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Using HPRT gene mutation assay for detection of reasons of thyroid disorders in patients at Al-Zuaaffaranya city

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Abstract: Thyroid disorders have a multifactorial etiology, and the right combination of genetic, environmental, and endogenous factors are required for the initiation of the disease process. This study was carried out to evaluate some hormonal and genetic parameters of some Iraqi patients from Al Zuaaffaranya city with thyroid disorders in order to find if the exposure to pollutant have a role in the increasement of this disorders, this study consisted of two parts The first part was conducted on 25 patients21(84%) of them females and 4(16%) were males suffered from thyroid disorders who have been referred to the Department of Radiation, Nuclear Medicine Hospital in Baghdad and 25 healthy control during the period from March to july2015. The age of patients and healthy individuals ranged between (13-60) years. The results showed that the most frequent thyroid disorders among patients were thyroid non-toxic goiter15:25 (60%)and hyperthyroidism 5:25 (20%) while hypothyroidism4:25 (16%) and thyroid cancer 1:25 (4%) in less frequent. Thyroid hormones thyroxin (T4), triiodothyronine (T3), and thyroid stimulating hormone(TSH) levels were determined in all subjects by enzyme linked fluorescent assay (ELFA). T3, T4 and TSH level significantly high p≤(0.01), (1.83±0.144nmol/L, 181.2±54.245 nmol/L, 0.38±0.12 µ IU/ml respectively) In Thyroid toxic goiter(TG), T4, T3 level significantly high p≤0.01(78 ±11.57,1.37±0.06)in hypothyroidism group. The second part of the study include the using of HPRT gene mutation assay as useful biomarkers for the detection of organism's exposure to ionizing radiation .The results of the average mutation frequency for HPRT (Mf-HPRT) gene revealed a significant increase ($p<0.05$) in patients with thyroid disorders comparing with the control group This indicate a possible exposure to pollutant which may be radiation pollution .

Key words: Thyroid disorders, HPRT gene, Thyroid hormones.

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Introduction:

Thyroid gland is the biggest purely endocrine tissue in the human body, the main function of the gland lies in the synthesis of hormones; specifically the thyroid hormone and also calcitonin to a lesser degree (1). These hormones act on cells in virtually every body tissue by combining with nuclear receptors and altering expression of a wide range of gene products. Thyroid hormones are required for normal brain and somatic tissue development in the fetus and neonate and in people of all ages, regulates protein, carbohydrate, and fat metabolism. Several different types of thyroid disorders may develop including an under reactive thyroid (hypothyroidism), overactive thyroid (hyperthyroidism, and growths on the thyroid that may be benign (nodules) or malignant (cancer) (2).

Thyroid disorders increase in an ubiquitous global phenomenon suspected to further rise in the

upcoming decades (3,4). According to the American Thyroid Association (ATA), More than 12 percent of the U.S. population will develop a thyroid condition during their lifetime (5) .

Thyroid disorders are also health problems in Iraq. (6) reported that disorders rate was(50.82%), the prevalence in female (55.24%)was higher, than in male (34.57%), these percentages reflect severe endemicity. In a recent study of convenience sample of students from AL- Russafa 2 sector, found that thyroid disorders prevalence was estimated to be 14.35%(7).

Because the thyroid affects the adult body's major systems, and it is crucial to fetal development, its disruption by exogenous chemicals is of intense interest. Moreover, the thyroid, along with breast tissue and bone marrow, is especially vulnerable to ionizing radiation, it can be affected by ionizing radiation through the skin by gamma radiation, including X rays, by fission products, such as cesium; or by ingestion or inhalation of iodine-131 (^{131}I), an isotope present in nuclear fission products. ^{131}I emits mostly beta radiation, which penetrates surfaces more shallowly than gamma radiation (8). The most common thyroid manifestations of radiation are hypofunction, in addition to thyroid nodules and thyroid cancer. Autoimmune thyroid disease has been linked to therapeutic medical radiation, as well as environmental radiation exposure (9; 10).

Pollutant refers to a substance, organism or energy form present inamounts that impair or threaten an ecosystem to the extent that its currentor future uses are precluded(11).

In Iraq, radioactive contamination was and still an environmental pollution problem since its levels raised after both Gulf wars I and II, (12). Therefore, it seemed necessary to study the relation between thyroid disorders and radiation exposure and also to determine individual predispositions to the development of certain pathological processes under the influence of hazardous factors, in this study this was based on the detection of HPRT gene mutation assay for the detection of radiation exposure in thyroid disorders patients at Al-Zuaaffaranya city.

