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Morphological and Immunohistochemical Changes in Thyroid Gland Due to Exposure of Formalin in Albino Rats

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Paiman Jamal Muhammed Ameen[1] **Nali AbdulQader Ma'aroof**[2]

Abstract:

Background: Formaldehyde is the most widely used chemical in daily life; thus, chronic exposure to formaldehyde has been revealed to have negative effects on different organs in humans and animals.

Objective: This research was designed to investigate variable thyroid changes arising from chronic formaldehyde exposure by measuring different histomorphometry parameters in accordance with estrogen receptor and S-100 protein expression.

Materials & Methods: In this experiment, two hundred (200) rats were used and divided into two groups (100 male and 100 female), each group was subdivided into control and experimental groups each with 50 rats. The rats were exposed to formaldehyde using (20 ml =92.6ppm formaldehyde), 5hrs / d, for 21 days. Animals were sacrificed, and thyroid sections were examined for histomorphometry using H&E stain and immunohistochemistry for localization of estrogen receptor and S-100 protein **Results:** Significant decrease obtained in histomorphometrical measurements in the area of the colloids, area of follicles and width, height and number of the follicles in the cells of both experimental groups with no significant effect of gender in both control and experimental groups except in the height of the cells which showed significant decrease in female more than male. Formaldehyde exposure showed no significant effect on localization of estrogen receptor but significant increase of S-100 protein localization in both male and female groups. **Conclusion:** Formaldehyde had similar effect on histological structure of thyroid gland in both sexes causing disruption of thyroid follicles. Exposure of

[1]**Hawler medical university-college of medicine**
[2]**Hawler medical university-college of medicine**

Formaldehyde had no effect on estrogen receptor but caused an increase in S-100 protein localization in both sexes.

Key words: Formaldehyde, Rat, Estrogen receptor, S-100 protein

Introduction

Formaldehyde is a colorless substance, flammable, polymerized as a gas at normal room temperature(1). The vapors are highly pungent, respiratory irritant and produces rhinorrhea and discharge of water from eye on direct exposure (2). Formaldehyde concentration is usually explained as parts per million (ppm; 1 ppm¼ 1.25 mg/m3), and 40%–50% of its aqueous solution is called formalin (3). A potential risk for occupational and environmental exposure is due to the widely uses of formaldehyde in building materials, textiles, insulation, and other industries(2). Extensive exposure of formaldehyde gas to human occurs in up to 1 ppm concentrations(4). The International Agency for Research on Cancer (IARC) has recently classified formaldehyde as "carcinogenic in humans" (class 1), but still widely used in anatomy and pathology departments in fixation and preservation process of biological tissues(1). The present study is based on fact that the formalin is being used universally in various field. Person working in rubber industries, dyeing, all laboratory workers, all medical students, teachers and staff working in Anatomy and Pathology department remain exposed to hazardous and deleterious effect of formalin.

The aim of the current study is to explore the histopathologic lesions in the rat thyroid gland by chronic exposure to formaldehyde vapor.

MATERIALS AND METHODS

Study Area:

The duration of the study was from October 2012 to June -2013, into two different locations; starting from housing and exposing the rats to formaldehyde vapor in the animal house of College of Medicine/Hawler Medical University; ended by scarifying and biopsy taking from thyroid glands, and stained with Hematoxylin and Eosin (H&E).

Animal model:

Two hundred Wister albino rats approximately 8 – 16 weeks (100 male and 100 female) weighting (200 -300) gm were used in this study under supervision of staff of Animal House. The animals were adapted for one week to the laboratory conditions before the experimentation which was approved by the local scientific committee in the college. The plastic cages with wooden chips were used in housing the rats. The rats were treated in according to the standard guides of laboratory animals (5). During the experimental period twelve large cages were used (each contained 15 rats) and four small cage (each contained 5

rats), 12:12 light/dark photoperiod (LD) at 22 ± 2 ºC (6). Standard rat pellets were given to the animals, which was formulated by using a computer program depending on Pico Lab. Rodent Diet 20 as the following: sun flower oil 4.4%, methionine 0.158%, wheat 66.6%, soya 25.6%, choline chloride 0.062%, lime stone 1.5%, salt 0.63%, and trace elements 0.05%.

