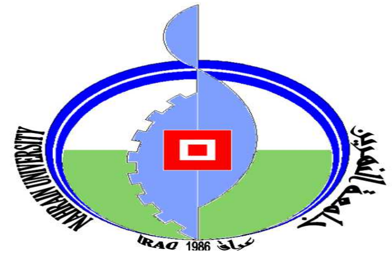
Ministry of Higher Education and Scientific Research
Al- Nahrain University
College of Science



The Relationship between Thyroid Disorders and *Helicobacter pylori* Infection

A Thesis

Submitted to the College of Science /Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology

By
Sarah Talib Kadhem Al- Mofarji
B.Sc. Biotechnology / College of Science /Al - Nahrian University
(2010-2011)

Supervised by
Dr.Abdulwahid B.Al-Shaibani (Professor)
Dr. Sanad B. Al A'araji (Assist. Prof.)

August 2013 Ramadan 1434

الاهــــــــــــــــداء

الى ينبوع العطاء الى من علمتني ابجدية الحروف

الى من كان دعائها سر نجاحي الى بسمة الحياة

الى من علمتني بأني خلقت للنجاح وليس للفشل الى من يركع العالم امام عطائها الى الغالية التي لا ارى اشراقة الامل الا في عينها

امـــــــي الغاليـــــة

الى من انعم عليه الله بالهيبة والوقار الى من علمني العطاء دون انتظار الى من احمل اسمه بكل افتخار الى من منحنا الحب والاحترام الى من لا تفيه كلمات الشكر والعرفان الى روحه الطاهرة شكرا وامتنانا

ابـــــــي الغالـــي

الى استاذيّ الفاضلين الدكتور عبدالواحد باقرالشيباني والدكتور سند الاعرجي بكل فخر اذكرهم بالوفاء والعرفان لدعمهم وتشجيعهم المتواصل.

الى من اعتبرها سندي في الحياة **اختي الغالية رشا**

الى من سرنا نشق طريق النجاح سويا الى من تحلو بالإخاءوتميزوا بالوفاء والعطاء

زميلاتي وزملائي

اهدي ثمرة جهدي المتواضع

ســـــــــارة

Acknowledgment

Praise to God the first cause of all causes the glorious creator of this Universe for his mercy and kindness .

I owe my thankful , respect and appreciation to my supervisors Professor Dr. Abdulwahid B. Al-Shaibani and Assistant Professor Dr. Sanad B. Al-A'araji for their great help and advices during this work .

I would like to express my great full thanks to Dr. Khalid Al-Lehibi and to the staff members of the Specialized Center for Endocrinology and Diabetes at Al-kindy Hospital in Baghdad for their help.

Sincere thanks to the staff members of the Immuonology lab at the Central Public Health Laboratories / Ministry of Health for their help and assistance .

I abundant appreciative to Dr. Abdul Khaliq Al-Naqeeb at the College of Health and Medical Technology for his efforts in modifying and approving the statistical analysis of the research data.

Deepest thanks to all staff of Biotechnology Department / College of Science / Al – Nahrain University .

Sarah

Chapter One

Introduction

and

Literatures Review

Chapter Two

Materials and Methods

Chapter Three

Results and Discussion

Chapter Four

Conclusions and Recommendations

References

Appendices

4.1 Conclusions:

- Thyroid disorders were increased with age of patients and females more susceptible than males.
- There was a correlation between thyroid disorders and high body weight.
- Cholesterol was found to be related to the high density lipoprotein (HDL) levels in patients of thyroid disorders.
- Thyroid disorders patients were more susceptible for *Helicobacter pylori* infection.
- *Helicobacter pylori* incidence rate increased in the hypothyroidism patients more than in hyperthyroidism ones.

4.2 Recommendations:

- More studies are needed on the association between peptic ulcer caused by *Helicobacter pylori* and thyroidism.
- Anti-TPO (thyroid peroxidase) enzyme in thyroidism patients and its role in *H. pylori* infection need to be more investigated.
- Studies are needed on the association between diabetic mellitus ,thyroidism and *H. pylori* infection.
- More studies are need on the association between subclinical hypothyr-oidism ,hyperthyroidism and *H. pylori* infection.
- Genetic studies are needed on the relationship between thyroid disorders and gastric carcinoma caused by *H. pylori.*

Appendix (I) Questionnaire form.

Questionnaire form for thyroid patients

Patient name: …………………………………….. Case No. : ……………….

Age: ☐ yrs. Weight: ☐ kg Height: ☐ cm

Gender: ☐ Female ☐ Male Smoking: ☐ yes No ☐

Thyroid disorders: ☐ yes ☐ No

If yes, receiving and medication : ………………………………………………

Chronic diseases : ☐ yes ☐ No

If yes ……………………………………………………….

Receiving any medication: ☐ yes ☐ No

If yes ……………………………………………………………………….

Finding and Statistical Analysis Data.

Appendix (II) Multiple comparisons between each contrast among the studied samples according to age parameter by applying (Least Significant Different-LSD) method .

(I) Group	(J) Group	P-value	Comparison Significant (*)
Healthy	Controlled	0.005	HS
	Hypothyroidism	0.000	NS
	Hyperthyroidism	0.058	NS
Controlled	Hypothyroidism	0.663	NS
	Hyperthyroidism	0.639	NS
Hypothyroidism	Hyperthyroidism	0.383	NS

(*) HS: Highly Significant at P< 0.01 ; NS: Non Significant at P>0.05

The results showed that significant different were reported at the contrasts (healthy, controlled) and (healthy, hypothyroidism) at P<0.01, while the leftover contrasts has no significant differences .For summarizing the preceding results, it can concluded that the studied samples had been unrestricted selection, since they were submitted to the random selection according to age parameter.

