The potential impact of oil pollution on Thyroid and Parathyroid Hormones in directly exposed workers in Al-Shaiba oil refinery

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ABSTRACT

Thyroid hormones are essential for growth, neuronal development, reproduction and regulation of energy metabolism. Hypothyroidism and hyperthyroidism are common conditions with potentially devastating health consequences that affect all populations worldwide. This study aimed to evaluate the effects of exposure to petroleum gases, vapors and pollutant materials on oil refinery workers in Al-Shaiba / Basra-Iraq. However, samples were collected from male workers Ages from (22 – 55) years and divided into four groups according to their years of experience in the same refinery and compared with healthy control. We highlight It also showed that T4, TSH, and PTH hormone more effected by the heavy metals than T3 hormone in comparison to the healthy control group. It showed that worker with direct exposure of pollution sources for more than 5 years of exposure is more affected with oil refinery pollutant than workers who only exposed for 1-5 years.

Keywords :Oil refinery workers, hyperthyroidism Para-thyroid

1. Introduction

The process of refining crude oil in the petroleum industry yields over 2500 refined products, such as gasoline, kerosene, liquefied petroleum gas, diesel fuel, fuel oils, aviation fuel, lubricating oils, and feedstocks that are utilized in the petrochemical industry (Al-Fartosy *et al.*2014 ;Al-Fartosy *et al.* 2017). The petroleum refinery process commences with the reception of crude oil for storage at the refinery, encompasses all petroleum treatment and refining procedures, and concludes with storage arrangements in readiness for the transportation of the refined products from the refinery (Adebiyi FM.,2022). The thyroid gland is an endocrine gland that synthesizes and releases the thyroid hormones, namely tri-iodothyronine (T3) and thyroxine

(T4) (Kleerekoper M., 2008). The thyroid gland is situated in the anterior region of the neck and exhibits a bilobular morphology, resembling that of a butterfly. According to (Clines *et al.*, 2011), the thyroid gland comprises two distinct cell types, namely para follicular and follicular cells. The synthesis of thyroid hormones is attributed to the follicular cells. The cells of the follicle encompass a compartment known as the colloid, which houses thyroglobulin, a glycoprotein that serves as a reservoir for the T3 and T4 precursors (AL-Slem B., 2013). The para-follicular cells, commonly referred to as C-cells, are responsible for the secretion of calcitonin hormone, which plays a crucial role in calcium regulation (Bertholf R., 2011; Zuñiga LF, *et al*.,2022).

The hormone known as Parathyroid hormone (PTH) is comprised of eighty-four amino acids and plays a crucial role in maintaining calcium homeostasis, as stated by (Ramasamy I. 2006). The parathyroid hormone (PTH) is known to elevate the levels of calcium ions ($Ca^{+2)}$ in the bloodstream through various mechanisms. These include enhancing the absorption of Ca^{+2} in the intestines and promoting its reabsorption in the kidneys, particularly in the distal tubules and possibly in Henle loops. Additionally, PTH stimulates the liberation of Ca^{+2} from bones. (Al-Mahdawi FK, *et al.,* 2018). In addition to its primary role, PTH has been found to have other functions such as promoting magnesium reabsorption by the loop of Henle and impeding phosphate reabsorption by the proximal tubule (Gaw A., 2006). The typical physiological range of calcium within the human body is between 1000 to 1200 grams (Al-Shams *et al.*,2022). The skeletal system accounts for approximately 99% of the total calcium content in the body, while the remaining 1% is distributed between the intracellular and extracellular compartments. According to (Blaine *et al*., 2015), although the majority of calcium in the human body is found in bone tissue, it is also an essential cation in both intracellular and extracellular compartments.

The release of diverse heavy metals, including arsenic (As), lead (Pb), and cadmium (Cd), during petroleum refining operations has been observed to impact the levels of parathyroid hormone (PTH). Several studies have demonstrated a reduction in parathyroid hormone (PTH) levels after exposure to cadmium (Cd). (Schutte *et al.* 2008) provided an explanation for the reduction in PTH levels following exposure to cadmium, attributing it to the direct osteotoxic impact of the metal (Babić Leko *et al*., 2021). This study aimed to evaluate the effects of exposure to petroleum gases, vapors and pollutant materials on oil refinery workers in Al-Shaiba / Basra-Iraq.