Experiment design

The rats divided into two groups (100 male and 100 female), each group was subdivided into control and experimental group each with 50 rats. One hundred healthy Wister albino rats (50 male and 50 female) were selected to study the effects of FA exposure on the thyroid gland, 20 ml = 92.6ppm of FA placed in flat glass and located 30 cm from the ground box avoiding to drink the solvent during exposure. The prisoned rats were allowed to eat and drink freely during the 5 hours of exposure time and FA exposure repeated for 21 days, 5 hours daily. The animals were sacrificed after exposure period, neck dissection done and thyroid gland removed, 10% formalin was used for overnight fixation.

In the control groups, the rats were bred under normal condition, one hundred normal healthy Wister albino rats (50 male and 50 female) were sacrificed and the samples compared with that of experimental animals.

Sampling method:-

Hematoxylin and eosin staining (H&E) :

At the end of the experiment, ketamine hydrochloride (100 mg/Kg) and xylazine were used to euthanize the animals. Thyroid specimens were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed. The tissues were embedded in Paraffin, then 5µm thickness of sections were obtained and stained with hematoxylin and eosin for detection of any abnormal lesions that have been formed as a result for formaldehyde exposure (7).

Immunohistochemical studies:

for the immunohistochemical detection of estrogen receptor and S-100 protein expression, the Dako cytomation EnVision(r) Dual link system-HRP (DAB+) or (AEC+) staining protocol was used for appliction to formalin fixed, paraffin embedded tissues. Positive expression of immunostaining gave clear cut nuclear or cytoplasm stained brown color. The determination of positive cells done by counting 1000 thyroid tissue. All significantly stained tissue cells considered as positive, to obtain the percentage (immunostaining index) the values divided by 10; for each section at least 10 HPFs were measured for the purpose of scoring. The extent of S100 immunostaining was assessed; Negative (cut of point) when ER or S100 index < 5, weak positive (cut off point) when ≤ 6-15 and strong positive when ≥ 16 (8).