Appendix (III) Multiple comparisons between each contrast among the studied samples according to gender parameter by applying (Crosstabs procedure forms association for two-way)

(I) Group	(J) Group	Contingency coefficients	P-value	Comparison Significant(*)
Healthy	Controlled	0.131	0.187	NS
	Hypothyroidism	0.399	0.000	NS
	Hyperthyroidism	0.323	0.002	HS
Controlled	Hypothyroidism	0.309	0.001	HS
	Hyperthyroidism	0.247	0.040	S
Hypothyroidism	Hyperthyroidism	0.050	0.650	NS

(*) **HS: Highly Significant at P< 0.01 ; NS: Non Significant at P>0.05**

The results showed there were non significant different were reported at the contrasts (healthy, controlled), and (hypothyroidism, hyperthyroidism) , while the leftover contrasts had significant differences at (p < 0.01). From the preceding results, it can observed that female constructed the vast majority of frequencies for the patients either who had (hypothyroidism or hyperthyroidism) disease, and they are accounted 52(91.23%) and 22(88.0%) respectively. For summarizing the preceding results, it can concluded that the studied samples had been unrestricted selection, since they were submitted to the random selection according to gender parameter.

Appendix (IV) Multiple comparisons between each contrast among the studied samples according to BMI parameter (Least Significant Different-LSD) method.

(I) Group	(J) Group	P-value	Comparison Significant (*)
Healthy	Controlled	0.844	NS
	Hypothyroidism	0.239	NS
	Hyperthyroidism	0.730	NS
Controlled	Hypothyroidism	0.389	NS
	Hyperthyroidism	0.632	NS
Hypothyroidism	Hyperthyroidism	0.212	NS

(*) NS: Non Significant at P>0.05

The results showed there were non significant different were reported at the studied contrasts , which indicating that the studied individuals having discerned too highly increases in their body's weights. For summarizing the preceding results, it can conclude that the studied samples had been unbiased selection, since they were similarly selected according to BMI parameter.

Appendix (V) One - Way ANOVA for testing equality of Means and Levene Test for equality of variances for Thyroids Function Parameters among the Studied samples

Parameters	Levene's Test for Equality of Variances		ANOVA for Equality of Means		Comparison Significant
	F-statistics	Sig. (*)	F - statistics	Sig. (*) (2-tailed)	
T3	1.182	0.321	5.179	0.002	HS
T4	2.450	0.066	4.066	0.008	HS
TSH	76.070	0.000	50.746	0.000	NS

(*) HS: Highly Significant at P< 0.01

Appendix(V) showed the results of ANOVA technique for testing equality of mean values of thyroids function (T3, T4, and TSH) parameters among different of the studied samples. The result showed that highly significant different at(P<0.01) were reported and that results is not enough to reject the statistical hypothesis, which says that the four groups having corresponding proportionally /or whether the different groups were seems to be drown from the same population, and for exploring the nature of real /or actual differences among that different samples, multiple comparisons through applying suitable method should be used in order to illustrated the probability levels of rejected the statistical hypothesis when it is true, and that could be concluded there is at least within one pair of samples are not equal, and with respect to that we needs to be continuing the test of comparisons by using the Least Significant Difference (LSD) test, which were illustrated in the next appendix(VI).

Appendix (VI): Multiple Comparison (LSD) among all pairs of Thyroids Function (T3, T4, and TSH) parameters According to different treated samples

(I) Group	(J) Group	T3	T4	TSH
		P-value(*)	P-value(*)	P-value(*)
Healthy	Controlled	0.009	0.077	0.829
	Hypothyroidism	0.001	0.781	0.000
	Hyperthyroidism	0.846	0.005	0.458
Controlled	Hypothyroidism	0.578	0.048	0.000
	Hyperthyroidism	0.053	0.210	0.386
Hypothyroidism	Hyperthyroidism	0.015	0.003	0.000

(*) HS: Highly Significant at P< 0.01 ; NS: Non Significant at P>0.05

Regarding to the subjects of T3 parameter, the results of shaded cells exploring significant different at (P<0.01) between (healthy, controlled), (healthy, hypothyroidism), and significant different at(P<0.05) between (hypothyroidism, and hyperthyroidism) samples, while the leftover were reported no significant . With respect to the subjects of T4 parameter, the results of the shaded cells exploring significant different at (P<0.01) between (healthy, hyperthyroidism), (hypothyroidism, and hyperthyroidism), and significant different at(P<0.05) between (controlled, hypothyroidism) samples, while the leftover were reported no significant .Relative to subject of TSH

parameter, the results of the shaded cells exploring significant different at(P<0.01) between (healthy, hypothyroidism), (controlled, and hypothyroidism), and (hypothyroidism, and hyperthyroidism) samples, while the leftover were reported no significant .

Appendix (VII): Summary Statistics of Lipids Profiles Parameters at different of the Studied Samples

Parameters	Groups	No.	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Min.	Max.
						L. B.	U. B.		
Cholesterol	Healthy	60	180.7	42.7	5.5	169.7	191.7	116	278
	Controlled	40	188.7	50.3	8.0	172.6	204.7	106	295
	Hypothyroidism	57	190.4	41.5	5.5	179.4	201.5	113	302
	Hyperthyroidism	25	157.3	24.2	4.9	147.3	167.3	113	197
Triglycerides	Healthy	60	173.5	99.8	12.9	147.7	199.3	79	609
	Controlled	40	176.3	61.6	9.7	156.6	196.0	97	325
	Hypothyroidism	57	166.6	40.9	5.4	155.8	177.5	77	327
	Hyperthyroidism	25	149.4	41.0	8.2	132.5	166.4	96	259
HDL	Healthy	58	55.8	23.3	3.1	49.7	61.9	21	125
	Controlled	40	51.2	20.8	3.3	44.6	57.9	26	118
	Hypothyroidism	55	44.9	15.9	2.1	40.6	49.2	24	98
	Hyperthyroidism	25	41.2	13.5	2.7	35.7	46.8	18	77
LDL	Healthy	58	88.8	40.1	5.3	78.2	99.3	13.8	193.6
	Controlled	40	101.7	43.0	6.8	87.9	115.4	31.4	187.4
	Hypothyroidism	55	112.7	41.9	5.6	101.4	124.0	20.8	208
	Hyperthyroidism	25	87.3	21.8	4.4	78.3	96.3	40	130
VLDL	Healthy	58	34.7	20.1	2.6	29.4	40.0	15.8	121.8
	Controlled	40	34.8	11.7	1.9	31.1	38.5	19.4	65.0
	Hypothyroidism	55	33.4	8.2	1.1	31.2	35.6	15.4	65.4
	Hyperthyroidism	25	29.1	7.8	1.6	25.9	32.3	15.4	49.2