2. Material and Methods

2.1 Subject:

In this study, 160 blood samples of Iraqi male workers were collected from the Al-Shaiba refinery in Basra province. All workers were working for over a year in the refinery. The samples were divided into two groups:

Group 1: 80 blood samples from male workers of Basra's southern refinery which were exposed to heavy elements, which includes two categories:

Category 1: 30 blood samples from workers directly exposed to heavy metals due to their direct routine exposure to petroleum products.

Category 2: 50 blood samples from workers indirectly exposed to heavy metals because they had little to non-direct exposure from emissions from the petroleum industry, and were either students or had administrator's jobs.

Group 2: 80 blood samples from male workers in non-oil facilities as a healthy control group.

All workers have been working in the petroleum refinery for over a year and their weight between (80 – 95) Kg and on a different side, the 80 volunteers were exposed to few or no direct emissions from the oil industry and their weight between (80 – 90) Kg.

All participants had no family history of chronic diseases like diabetes and respiratory disease. Taking into consideration that all participants were non-alcoholic drinks. The worker's age, functional services, and time of exposure to pollution (working hours) were recorded as in table (1). The questionnaire illustrated in Table (1) was used for interviews.

Table (1): Baseline characteristics for individuals in this study.

Groups		Number of cases	Age range	Age (mean±SD)	Minimum working years	Minimum working hours
Group 1: Al-Shaiba Oil refinery Workers direct exposed	Total number (G_T)	80	22 – 55	45	One year	6 h
	Duration (1-5 years, G_1)	50	22 – 45	37	One year	6 h
	Duration (> 5 year,G_2)	30	22 – 55	52	One year	6 h
Group 2: Control	C	80	22 – 50	45	–	–

Exclusion criteria

The study excludes any samples that have other interfering factors that may affect the accuracy of the study. Persons who were excluded from the control and patients group were:

1- Female workers.

2- Workers with diabetes or hypertension which may affect the accuracy of the study.

3- Workers with respiratory diseases.

2.2 Place of Work

This study was conducted in the Department of Chemistry, College of Science, University of Basra, and Al-Byaan laboratory in the province of Basra.

2.3 Samples collection

A volume of 8ml of blood samples was kept in gel tubes and allowed to clot at 37 ^{0}C for 30 minutes, then centrifuged at 3000 rpm for 5 minutes to collect serum samples for biochemical analysis.

The sera were stored in deep freeze (-20°C) and became ready to use in the measurement of biochemical parameters and elements. The rest of the whole blood samples (2 ml) was kept in tubes containing EDTA as an anticoagulant and became ready to measure complete blood count (CBC) and erythrocyte sedimentation rate (ESR).

2.4 Determination of hormonal biomarker

The concentration of serum samples of the hormonal biomarkers (thyroid-stimulating hormones, triiodothyronine and thyroxine, and parathyroid hormone) was measured for both patients and controls. Before proceeding with the assay, all serum and reagents samples were brought to room temperature at (20-25°C), and gently mixed before the use.

2.5 Determination of serum triiodothyronine (T3)

Triiodothyronine (T3) ELISA kit was used to determine serum T3 in each cases-controls participant enrolled in this study.

A- **Principle**

T3 ELISA is a type of competitive immunoassay. T3 in calibrators, controls, specimen samples, and an enzyme-labeled antigen (HRP conjugate) compete for a limited number of anti-T3 antibody binding sites on the microplate wells.

B- Reagent preparation

T3-enzyme Conjugate Solution

In a suitable container, the T3-enzyme conjugate diluted 1:11 with assay diluent.

C- Wash Buffer

1X Wash buffer was prepared by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Then Stored at room temperature (20-25°C).

D- Procedure

The procedure for determining this parameter was achieved according to (Roller *et al*., 1982; Asaad, 2011).

1. Microplate wells were formatted for serum, control, reference and duplicate sample.the unused microwell strips were replaced into the aluminum bag, sealed, and then stored at 2-8°C.

2. 50 µl aliquots of control, calibrators or were pipetted into the assigned wells, then 50 µl of T3 enzyme conjugate solution and 50 µl of working solution. T3-biotin antibodies were added to all wells, respectively.

3. The microplate was gently shaken for 20 seconds to mix the components, then covered and incubated for one hour at room temperature.

4. Each well contents were removed, rinsed three times with 300µl of washing buffer (1X), then 100µl of TMB substrate was added solution to all well.