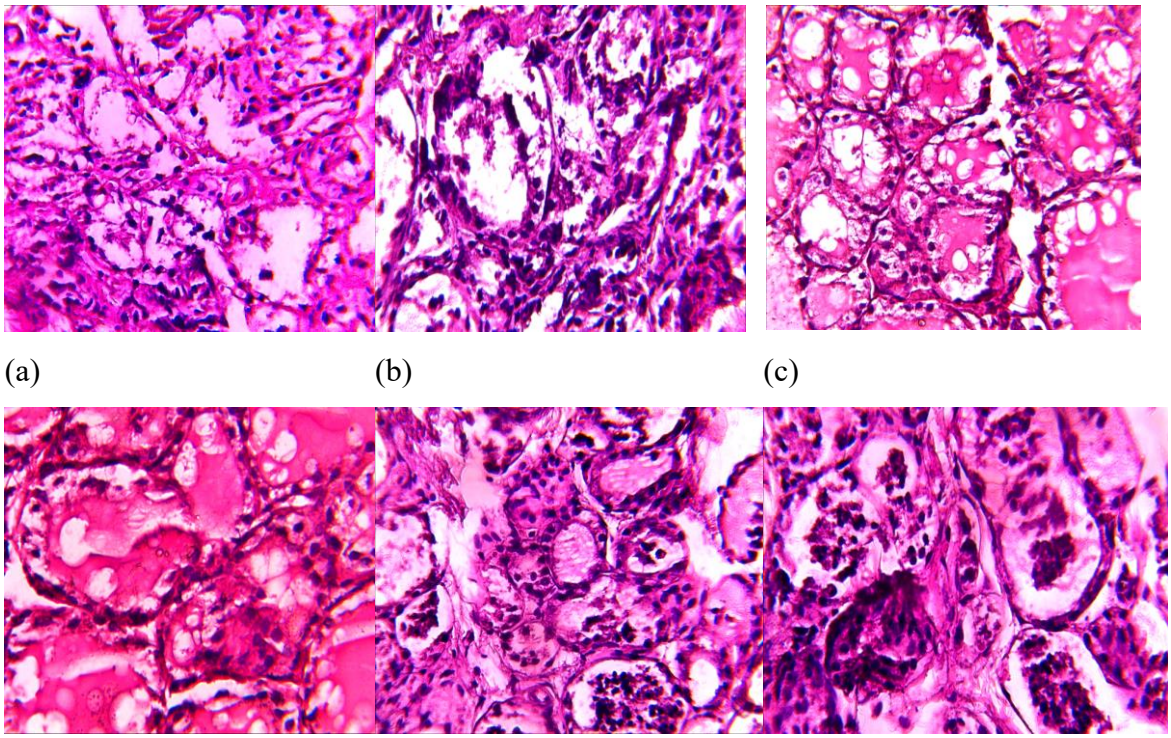
Statistical Analysis

Statistical analysis performed using statistical package for social sciences (SPSS) version 21, using independent samples t test. p value of ≤ 0.05 was regarded as statistically significant.

Results:

Histomorphomertical changes:

By using light microscope thyroid sections from the experimental group showed marked distortion of follicular architecture, follicles had exfoliated cells in their lumens which were more obvious in the central group of follicles. Vacuolization of the colloid and the follicular cell was noted and some follicles were devoid of colloid, as shown in figure (1).



(a) (b) (c)

(d) (e) (f)

Figure 1:

Cross section from rat thyroid gland, H&E X400; (a) in male rat, after FA exposure showing loss of normal follicular pattern. (b) in female rat, after FA exposure showing loss of normal follicular pattern. (c) male rat in experimental group, showed with vacuolated follicular cells lining thyroid follicles (blue arrows) with vacuolated colloid (yellow arrow). (d) in experimental group female rat, showing thyroid follicles lined with vacuolated follicular cells (blue arrows) and having vacuolated colloid (yellow arrow). (e) in experimental male rat, showing exfoliated cells in the lumen of the follicles (green arrows). (f) in experimental female rat, showing exfoliated cells in the lumens of the thyroid follicles (green arrows).

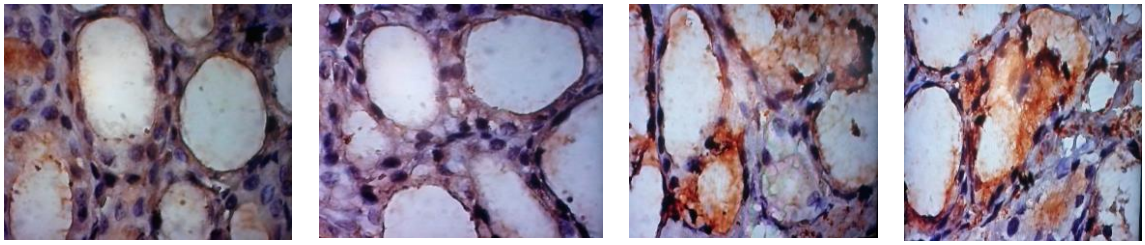
The most conspicuous morphological changes within the thyroid gland in animals sacrificed after three weeks exposure to FA 5hrs/d as compared to control group there was highly significant decrease in the area of colloid, area of the follicle and width of the cells with a highly significant increase in the height of the cells and number of the follicles in comparison to the control group in both male and female as delineated in table (1).

Table 1: The effect of FA on the morphological variable in both male and female groups of rats.