Materials and Methods:

Subjects:

Thyroid Disorders Groups:

Twenty five patients of thyroid disorders who attend the endocrinologist in Nuclear -Medicine Hospital in Baghdad, were selected. Clinical, ultrasonication and serum thyroid hormones were used for diagnosis. Patients' ages ranged from 13-71 years. All patients were suffering from thyroid disorders (hyperthyroidism, thyroid non-toxic goiter, thyroid hypothyroidism and thyroid cancer)who have been referred to the Department of Radiation, Nuclear Medicine Hospital in Baghdad during a period from June 2013to October 2014 .

Control Groups:

A total of twenty five healthy control were included in the study ages from 13-60 years, 21were female and 4 males in order to compare with thyroid disorders groups.chosen from

population living in Baghdad far away from Al-Zaafaranyia, individuals with negative antithyroid antibodies , no family history of thyroid and other autoimmune diseases.

Sample Collection:

Five milliliters (ml) of venous blood were collected from patients as well as controls. The aspirated blood was immediately transferred into plain tubes to be used in different tests as shown below:

Laboratory Investigations:

A. The serum was obtained by putting the blood samples in a clean dry plain plastic tube and was allowed to clot at 37°C for 10-25 minutes before centrifugation. Centrifuged at 3000 rpm for 15 minutes, for serological markers which include the following Biochemical Parameter:

- Total T3, T4 and TSH in serum.

Determination of Thyroid Hormones:

The hormones T_3, T_4 and TSH were determined by using VitekImmuno Diagnostic assay System(VIDAS) VIDAS T_3, VIDAS T_4 , and VIDAS TSH kits, which are enzyme immunoassay for detection of total T_3, T_4 and TSH in serum using the ELFA technique (enzyme linked fluorescent assay). These systems enable us to estimate total T_3, T_4, and TSH in serum or plasma.(Biersack and Hotze, 1991).

The Result analysis included:

Measurement of hormones at normal range
T_3= (1.2-2.8) nmol/l
T_4= (60-155) nmol/l
TSH= (0.4-4.0) µIu/l.

Blood Sampling:

Five ml of peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

Blood Culturing:

The *HPRT* gene mutation assay was performed according to the description by(16). Two sets of cultures were prepared, each set of culture contained 0.5 ml heparinized blood and 4.5 ml RPMI 1640 with 20% fetal calf serum and 0.2 mg/ml PHA-M. One set of culture was added with 0.2 mM 6-thioguanine (Sigma). At 33 h of incubation, cytochalasin B (the final concentration,4.5 mg/ml) was put into two sets of cultures. At 72 h of incubation,the lymphocytes were harvested by centrifugation and fixed with methanol: acetic acid (3:1). The slides were prepared and stained with 10% pH 6.8 Giemsa solution.

Microscopic examination:

The binucleated and multinucleated cells per 1000 lymphocytes in two sets of cultures were scored under light microscopy (magnification 1000X). Mutant frequency of HPRT gene (Mf-HPRT) was calcula ted with the following formula (14):

$$\text{Mf-HPRT}(‰) = \frac{\text{binucleated and multinucleated cells in culture with 6 - TG per 1000}}{\text{binucleated and multinucleated cells in culture without 6 - TG per 1000}}$$

Results and Discussion:

Demographical Distribution:

Thyroid disorders are common in all parts of the world , these disorders can plague the thyroid gland, including autoimmune disorders, benign and malignant tumors, and goiter.From twenty five of patients from Al-Zuaaffaranya city who were involved in the first part of this study The results showed that 11 of them have a relatives with thyroiddisorders and the most frequent disorders were thyroid non-toxic goiter (60%) and hyperthyroidism (20%) while hypothyroidism (16%) and thyroid cancer (4%) in less frequent , these disorders were distributed highly among the age group(30-50) yearsas shown in table (1)Also this table showed that females had the superior total number than males; they were 21 females (84%), and 4male (16%) from the total25 cases number patients.