5. The plate was covered then incubated at 25C for 15 minutes, then 50µl of stop solution was added to each well and mixed gently for about (15-20) sec.

6. The Absorbance at (450nm) of each well was researched by using an ELISA reader within 5 minutes after addition of stop solution.

E- Calculations:

The amount of buffer needed is equal to the number of wells multiplied by 0.5. The amount of enzyme compound solution needed is equal to the number of wells multiplied by 0.005.

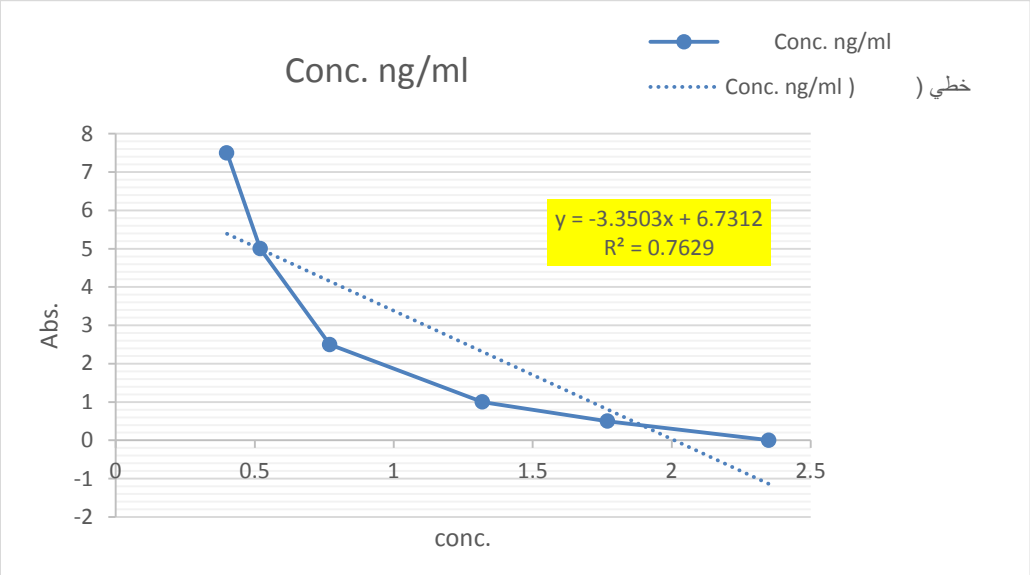

Figure (1): standard curve of T3.

2.6 Determination of serum thyroxine

Thyroxine ELISA kit was used to determine serum T4 in each cases-controls participants enrolled in this study.

A- Principle

The Thyroxine (T4) Competitive ELISA Kit is a competitive Enzyme-Linked Immunosorbent Assay (ELISA) in solid phase. This assay is intended to detect and quantify thyroxine levels in serum. The assay detects thyroxine regardless of species.

B- Reagent preparation

T4-enzyme Conjugate Solution

Dilute the T4-enzyme conjugate 1:11 with assay diluent in a suitable container. For assistance, dilute 80µl of enzyme conjugate with 0.8ml of assay diluent for 16 wells.

C-Wash Buffer

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

D- procedure

The procedure for determining this parameter was achieved according to (Walker, 1997):

1. Microplate wells were formatted for serum reference, control and patient sample in duplicate.Any unused microwell strips were replaced back into the aluminum bag, seal, and store at 2-8°C.
2. Aliquots of 25µl of the standards, control, or specimen were pipetted into the assigned wells.
3. Aliquot of 50µl of working T4-enzyme conjugate solution were added to each wells, then 50µl of T4-Antibody-Biotin solution was also added to each wells.
4. The microplate was gently swirled for 20-30 seconds to mix the contents, then covered and incubated for one hour at room temperature (25°C).
5. Each well's contents were removed, and rinsed three times with 30ml of 1X washing buffer, then 100ml of TMB (3,3′,5,5′-Tetramethylbenzidine) solution was added to each well.
6. The plate was covered and incubated at room temperature for 15 minutes, then 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
7. Absorbance (450nm) of each well was researched by using an ELISA reader within 5 minutes after the addition of the stop solution.