Sex		Groups	N	Mean	Std. Error	Std. Deviation	t-test	d.f	P-Value
Male	Area of colloid	Control	500	100%	0.000	0.000	78.84	998	0.00 HS
		Experimental	500	52.9%	0.597	13.35			
	Area of follicle	Control	500	2359.6	24.04	537.5	10.18	998	0.00 HS
		Experimental	500	1810.9	48.24	107.87			
	Width of cell	Control	500	7.95	0.027	0.620	36.75	998	0.000 HS
		Experimental	500	6.12	0.414	0.925			
	Height of cell	Control	500	8.41	0.183	4.099	-7.184	998	0.000 HS
		Experimental	500	9.76	0.4412	0.986			
	Number of follicle	Control	50	9.68	0.277	1.963	-9.164	98	0.000 HS
		Experimental	50	14.52	0.45	3.176			
Female	Area of colloid	Control	500	100%	0.000	0.000	76.86	998	0.000 HS
		Experimental	500	53.69	0.602	13.47			
	Area of follicle	Control	500	2393.3	24.47	547.1	10.34	998	0.000 HS
		Experimental	500	1809.4	48.24	107.8			
	Width of cell	Control	500	8.08	0.533	1.193	28.966	998	0.000 HS
		Experimental	500	6.13	0.415	0.924			
	Height of cell	Control	500	7.54	0.049	1.103	-23.18	998	0.000 HS
		Experimental	500	9.14	0.045	1.019			
	Number of follicle	Control	50	9.72	0.271	1.917	-9.45	98	0.000 NS

Immunohistochemistry:

The result indicated that the estrogen receptor was not expressed in the epithelial cell of male and female thyroid tissue in both control and experimental groups of rats, as shown in figure: 2.



(a) (b) (c) (d)

Fig 2: thyroid gland cross sections (X1000); (a) male rat in control group, with IHC stain showing no localization for estrogen receptor. (b) control group female, with IHC stain showing no localization for estrogen receptor. (c) experimental group male, with IHC stains showing no localization for estrogen receptor. (d) experimental group female, with IHC stains showing no localization for estrogen receptor.

Localization of S-100 protein

The result of the study showed that there was significant increase in S-100 protein expression in thyroid gland sections of experimental group which showed strong positive in both male and female with mean value (37.5%) and (35.5%) respectively when compared with control group which showed weak positive with mean value (9.7%), (10.1%) respectively, as shown in table (2); figure (3). Also the result shows that there was no significant effect of gender on S-100 protein expression, as shown in table (3) .

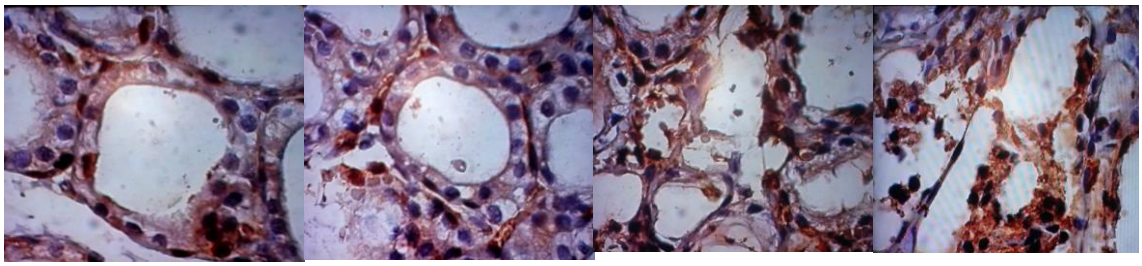
Table 2 : The expression of S-100 protein in control and experimental groups

Sex	Groups	N	Mean	Std. Deviation	Std. Error Mean	t-test	d.f.	P-Value
Male	Control	50	9.7	1.35	0.19	-22.0	98	0.00
	Experimental	50	37.5	8.8	1.24			
Female	Control	50	10.1	1.89	0.26	- 18.1	98	0.00

Table 3 : The effect of gender on S-100 protein expression

Groups	Sex	N	Mean	Std. Deviation	Std. Error Mean	t-test	d.f.	P-Value
Control	Male	50	9.7	1.89	0.26	1.33	98	0.18