Appendix(VII) showed the summary statistics of different Lipids Profiles parameters readings at different of the studied samples. Relative to subject of BMI parameter, which indicating that the most of the studied individuals having discerned too highly increases in their body's

weights, the results indicated that Lipid Profiles parameters had reported dissent in somewhere statistics. Cholesterol parameter has reported some of readings at the studied samples full outside standard of normal interval at the two sided however mean value and their 95% confidence interval were reported normal estimates, except at the hyperthyroidism samples, which were registered dissent readings at the downstairs side only. Triglycerides parameter has reported some of readings at the studied samples full outside standard of normal interval at the upstairs side, as well as mean value and their 95% confidence interval were reported normal responding, and at the hyperthyroidism samples, which were registered normal estimate also .

HDL parameter has reported some of readings at the studied samples full outside standard of normal interval at the two side, as well as mean value and their 95% confidence interval were reported normal estimates, except at the hyperthyroidism sample, which were registered abnormal estimate at the lower bound of the confidence interval .LDL parameter has reported some of readings at the studied samples full outside standard of normal interval at the two side, as well as mean value and their 95% confidence interval were reported normal responding, except at the healthy, controlled, and hyperthyroidism samples, which were registered abnormal responding at the lower bound of the confidence interval estimates. Finally, VLDL parameter has reported some of readings at the studied samples full outside standard of normal interval at the upstairs side, as well as mean value and their 95% confidence interval, which were reported abnormal responding at the upper bound of the confidence interval estimates, except at the hyperthyroidism samples, which were registered normal estimate.

Appendix (VIII): One - Way ANOVA for testing equality of Means and Levene Test for equality of variances for Lipid Profile Parameters among the Studied samples

Random Periods	Levene's Test for Equality of Variances		ANOVA for Equality of Means		Comparison Significant(*)
	F-statistics	P-Value	F – statistics	Sig. (2-tailed)	
Cholesterol	**4.683**	**0.004**	**3.965**	**0.009**	**HS**
Triglycerides	**4.878**	**0.003**	**0.901**	**0.442**	**NS**
HDL	**3.414**	**0.019**	**4.661**	**0.004**	**HS**
LDL	**3.518**	**0.016**	**4.319**	**0.006**	**HS**
VHDL	**4.507**	**0.005**	**1.103**	**0.349**	**NS**

(*) HS: Highly Significant at P< 0.01 ; NS: Non Significant at P>0.05

Appendix(VIII) showed the results of ANOVA technique for testing equality of mean values of Lipid Profile (Cholesterol, Triglycerides, HDL , LDL, and VHDL). The results showed that there were non significant were reported with Triglycerides and VLDL parameters among different of the studied samples, rather than violations of the equal variance assumption had been occurred.

Relative to subject of the leftover parameters, highly significant differences were reported at P<0.01, and that result was not enough to reject the statistical hypothesis, which says that the four groups having corresponding proportionally /or whether the different groups were seems to be drown from the same population, and for exploring the nature of real /or actual differences among that different samples, multiple comparisons through applying suitable method should be used in order to illustrate the probability levels of reject the statistical hypothesis when it is true, and that could be concluded there was at least within one pair of samples are not equal, and with respect to that it needs to be continuing the test of comparisons by using the Least Significant Difference (LSD) test, which were illustrated in the next appendix(IX).

Appendix (IX) : Multiple Comparison (LSD) among all pairs of Lipid Profile parameters According to different treated samples

(I) Group	(J) Group	Cholesterol	Triglycerides	HDL	LDL	VLDL
		P-value(*)	P-value(*)	P-value(*)	P-value(*)	P-value(*)
Healthy	Controlled	0.358	0.841	0.258	0.113	0.979
	Hypothyroidism	0.214	0.598	0.003	0.001	0.612
	Hyperthyroidism	0.021	0.151	0.002	0.878	0.092
Controlled	Hypothyroidism	0.837	0.502	0.117	0.179	0.628
	Hyperthyroidism	0.004	0.133	0.045	0.155	0.109
Hypothyroidism	Hyperthyroidism	0.001	0.307	0.440	0.008	0.201

(*) **HS: Highly Significant at P< 0.01 ; NS: Non Significant at P>0.05**

Regarding to the subjects of Cholesterol parameter, the results of the shaded cells exploring significant different at (P<0.01) between (controlled, hyperthyroidism), (hypothyroidism, hyperthyroidism), and significant different at (P<0.05) between (healthy, and hyperthyroidism)

samples, while the leftover were reported no significant differences. With respect to the subjects of Triglycerides parameter, the results were exploring a non significant different among all multiple comparisons. Relative to subject of HDL parameter, the results of the shaded cells exploring significant different at (P<0.01) between (healthy, hypothyroidism), and (healthy, and hyperthyroidism) samples, and significant different at(P<0.05) between (controlled, and hyperthyroidism) samples, while the leftover were reported non significant differences.

Regarding to the subjects of LDL parameter, the results of the shaded cells exploring significant different at (P<0.01) between (healthy, hypothyroidism), (hypothyroidism, hyperthyroidism) samples, while the leftover were reported non significant differences.