E. Calculations

The number of wells multiplied by 0.5 equals the amount of buffer required. The number of wells multiplied by 0.005 equals the amount of enzyme compound solution required. Standard curve was prepared

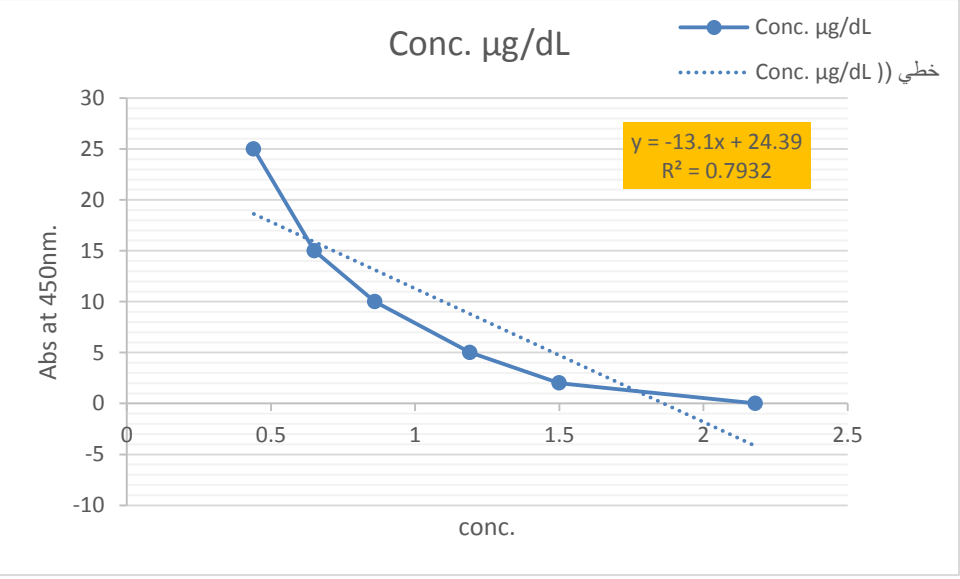

Figure (2): calibration curve of T4.

2.7 Thyroid stimulating hormone ELISA kit

A- Principle

The CBI TSH is a solid phase sandwich ELISA method. The samples, and anti-TSHHRP/Biotin conjugate are added to the wells coated with Streptavidin. TSH in the patient's sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. A standard curve is prepared relating color intensity to the concentration of the TSH

B- Procedure:

The procedure for determining this parameter was achieved according to (Wild, 1994).

1. The microplate wells were formatted for each serum reference, control, and patient specimen to be assayed in duplicate. Any unused microwell strips were replaced into the aluminum bag, sealed, and stored at 2-8°C.
2. Aliquots of 50µl of standards, control, or specimen were pipetted into the assigned wells, then 50µl of the working TSH-enzyme conjugate solution and 50 µl of TSH-Antibody-Biotin solution were added to all wells, respectively.
3. The microplate was swirled gently for 20-30 seconds to mix the components, then covered and incubated for one hour at room temperature (25°C).
4. Each well contents were removed, rinsed three times with 300µl of washing buffer (1X), then 100µl of TMB substrate was added solution to all well.
5. The plate was covered and incubated at room temperature for 15 minutes, then 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
6. Absorbance (450nm) of each well was researched by using an ELISA reader within 5 minutes after addition of the stop solution.

C- Calculations:

- The Amount of Buffer required equal to the Number of wells multiplied by 0.05

The Quantity of the solution of enzyme conjugate necessary equal to # of wells multiplied by 0.005 standard curve in this study.