	Female	50	10.1	1.35	0.19			
Experimental	Male	50	37.5	9.72	0.26	- 1.01	98	0.28
	Female	50	35.5	8.82	1.37			



(a) (b) (c) (d)

Fig 3: Section from thyroid gland (X1000); (a) male rat, control group showing weak positive cytoplasmic localization of S-100 protein with brown color (blue arrow). (b) female, control group showing brown color (blue arrow), weak positive cytoplasmic localization of S-100 protein. (c) male rat, experimental group showing strong positive cytoplasmic localization of S-100 protein with brown color (blue arrows). (d) female rat, experimental group showing strong positive cytoplasmic localization of S-100 protein with brown color (blue arrows).

Discussion

The light microscopical examination of thyroid glands of the experimental group revealed marked distortion of follicular structure showing loss of normal architecture of thyroid gland, exfoliated cells have been seen in lumen of some follicles. due to destruction of thyrocytes, in some areas there was loss of the follicular pattern. Thyroid gland morphometrical analysis of both male and female rats exposed to FA have revealed a marked decrease in the area of colloid, area of follicle, and width of the cell with a marked increase in the height of the cell and number of the follicles in comparison to the control group in both male and female rats. These changes were more obvious in central group of follicles. These results are corresponding with that of other studies which were conducted by (9, 10, 11). A study by (11) stated that the localization of the C cells in the central regions of the thyroid gland lobes, where thyroid hormone synthesis and secretion seems to be higher than in the periphery of the lobes, because of being C cells as APUDE, which has a role in synthesizing amino acids in addition to their role in calcium homeostasis, somehow regulate (stimulate and/or suppress).

In the present study the central region of the thyroid was more affected than the periphery; this may be due to the presence of C cells in the central region of the thyroid gland. The hypothesis concerning

the mutual cooperation between parafollicular and follicular cells in both physiological and pathological conditions supported by several studies(12, 13, 14).

Examination of histological sections of all control rats showed that follicles of the thyroid glands varied in size and shape and each follicle surrounded by a layer of simple cuboidal to flat epithelium enclosing a cavity filled with colloid, and also there was no sex difference in microscopic appearance of the thyroid glands, except in height of epithelium. The result of the present study is in accordance to that described by other authors (15, 16, 17, 18, 19).

In this study IHC for detection of estrogen receptor in normal and experimental rats and evaluated the effect of genders. It was observed in the present study that there was no detection of ER in both control and experimental groups and there was no effect of gender. This result is in agreement with the results of studies achieved by (20, 21, 22), and in disagreement with (23).

Hiasa (24) concluded that the incidence of ER does not significantly differ in males and females thyroid gland. Also, Valle (25) mentioned that ER concentration in the human thyroid gland is very low as to make a direct responsiveness to circulating estrogens questionable. So, the effect of estrogen on thyroid gland may be indirect.

In this study, S-100 protein stained focally with weak positive in cytoplasm of healthy thyroid follicular cells and strong positive cytoplasm in experimental thyroid follicular cell in both male and female groups with no significant effect of gender. This result was in agreement with (26, 27, 28).The expression of S-100 protein most probably to be up regulated or down regulated in different pathological conditions of the thyroid. However, extract function and mechanism of action of S-100 protein remained largely unknown, but it was suggested that S100 played a vital role in the progression of inflammation of thyroid follicles, so the inflammation of the thyroid gland due to FA exposure may cause increase in the number S-100 protein in the thyroid follicles. Also, the hyper active state of thyroid gland after FA exposure may be another factor caused increase S-100 protein (26).

Conclusion:

FA had similar effect on histological structure of thyroid gland in both sexes causing disruption of thyroid follicles. Exposure of FA had no effect on estrogen ER but caused an increase in S-100 protein localization in both sexes.

Conflict of Interest

The author declare that there is no conflict regarding this publication.

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