With respect to the subjects of VLDL parameter, the results were exploring non significant different among all multiple comparisons

.

Appendix (X) Distribution of the *Helicobacter Pylori* responding at the different of the studied samples with comparison significant

Group	Freq. & Percents	*Helicobacter pylori*		Total	C.S. P-value
		Neg.	Pos.		
Healthy	Freq.	20	40	60	LRT=16.939 P=0.001 HS CC=0.272 P=0.002 HS
	% Group	33.3%	66.7%	100%	
	% *Helicobacter pylori*	51.3%	28%	33%	
Controlled	Freq.	9	31	40	
	% Group	22.5%	77.5%	100%	
	% *Helicobacter pylori*	23.1%	21.7%	22%	
Hypothyroidism	Freq.	3	54	57	
	% Group	5.3%	94.7%	100%	
	% *Helicobacter pylori*	7.7%	37.8%	31.3%	
Hyperthyroidism	Freq.	7	18	25	
	% Group	28%	72%	100%	
	% *Helicobacter pylori*	17.9%	12.6%	13.7%	
Total	Freq.	39	143	182	
	% Group	21.4%	78.6%	100%	
	% *Helicobacter pylori*	100%	100%	100%	

To predicting /or to find out the relationship /or association of *Helicobacter pylori* response's distribution among different of the studied samples, through the contingency coefficient had been constructed in appendix(X) , which were illustrated the contingency correlation coefficient with probability level (P-value) .The results showed that there were highly significant differences at (P<0.01) along the distribution of the two categories of *Helicobacter pylori* responding throughout the different of the studied samples.

Nevertheless of preceding result of exploring the meaningful relationship between the distribution of *Helicobacter pylori* responses throughout the studied samples, since that results were indicating in at least within one pair of samples that correlation had been obtained, and with respect to that it needs to be continuing for searching in which pairs of contrasts the studied samples constructing that correlation significant.

Appendix (XI): Distribution of the *Helicobacter Pylori* responding at the different contrasts of the studied samples with comparison significant

Contrasts	Contingency Coefficients	P-value	Odds Ratio	95% Confidence Intervals
Healthy X Controlled	**0.116**	**0.242**	**1.722**	**(0.689 ; 4.304)**
Healthy X Hypothyroidism	**0.333**	**0.000**	**9.000**	**(2.501 ; 32.39)**
Healthy X Hyperthyroidism	**0.052**	**0.630**	**1.286**	**(0.461 ; 3.583)**
Controlled X Hypothyroidism	**0.250**	**0.011**	**5.226**	**(1.316 ; 20.757)**
Controlled X Hyperthyroidism	**0.062**	**0.617**	**0.747**	**(0.237 ; 2.348)**
Hypothyroidism X Hyperthyroidism	**0.305**	**0.004**	**0.143**	**(0.033 ; 0.611)**

Regarding to the subjects of contrast (healthy X controlled) samples, the results exploring non significant differences, whereas an Odds Ratio in relation of that contrast shows that Neg. outcomes of *Helicobacter pylori* parameter increasing 1.722 times at the healthy samples compared with that reported at the controlled samples , and that estimate had a wide probable of the preceding outcomes according to the upper bound of the confidence interval.

With respect to subjects of contrast (healthy X hypothyroidism) samples, the results exploring highly significant differences at(P<0.01), and an Odds Ratio in relation of that contrast

showed that Neg. outcomes of *Helicobacter pylori* parameter increasing 9 times at the healthy samples compared with that reported at the hypothyroidism samples , and that estimate had too wide probable of the preceding outcomes according to the upper bound of the confidence interval.

Relative to the subjects of contrast (healthy X hyperthyroidism) samples, the results exploring non significant differences, whereas an Odds Ratio in relation of that contrast showed that Neg. outcomes of *Helicobacter pylori* parameter increasing 1.286 times at the healthy samples compared with that reported at the hyperthyroidism samples, and that estimate had a wide probable of the preceding outcomes according to the upper bound of the confidence interval.

With respect to subjects of contrast (controlled X hypothyroidism) samples, the results exploring highly significant differences at(P<0.01), and an Odds Ratio in relation of that contrast showed that Neg. outcomes of *Helicobacter pylori* parameter increasing 5.226 times at the controlled samples compared with that reported at the hypothyroidism samples , and that estimate had a wide probable of the preceding outcomes according to the upper bound of the confidence interval. Relative to the subjects of contrast (controlled X hyperthyroidism) samples, the results exploring non significant differences , and an Odds Ratio in relation of that contrast showed that Neg. outcomes of *Helicobacter pylori* parameter decreasing 0.747 times at the controlled samples compared with that reported at the hyperthyroidism samples, and that estimate had a revered wide probable of the preceding outcomes according to the upper bound of the confidence interval.

Finally, regarding to the subjects of the contrast (hypothyroidism X hyperthyroidism) samples, the results exploring highly significant differences at (P<0.01), and an Odds Ratio in relation of that contrast showed that Neg. outcomes of *Helicobacter pylori* parameter decreasing to 0.143 times at the hypothyroidism samples compared with that reported at the hyperthyroidism samples (i.e. increasing of Pos. outcomes of *Helicobacter pylori* parameter to 6.9993 times compared with that reported at the hyperthyroidism samples), and that estimate had too short probable of the preceding outcomes according to the lower bound of the confidence interval.