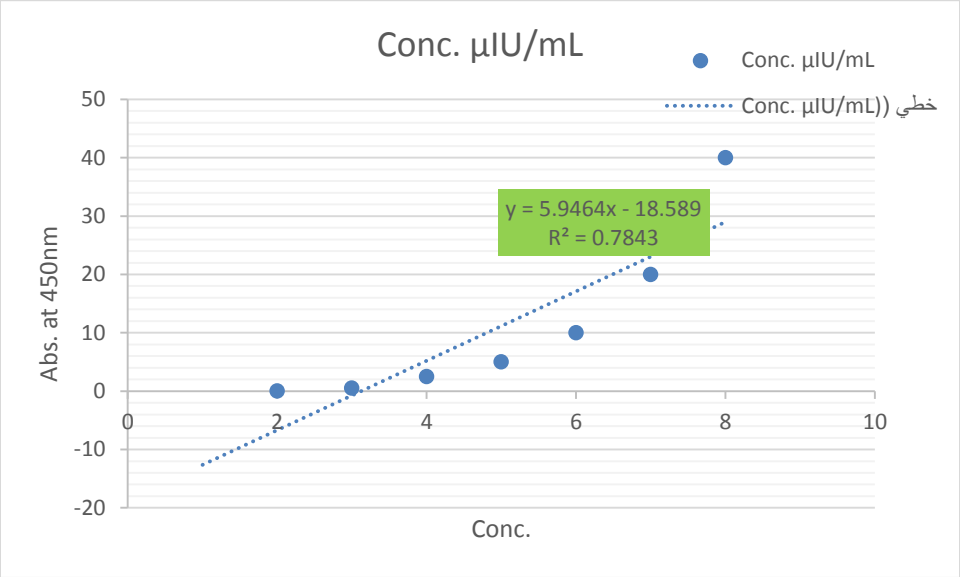

Figure (3): standard curve of TSH.

2.8 Estimation of parathyroid hormone (PTH) level

A- The Principle:

The level of PTH in the serum was estimated by a ready-made test kit from the German company Demeditec (Mukdad, 2021; Segre, *et al*., 1974 ; and RAISZ, *et al*., 1979) where the plate pits covered with PTH antibody are incubated with PTH present in the serum, Reangent1 (biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) at room temperature for three hours with stirring, during the incubation period a constant amount of PTH paired with PTH competes in the sample serum to monitor a specific number of binding sites on the PTH antibody and after the expiration of the three hours we wash the pits and after the washing step the PTH associated with the use of Reagent B (TMB Substrate) The coupling of Reagent B (TMB Substrate) associated with the drill is gradually accompanied by an increase in the concentration of PTH, and after waiting 30 minutes with stirring, the color development stops by adding the stop solution, a standard curve of the concentration of the standard against the absorbency will be obtained .The color intensity will be directly proportional to the amount of PTH in the sample.

B- Procedure:

The reagents and samples reached room temperature then solutions gently mixed without foam.

1. 25µl micropipette of titration solutions and serum, is added in each calibration drill

2. 50µl micropipette from REAGENT1, the calibration container, constants and serum are added in the calibration pits of the calibration container, constants and serum.

3. 50µl micropipette from REANGET 2, is added in the calibration drill, calibration container, constants and serum

4. The plate is incubated for 3 hours at room temperature (18-25 ° C) using a plate vibrator at 700 rpm ± 100, the any adhesive agent of the plate was removed.

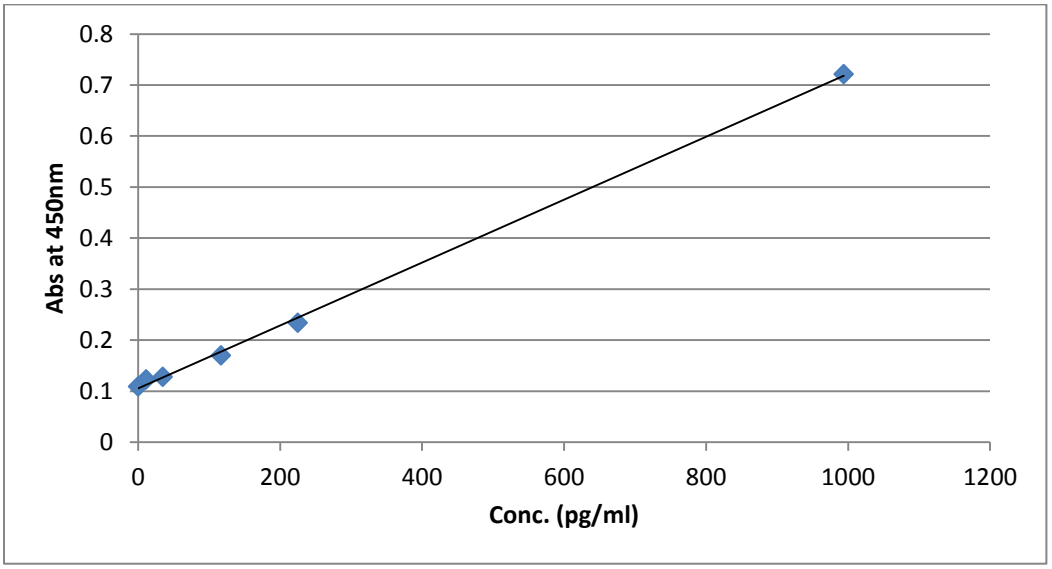

Figure (4) Standard Curve of Parathyroid Hormone (PTH).