Table (1): Characteristics of the 25 patient Who Tested Positive for Thyroid disorders

Patient NO.	Age	Sex	Relatives	T3	T43	TSH	Diagnosis
1	48	F	NO	1.9	88	0.7	NTG
2	32	F	Yes	2	79	1.4	NTG
3	35	F	NO	1.4	60	3.7	Hypo
4	45	F	Yes	2.6	91	1.6	NTG
5	28	F	Yes	1.4	60	5.3	Hypo
6	29	F	Yes	1.4	88	4	TG
7	60	F	NO	1.9	105	1	NTG
8	29	F	NO	5	285	0.1	TG
9	22	F	NO	2	128	1.2	NTG
10	25	F	NO	2.1	98	1.3	NTG
11	50	F	NO	1.5	98	6.2	Hypo
12	13	F	Yes	2.9	80	1.2.	NTG
13	38	F	NO	2.08	103	3.15	Ca
14	27	F	Yes	1.8	80	1.5	NTG
15	40	M	Yes	2.1	89	2	NTG
16	35	F	Yes	1.8	123	2	NTG
17	50	F	NO	1.6	81	1	NTG
18	45	F	Yes	1.4	88	1.9	NTG
19	35	F	NO	2.2	85	0.9	NTG
20	52	M	Yes	2.1	87	0.647	TG
21	19	F	NO	1.6	58	0.7	TG
22	40	F	NO	2.2	85	2.1	NTG
23	30	F	NO	1.3	90	2.3	NTG
24	41	M	NO	5.2	265	0.1	TG
25	45	M	Yes	1.2	56	4.8	Hypo

TG :Toxic goiter ,NTG: thyroid non-toxic goiter, Hypo : Hypthyroidisim, Ca: Thyroid cancer

Biochemical Parameters:

Serum TSH, T4 and T3 levels:

Thyroid hormones thyroxin(T4) , triiodothyronine (T3), and thyroid stimulating hormone (TSH) levels were determined in all subjects by enzyme linked fluorescent assay (ELFA). T3,T4and TSH level significantly high $p \leq 0.01$),(1.83±0.144nmol/L,181.2±54.2 45nmol/L, 0.38±0.12 µ IU/ml respectively) In Thyroid toxic

goiter(TG)AlsoT4 level significantly high p≤0.01(92.46 ±3.90)in thyroid non toxic goiter group Furthermore TSH level were significantly increase p≤0.01(5.00±0.5) in hypothyroidisim patients.

Table (2): TSH, T4 and T3 levels for thyroid disorders patients and control groups

		T3		T4		TSH	
Group	**No.**	**Mean±SE**	**P value**	**Mean±SE**	**P value**	**Mean±SE**	**P value**
Control	25	3±0.14 1.8		±1.779.4	0.01**	±0.222.02	0.01**
Hyperthyroidsim	5	3.17±0.45	0.01**	±54.2181.2		0.38±0.12	
Thyroid non toxic goiter	15	1.9±0.10	0.04	±3.992.4	0.01**	±0.131.4	0.08
Hypothyroidsm	4	1.37±0.06	0.2	±11.5778	0.8	±0.55	0.01**

****: Highly significant (p≤0.01),*: Significant differences at (p<0.05)**

Serum TSH, T4 and T3 levels for thyroid disorders patients and control groups are presented in table (2). TSH level significantly (p≤ 0.01) increased ,(5±0.5 µ IU/ml,) in hypothyroidsim group while there were significant(p≤ 0.01) decrease inTSH level in hyperthyroidism group T4 levels reveal a highly significant (p≤ 0.01) difference (181.2± 54.2,92.4±3.9nmol/L)in hyperthyroid group and thyroidnon toxic goiter group,. Also there was significant (p≤0.01) increase in T3 level (3.17±0.45nmol/L) in Hyperthyroidism group when compared with other groups. Thyroid disorders seems to have a multifactorial etiology where interactions between the effects of multiple genes and environmental factors are important, and the right combination of genetic, environmental, and endogenous factors is required for the initiation of the disease process (15).(16) concluded that low-dose environmental radiation exposure may be associated with the development of autoimmune Thyroid disease.

The variations in the mean values of TSH, T4, and T3 levels were common, among thyroid disorders patients , (17) reported that the key test for the diagnosis and management of hypothyroidism and hyperthyroidism is the TSH assay. Current TSH assay have high sensitivity and are recommended as first – line strategy and most important measurement for identifying changes in thyroid function (18; 19). Researchers interested in the study of correlation of TSH, T4, and T3 levels and thyroid disorders, they usually found TSH levels in hypothyroidism were more than normal range, which confirmed our result (20 and 21), and that opinion is also corroborated by (22) who stated that Hypothyroidism is caused by thyroid under activity, and this may be primary (caused by disease of the thyroid), secondary (in response to decreased secretion of TSH), or tertiary (in response to decreased secretion of TRH).