Appendix (XII): Person's Correlation Coefficients between (Age, BMI, T3, T4, and TSH) Parameters at the studied samples with comparison significant

Contrast	Correlation Coefficient & P-vale	Parameters	BMI	T3	T4	TSH
Healthy	Correlation	Age (Per year)	0.314	-0.432	-0.351	-0.095
		BMI		-0.182	-0.245	0.038
		T3			0.321	0.054
		T4				0.075
	Sig. (1-tailed)	Age (Per year)	0.052	0.011	0.034	0.315
		BMI		0.177	0.104	0.423
		T3			0.048	0.392
		T4				0.352
Controlled	Correlation	Age (Per year)	0.417	-0.056	-0.070	0.090
		BMI		-0.010	-0.019	-0.268
		T3			0.410	-0.077
		T4				-0.196
	Sig. (1-tailed)	Age (Per year)	0.017	0.392	0.367	0.331
		BMI		0.481	0.463	0.093
		T3			0.019	0.355
		T4				0.169
Hypothyroidism		Age (Per year)	0.505	-0.095	0.142	-0.247
		BMI		-0.239	-0.100	-0.097
		T3			0.300	-0.330
		T4				-0.659
	Sig. (1-tailed)	Age (Per year)	0.001	0.300	0.215	0.083
		BMI		0.091	0.289	0.296
		T3			0.045	0.030
		T4				0.000
Hyperthyroidism	Correlation	Age (Per year)	0.855	-0.238	-0.073	-0.277
		BMI		-0.127	-0.014	-0.075
		T3			0.607	0.170
		T4				0.260
	Sig. (1-tailed)	Age (Per year)	0.000	0.216	0.407	0.180
		BMI		0.340	0.482	0.404
		T3			0.014	0.290
		T4				0.195

The results showed that the shaded cells of each contrasts of two parameters at the Appendix (XII) illustrated the meaningful /or significant differences in at least ($P<0.05$), while the leftover of were reported no significant correlation coefficients and as follows :

Regarding to the subjects of the Age parameter, there was a significant reversed correlation had been reported with (T3, and T4) in the healthy samples, then followed significant correlation with (BMI) in the controlled samples , then significant correlation with (BMI) in the hypothyroidism and hyperthyroidism samples. With respects to the subjects of T3 parameter, there was a significant correlation had been reported with T4 in the studied samples, as well as reversed correlation with TSH parameter in the hyperthyroidism samples. Finally, T4 parameter was reported reversed significant correlation with TSH parameter in the hypothyroidism samples.

Appendix (XIII): Person's Correlation Coefficients between (Age, BMI, Cholesterol, Triglycerides, HDL, LDL, and VLDL) Parameters at the studied samples with comparison significant

Contrast	Correlation Coefficient and P-vale	Parameters	BMI	Cholesterol	Triglycerides	HDL	LDL	VLDL
Healthy	Correlation	Age (Per year)	0.321	0.518	0.408	0.237	0.229	0.408
		BMI		0.075	0.098	0.212	-0.073	0.098
		Cholesterol			0.374	0.353	0.687	0.374
		Triglycerides				0.055	-0.134	1.000
		HDL					-0.247	0.055
		LDL						-0.134
	Sig. (1-tailed)	Age (Per year)	0.007	0.000	0.001	0.037	0.042	0.001
		BMI		0.289	0.231	0.055	0.294	0.231
		Cholesterol			0.002	0.003	0.000	0.002
		Triglycerides				0.341	0.158	0.000
		HDL					0.031	0.341
		LDL						0.158
Controlled	Correlation	Age (Per year)	0.587	0.409	0.367	0.050	0.341	0.364
		BMI		0.211	0.161	-0.150	0.256	0.158
		Cholesterol			0.475	0.384	0.849	0.414
		Triglycerides				0.277	0.162	0.966
		HDL					-0.091	0.257
		LDL						0.089
	Sig. (1-tailed)	Age (Per year)	0.000	0.004	0.010	0.381	0.016	0.010
		BMI		0.096	0.161	0.178	0.055	0.165
		Cholesterol			0.001	0.007	0.000	0.004
		Triglycerides				0.042	0.159	0.000
		HDL					0.288	0.055
		LDL						0.293
Hypothyroidism	Correlation	Age (Per year)	0.351	0.174	0.214	0.136	0.099	0.199
		BMI		0.037	0.359	0.073	-0.003	0.356
		Cholesterol			0.290	0.039	0.904	0.293
		Triglycerides				-0.012	0.104	0.996
		HDL					-0.310	-0.005
		LDL						0.103
	Sig. (1-tailed)	Age (Per year)	0.004	0.103	0.059	0.161	0.237	0.073
		BMI		0.394	0.004	0.298	0.491	0.004
		Cholesterol			0.016	0.390	0.000	0.015
		Triglycerides				0.465	0.226	0.000
		HDL					0.011	0.487
		LDL						0.228
Hyperthyroidism	Correlation	Age (Per year)	0.645	0.059	0.152	0.205	-0.149	0.202
		BMI		0.034	0.200	-0.035	0.021	0.289
		Cholesterol			0.104	0.425	0.758	0.141
		Triglycerides				0.021	-0.242	0.941
		HDL					-0.154	0.075
		LDL						-0.230
	Sig. (1-tailed)	Age (Per year)	0.000	0.390	0.234	0.163	0.238	0.167
		BMI		0.435	0.168	0.433	0.461	0.081
		Cholesterol			0.310	0.017	0.000	0.251
		Triglycerides				0.460	0.122	0.000
		HDL					0.231	0.362
		LDL						0.134

The results showed that the shaded cells of each contrasts of two parameters at the Appendix (XIII) illustrated the meaningful /or significant correlation in at least $P<0.05$, while the leftover of were reported non significant correlation coefficients and as follows :

Regarding to the subjects of the Age parameter, there were a significant correlation had been reported with (BMI, Cholesterol, Triglycerides, LDL, and VLDL) in the healthy and controlled samples, and with (BMI) in the hypothyroidism and hyperthyroidism samples.