3. Results and Discussion

3.1 Determination of Triiodothyronine (T3) levels

Results showed that there is a significant decrease in the G2 group (Worker with direct exposure to pollution sources for more than 5 years of exposure) than the G1 group (Worker with direct exposure to pollution sources for only 1-5 years of exposure), GT group (all workers) and healthy control group as indicated in figure (5) and table (1) these results might be due to Certain peoples have a greater risk of exposure to vapors of gasoline; these include worker of petroleum refineries, attendants of station service, illing-station workers, drivers of gasoline trucks and refinery workers (Periago JF, 2005). The oil industry holds a major potential of hazards for the environment, and may impact it at different levels: air, water, soil, and consequently all living beings on our planet (*Mariano J, and La Rovere E., 2017*). A small study of 42 petrol station

workers and 37 control subjects reported thyroid effects in the form of decreased T3, increased T4, and decreased TSH levels as a function of years worked (Uzma *et al*., 2008). These authors concluded that the solvent vapors from long term exposures at or near the petrol station were likely the cause of the thyroid effects, although a specific hypothesis with biological plausibility was not presented.

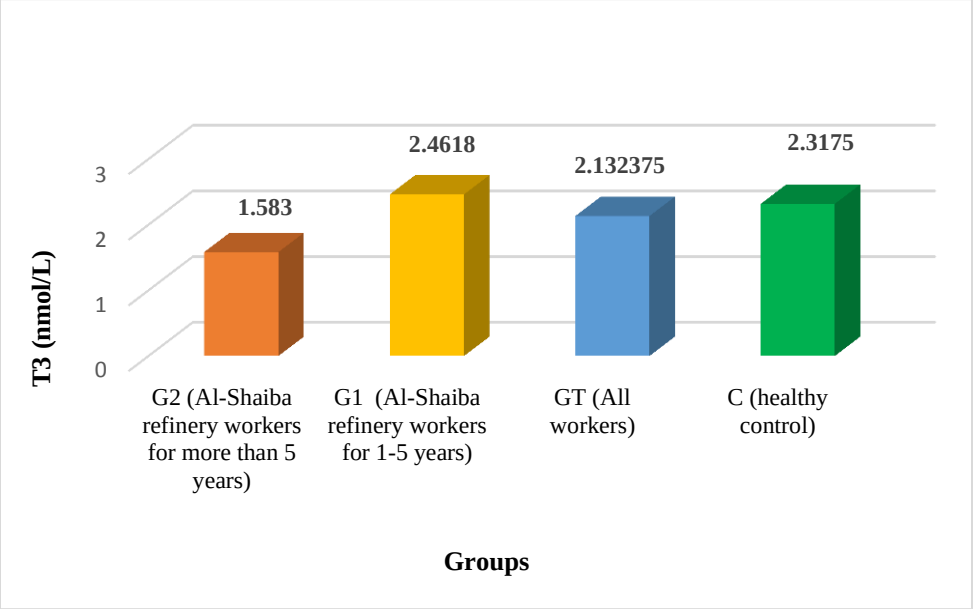

Figure (5): Serum T3 level of Alshaiba oil refinery worker and healthy control.