In the current study T4 andT3 levels significantly(p≤ 0.01) increased in Hyperthyroid group, these finding are supported by (23) who found that in Hyperthyroidism, TSH levels decreased to counteract the high TH level. A serum TSH level below 0.05 mU/L, in combination with an increased T4 level, indicates primary hyperthyroidism, hyperthyroidism usually starts as a non-toxic multi nodular goitre, which is formed via the hyper plastic response of

the thyroid gland to iodine deficiency, over time, the non-toxic goitr becomes functional and begins to secrete TH hormone(24).

The mutant frequencies of *HPRT* (Mf-*HPRT*) gene were performed on peripheral blood lymphocytes which were obtained from 25 individuals of resident living the Al-zaafaraniya region then compared with 25 individuals control living in Baghdad a way from Al- Al-zaafaraniya . The *HPRT* gene mutation assay was performed according to the description by(14).

The Mf-HPRT gene was calculated binucleated, trinucleated and quadrinucleated lymphocyte cell per 1000 lymphocytes in tissue culture with and without 6-thioguanine (Fig.1). The results of HPRT gene mutation assay in residents living this city are shown in table (3). The average Mf-HPRT (Mean ± SE) for zaafaraniya city significant increase ($P< 0.05$) in this region as compared with the control. These results suggest that the HPRT mutation spectrum can be used as a potential biomarker for assessing a specific environmental risk. Furthermore, studies of mutations at HPRT gene have provided insights into several aspects of somatic mutations in vivo, including molecular mechanisms of mutagenesis, the relationship between DNA damage and mutation, as well as individual susceptibility factors such as DNA repair capacity(10).

HPRT is the most commonly used genetic locus in mammalian cells mutational studies. The gene encoded for the purine salvage pathway enzyme HPRT. Hence, this locus is reasonably important to be tested because it is X-linked and mutation at the HPRT (15). This result is similar to those of (25) in which the evaluation of HPRT mutant assay as a biomarker monitoring the specific environmental mutagen. According to our result and due to the high susceptibility of the thyroid gland to radiation damage, a detailed long-term follow-up of thyroid function during the next several decades would be of great importance in identifying thyroid malignancies and deviation of its function for suspected exposed peoples to radioactive materials (especially for contaminated areas residents).

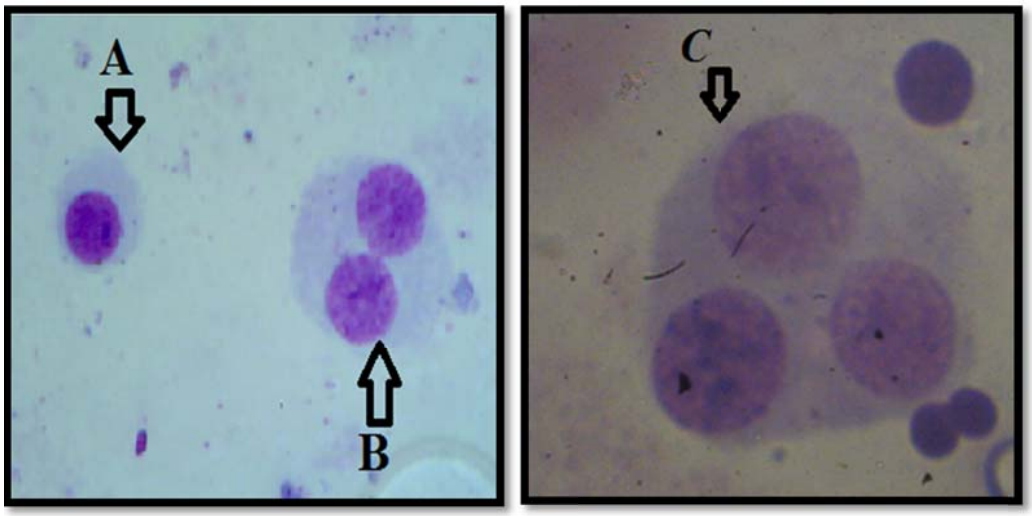

Figure (1): Cytokinesis blocked human lymphocyte cell ,(A) :Mononucleated lymphocyte cell , (B): Binucleated lymphocyte cell, (C Trinucleated lymphocyte cell.

Table (3): The average Mf-HPRT (Mean ± SD) for Decommission workers and Control group.

Group	Age Year (mean±SD)	No. of sample	Mutant Frequency (mean+SD)
Control	44.46±7.99	30	0.538±0.07[b]
Group A	43±8.46	16	0.622±0.14[a]

Similar latter in a column mean there is no significant difference ($p < 0.05$)

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