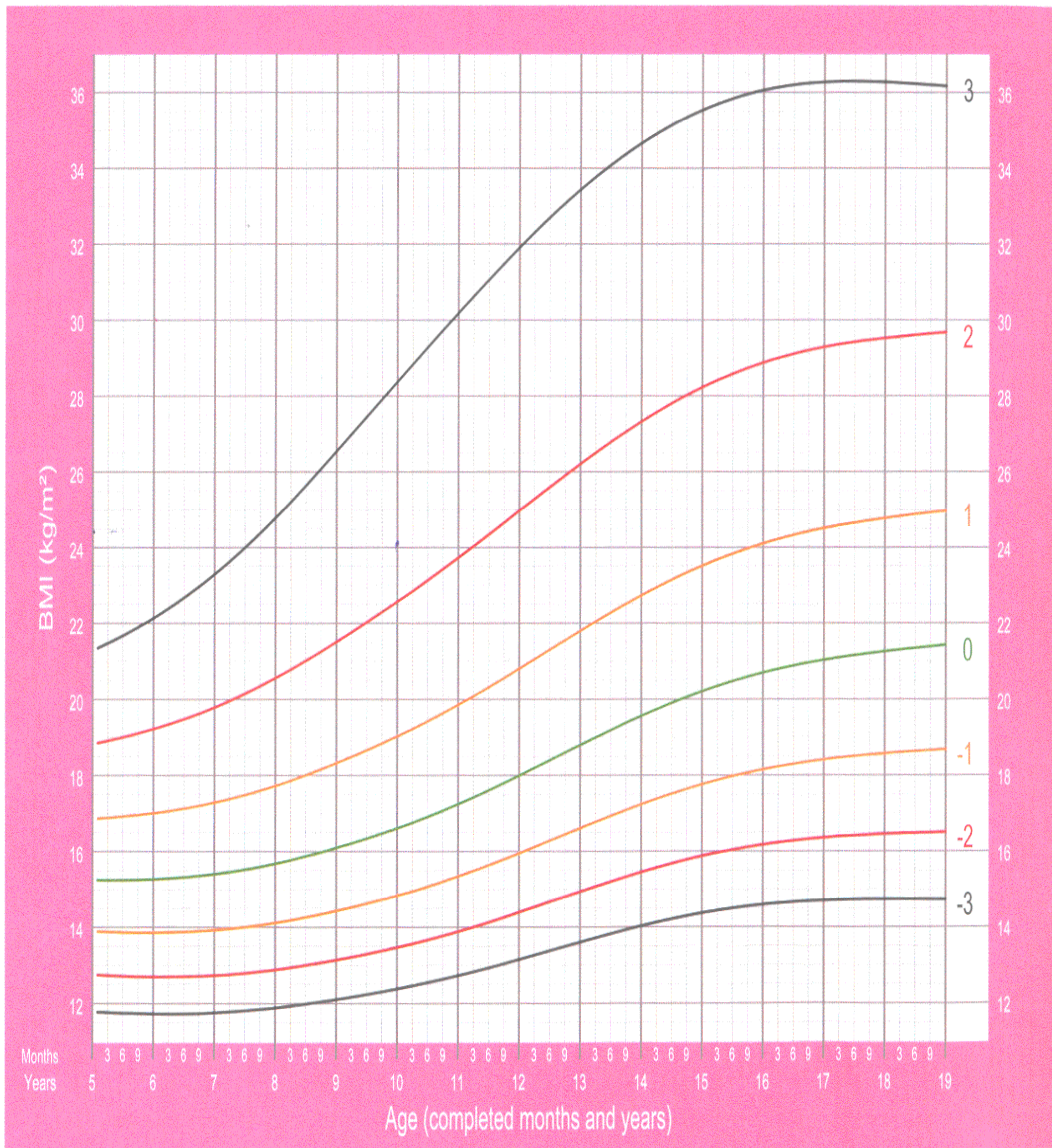
With respects to the subjects of the BMI parameter, there were significant correlation had been reported with (Triglycerides, and VLDL) in the healthy samples, then followed significant correlation with (BMI) in the hypothyroidism samples.

Related to the subjects of the Cholesterol parameter, there were significant correlation had been reported with (Triglycerides, HDL, LDL, and VLDL) in the healthy and controlled samples, then followed significant correlation with (HDL) in the hyperthyroidism samples.