3.2 Determination of T4 levels

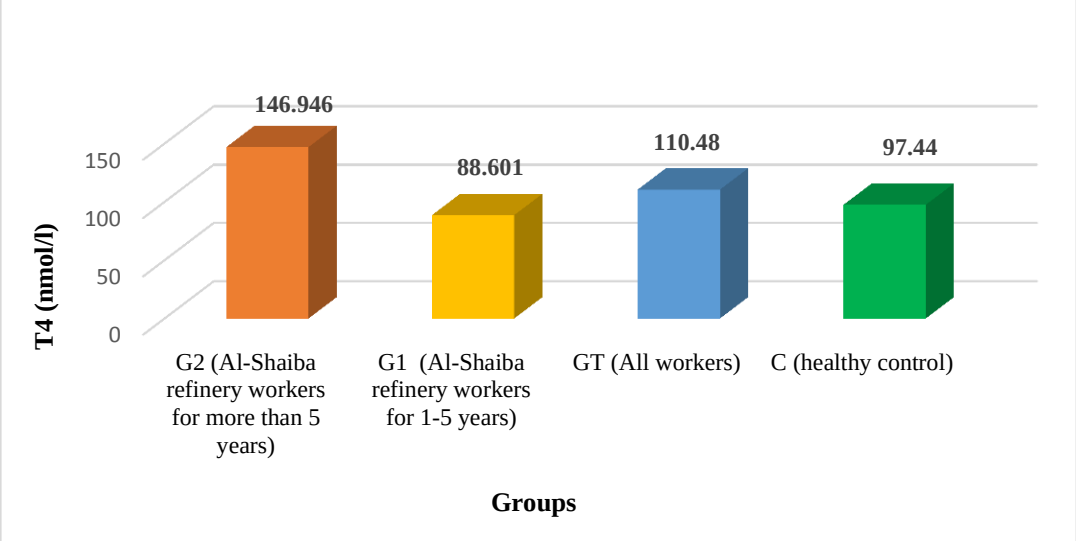

Figure (6): Serum Thyroxin (T4) level of Alshaiba oil refinery worker and healthy control.

Results indicated in figure (6) and table (1) showed that there is a significant increase in T4 levels in G2 group than G1 group and healthy control which might be due to the effect of direct exposure to heavy metals for more than 5 years on the thyroxin hormones. The result also showed a fluctuation in T4 levels among study groups as it showed that there is a significant increase in T4 levels in GT group than in healthy control groups.

3.3 Determination of Thyroid stimulation hormone (TSH) levels.

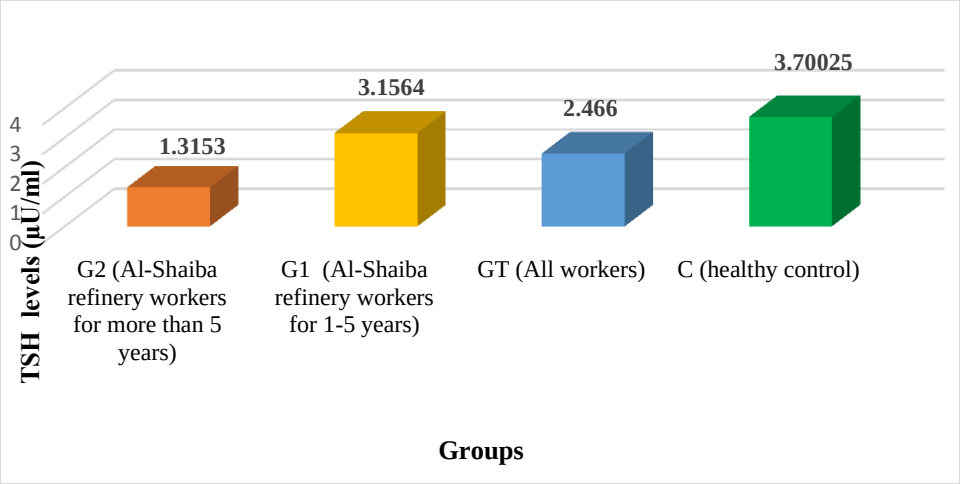

Figure (7): Serum TSH level of Alshaiba oil refinery worker and healthy control.

Results indicated in table (1) and figure (7) showed that there is a significant decrease in TSH level in Worker with direct exposure of pollution sources for more than 5 years of exposure than worker with direct exposure to pollution sources for only 1-5 years of exposure. Results showed that there is a significant effect in G2 group and GT group workers than G1 group who had a little year of exposure to oil pollutant and healthy control group. Health effects of occupational exposure to petrol chemicals are fairly unfamiliar among petrol filling workers. However, continued exposures for petrol chemicals might be the cause of well-known consequence of bone marrow suppression, hematological lethal effect (Getu *et al.*, 2020). A comparison was made by 99. Al-Hulfi, (2022) regarding smoking habits between the oil refinery and gas stations with the control group. The results showed a high significant decrease in TSH hormone in non-smokers in both the oil refinery and gas station when being compared with the

control group. Also, a highly significant decrease was shown when comparing smokers in both oil refinery and gas workers' hormones TSH in comparison to control group hormones.

