood pressure in smokers of present investigation, might be related to their lowered BMI as is reported earlier that, in

smokers, the features of insulin resistance, *i.e.*, obesity and hypertension were significantly less frequent as compared to non smokers (Masulli *et al.*, 2006).

The blood pressure, both systolic and diastolic, in the present investigation, was found to be significantly decreased in smokers as compared to control group. The trend persisted although less intensified in former than never smokers control subjects.

Similar findings, *i.e.*, lower blood pressure (BP) in smokers than in non-smokers have also been reported earlier (Green *et al.*, 1986), whereas, ex-smokers tended to have BPs similar to non-smokers (Savdie *et al.*, 1984; Seltzer, 1974). The lower average BP found in smokers has occasionally been attributed to differences in their body weight. However, BP differences among smokers and nonsmokers tended to persist even after body weight is controlled (Savdie *et al.*, 1984). The quitters for more than 3 years and current non-smokers showed larger increases in BP than the current smoker. However, the quitters for less than 1 year showed a smaller increase than the current smokers (Duk-Hee *et al.*, 2001).

A progressive increase in blood pressure with the prolongation of smoking cessation in men has been studied (Duk-Hee *et al.*, 2001). The study implies that the cessation of smoking may result in increase of blood pressure.

Heart rate in current smokers and former smokers, in the present investigation, was found to be significantly elevated as compared to never smokers. The elevation in heart rate may be related to the use of nicotine and consumption of caffeine that may affect the blood pressure, heart rate and stress hormone excretion rates (Raggat and Morrissey, 1997).

The investigation elucidates a marked influence of smoking on thyroid and its regulatory hormonal levels. Moreover, a strong association of smoking with body mass index, blood pressure and heart rate has also been worked out. The study should be extended on a larger scale and various other parameters must be investigated to explore the influence of smoking on health status of local population.

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