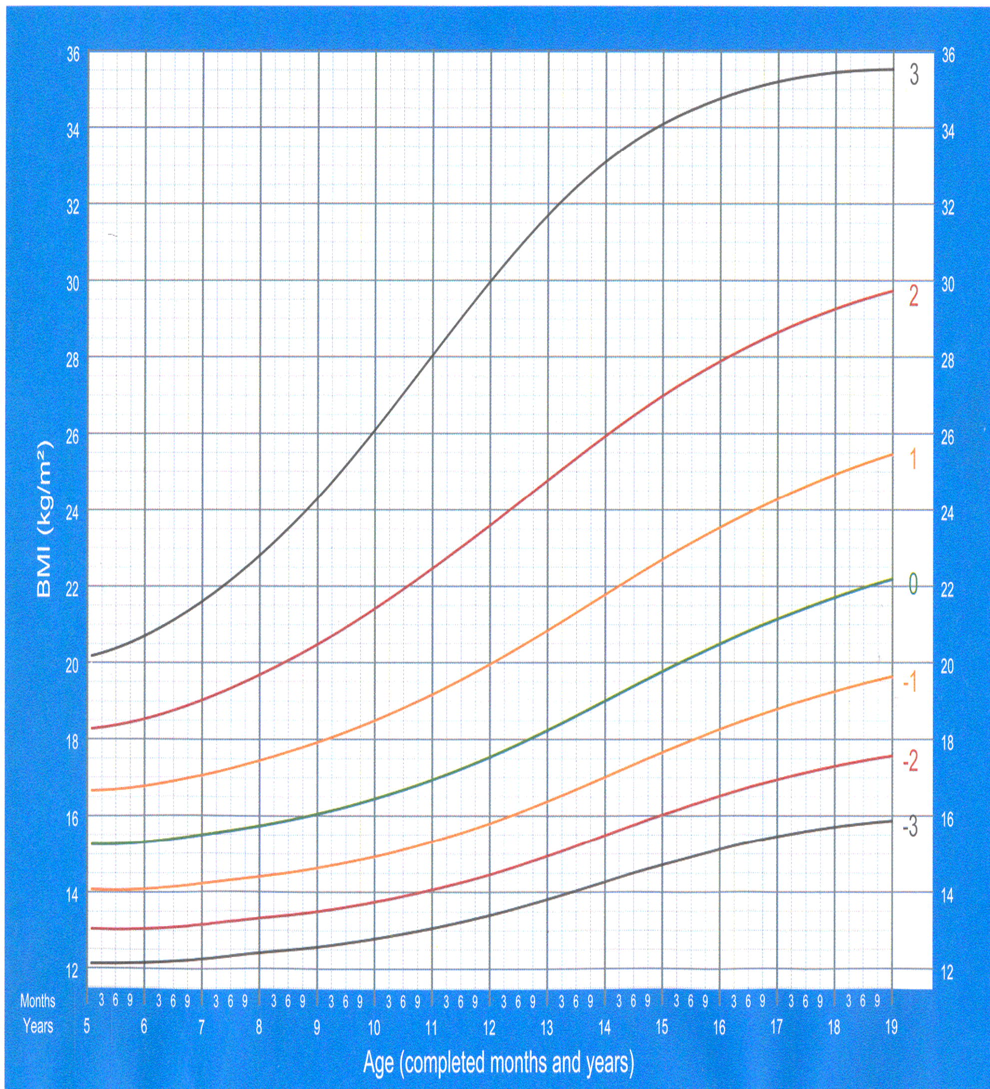
With respects to the subjects of the Triglycerides parameter, there were significant correlation had been reported with (VLDL) in the studied samples.

Regarding to the subjects of the HDL parameter, there were significant correlation had been reported with (LDL) in the healthy, and hypothyroidism samples.

Appendix(XIVa) Body Mass Index chart



Appendix (XIV b) Body mass index chart



Appendix(XV) The International Classification of adult underweight, overweight and obesity according to BMI

Classification	BMI(kg/m^2)	
	Principal cut-off points	**Additional cut-off points**
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Source: Adapted from WHO, 1995, WHO, 2000 and WHO 2004

1. Introduction and Literature Review

1.1 Introduction

Thyroid gland is one of the important organs in the human body that produces important hormones:triiodothyronineT3 and tetra-iodothyroxine T4 which have an important role in regulation of metabolic functions, development and growth. Thyroid dysfunction affecting various vital activities; those resulting from hypo or hyper thyroid gland activity leading to increase or decrease thyroid hormones T3 and T4 (Karnath and Hussain 2006) .

Hypothyroidism(Hashimotos' thyroiditis) and hyperthyroidism (Graves' disease) are the most common autoimmune thyroid disorders as one of most complications of thyroid dysfunctions. Autoimmune diseases occur when immune system begins to attack its own self antigens, so, that the best feature of autoimmune thyroid disease is the presence of auto- antibodies against thyroid antigens , such diseases are triggered by factors including infectious agents ,just like as infection with *Helicobacter pylori* (Swain *et al.,* 2005 ; Lazurova and Benhatchi 2012).

De luis *et al*.,(1998); Larizza *et al.,*(2006) and many other researchers found that a high percentage of individuals who have been diagnosed as thyroid patients were also infected with *H. pylori* which means that these bacteria plays an important role in the pathogenesis of such diseases . *Helicobacter pylori* is one of the most common bacterial pathogens that infects human around the worldwide, which acquired in the early childhood and is carried throughout lifetime if not treated with antimicrobial agents (Luther *et al* ., 2010).

In order to link *H. pylori* infection with the development of autoimmune thyroid diseases, a sensitive assay and careful population studies are required .

Due to the important role of *H. pylori* in the development of autoimmune thyroid diseases and malfunctions of human in Iraq as well in other parts of the world , studies are needed in this aspect especially those correlating dysfunction of thyroid gland and ulcer caused bacteria *H. pylori* so , this study was suggested to fulfill the aims of :

- Investigating the levels of T3, T4 and thyroid stimulating hormone TSH secreted by patients suffering from hyperthyroidism and hypothyroidism.
- Investigating the association between occurrence of thyroid disorders and *H. pylori* .

2 . Materials and Methods

2.1 Materials

2.1.1 Apparatus

Apparatus	Company /Origin
Centrifuge	Universal / Germany
ELISA Reader	Bioelisa reader ELx800 / Italy
ELISA Printer	Epson LX-300 / Italy
ELISA Washer	OrganonTeknlka / Australia
Mini VIDAS	BioMéreux / France
Spectrophotometer (UV-VIS)	Ce Cecil / England
Vortex mixer	KorlKolk / Holland
Water bath	Memmert / Germny

2.1.2 Kits

Kit	Company	Country
Tri-iodothronine (T3)	BioMérieux	France
Thyroxine (T4)		
Thyroid stimulating hormone (TSH)		
Anti – *H. pylori* (IgG)	NovaTec	Germany
Cholesterol Kit	Linear chemicals	Spain
Triglyceride kit		
HDL –Cholesterol Kit		

2.2 Methods

2.2.1 Sample collection

A total of 182 samples were collected from Iraqi individuals who attended to the Specialized Center for Endocrinology and Diabetes at Al Kindy teaching Hospital in Baghdad during the period from October 2012 to January 2013. Samples included 60 healthy individuals (31 females and 29 males) and

122 thyroid patients (100 females and 22 males) .Thyroid patients were divided into three subgroups : 40 patients were considered as a controlled group(individuals who have been under thyroid drug treatment either thyroixne or carbimazole) contains 40 patients (26 females and 14 males) , 57 patients belong to hypothyroid group (52 females and 5 males) and 25 patients hyperthyroid group (22 females and 3 males). All of them were subjected to a personal interview to fill specialized designed questionnaire form with a personal and medical history aspect [appendix I].