3.4 Determination of Parathyroid hormone (PTH) levels.

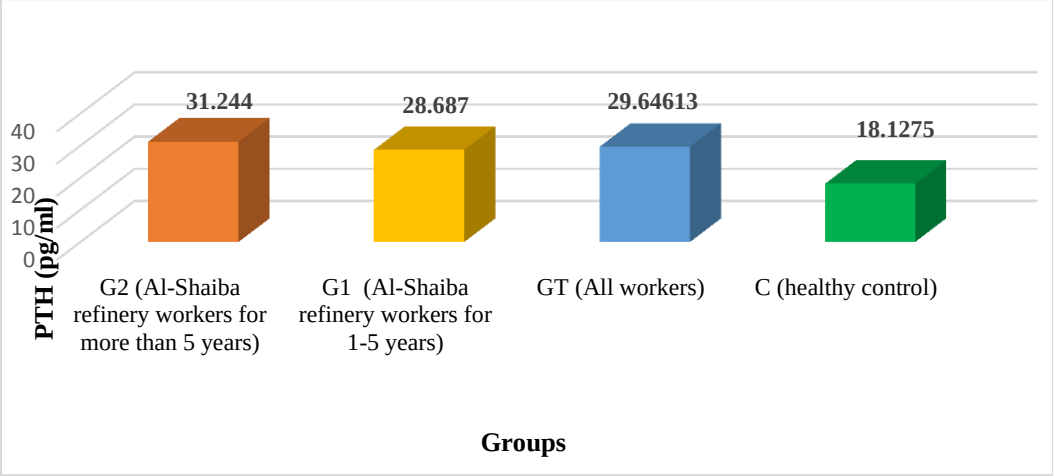

Figure (8): Serum PTH level of Alshaiba oil refinery worker and healthy control.

Results indicated in Figure (8) showed that there is a significant increase in PTH levels in all worker groups than in healthy control. It showed that the maximum increase was shown in G2 group which exposed directly to oil pollutant for more than 5 years than G1 and GT groups. Which might be due to the effect of pollution on parathyroid metabolism.

Table (2): The levels of T3, T4, TSH, and PTH levels in Al-Shaiba oil refinery workers and healthy control

Groups	Hormonal study			
	T3 (nmol/L)	T4 (nmol/l)	TSH (µU/ml)	PTH (pg/ml)
G2 Worker with direct exposure of pollution sources for more than 5 years of exposure	1.583 a	146.946 a	1.3153 a	31.244 a
G1 Worker with direct exposure to pollution sources for only 1-5 years of exposure (G1)	2.4618 b	88.601 b	3.1564 b	28.687 b
GT Workers with direct exposure to pollution elements (GT) (All workers)	2.132 b	110.480 c	2.466 c	29.646 b
C Healthy Control (without any exposure to pollutant sources)	2.317 b	97.441 d	3.7002 d	18.127 c
LSD value	0.483 **	32.378 **	0.893 **	7.503 **
P-value	0.0096	0.0001	0.0006	0.0001
Means having with the different letters in same column differed significantly, ** (P≤0.01).				

4. Conclusion

The study concluded that Workers with direct exposure of pollution sources for more than 5 years of exposure has direct effect of oil refinery pollutants than worker with direct exposure of pollution sources for only 1-5 years of exposure.

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دراسة حيوية كيمائية لهرمونات الغدة الدرقية والجار درقيه في دم العاملين في مصفى الشعيبة

لمى صبري مجيد ، فاطمة صيوان صباح

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الخلاصة

تعتبر هرمونات الغدة الدرقية ضرورية للنمو وتطور الخلايا العصبية والتكاثر وتنظيم طاقة الايض. يعتبر قصور الغدة الدرقية وفرط نشاط الغدة الدرقية من الحالات الشائعة التي لها عواقب صحية مدمرة يمكن أن تؤثر على جميع السكان في جميع أنحاء العالم. هدفت هذه الدراسة إلى تقييم تأثيرات التعرض للغازات والأبخرة والمواد الملوثة على العاملين في مصفاة النفط في الشعيبة / البصرة-العراق. إلا أنه تم جمع عينات من العمال الذكورتتراوح أعمارهم (55 – 22) سنة وقسمت إلى أربع مجموعات حسب سنوات خبرتهم في نفس المصفاة ومقارنتها بالسيطرة الصحية. أبرزنا أيضًا أن هرمون الغدة الدرقية (T4 ، TSH) و PTH يتأثر بالمعادن الثقيلة أكثر من هرمون T3 مقارنة بمجموعة التحكم الصحية. وأظهرت الدراسة أن العامل الذي يتعرض بشكل مباشر لمصادر التلوث لأكثر من 5 سنوات من التعرض أكثر تأثراً بملوثات مصفاة النفط من العمال الذين يتعرضون له لمدة 1-5 سنوات فقط.