2.2.2 Sample treatment

Approximately 5 ml of human blood was collected from each individuals and transfer red into sterilized test tubes and allowed for 30 min. to clot at room temperature , sample was centrifuged for 5 min. at 2500 rpm (rotation per minute)and the serum was separated and stored at (- 20 ^{0} C) for investigation for triiodothyronine T3 ,teraiodothyroxine T4 , thyroid stimulating hormone TSH , Anti – *Helicobacter pylori* (IgG) and lipid profile tests .

2.2.3 Measurement of Tri-iodothyronine (T3) by Enzyme Linked Fluorescent assay (ELFA) (Caryon *et al.,*2002).

2.2.3.1 Principle

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).The solid phase receptacle(SPR) serves as the solid phase and the pipetting device for the assay .

The T3 kit contained ready - to- use reagents which predispensed in the sealed reagents strips . All assay steps were performed automatically by the

VIDAS (Vitek Immuno Diagnostic Assay System) instrument . The reaction medium was cycled in and out of the SPR several times . The sample was taken, then , transferred into the wells containing the T3 antigens labeled with alkaline phosphatase (conjugate) . Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti- T3antibodies (sheep) coated on the interior of the SPR. Unbound components are eliminated during washing steps. During the final detection step ,the substrate (4-Methylumbelliferyl phosphate) was cycled in and out of the SPR .

The conjugated enzyme catalyzes hydrolysis of this substrate into fluorescent product(4- Methylumbelliferone); the fluorescence of which was measured at 450 nm . The intensity of fluorescence is inversely proportional to the concentration of antigen present in the sample . At the end of the assay results were automatically calculated by the VIDAS instrument in relation to the calibration curve stored in its memory and then printed out .

2.2.3.2 Test procedure

- The required reagents were removed from the refrigerator and allowed to come to the room temperature for at least 30 min .
- For each sample to be tested, one T3 strip and one T3 SPR were used .The storage pouch has been carefully released after the require SPRs have been removed .
- It was mixed 100µl from calibrator control and samples using a vortex type mixer .

- T3 SPRs and T3 strips were inserted into VIDAS instrument then the color labels with assay code be matched .All assay steps were done automatically by VIDAS instrument .
- The assay was completed within approximately 40 min. , then, the SPRs and strips were removed from the VIDAS instrument and disposed into the appropriate recipient .

2.2.3.3 Interpretation of results

As the assay was completed , results were analyzed automatically by the computer . Fluorescence was measured twice in the reagent strip's reading cuvette for each sample tested . The first reading was a background reading of the substrate cuvette before SPRs was introduced into substrate . The second reading was taken after incubating the substrate with enzyme remaining on the interior of the SPRs . Then , RFV (Relative Fluorescence Value) was calculated by subtracting the background reading from the final result .

This calculation usually appears on the result sheet . Results were calculated automatically by the VIDAS instrument in relation to the calibration curve stored in its memory(4 - parameter logistic model) and were expressed in nmol /l . Samples with a concentration greater than 9 nmol /l , may will be diluted by ½ in C1 control or normal serum . The result will be calculated taking into account the dilution factor and the concentration of C1 or normal serum used . Results of VIDAS T3 assay must be interpreted as a part of complete clinical profile and in association with thyroid function tests including at least a TSH assay .

2.2.3.4 Content of T3 (triiodothyronine)Kit:

Content	Composition
60 T3 strips(STR)	Ready to use
60 T3 SPRs (solid phase receptacle) 2× 3	Ready to use. Interior of SPRs coated with anti T3 monoclonal antibodies (sheep)
T3 control (C1) 1×2 ml (liquid)	Ready to use . Human serum + L – triiodothyronine + 1g/l sodium azide . The confidence interval in nmol / l is indicated on the MLE card after the following mention : control C1 Dose Value Range
T3 calibrator (S1) 1× 2 ml (liquid)	Ready to use . Human serum + L tri – iodothyronine + 1 g/l sodium azide . The concentration in nmol /l is indicated on the MLE card after the following mention : Calibrator (s1) RFV Range
1 MLE card (Master Lot Entry)	Specifications for the factory master data required to calibrate the test : to read the MLE data

2.2.4. Measurement of Thyroxine (T4) by Enzyme Liked Fluorescent Assay (ELFA). (Caryon *et al.*,2002).

2.2.4.1 Principle

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).The solid phase receptacle (SPR) serves as the solid phase and the pipetting device for the assay. The T4 kit contained ready-to-use reagents which predispensed in the sealed reagents strips .

All assay steps were performed automatically by the VIDAS(Vitek Immuno Diagnostic Assay System) instrument . The reaction medium was cycled in and out of the SPR several times. The sample was taken, then , transferred into the wells containing the T4 antigens labeled with alkaline phosphatase (conjugate).

Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti- T4 antibodies (sheep) coated on the interior of the SPR . Unbound components were eliminated during washing steps .

During the final detection step, the substrate (4-Methyl umbelliferyl phosphate) was cycled in and out of the SPR .The conjugated enzyme catalyzes hydrolysis of this substrate into fluorescent product (4-Methyl umbelliferone) , the fluorescence of which was measured at 450 nm .The intensity of fluorescence was inversely proportional to the concentration of antigen present in the sample . At the end of the assay, results were automatically calculated by the VIDAS instrument in relation to the calibration curve stored in its memory and then printed out .

2.2.4.2 Test procedure

- The required regents were removed from the refrigerator and allowed to come to the room temperature for at least 30 min .
- For each sample to be tested , one T4 strip and one T4 SPR were used . The storage pouch has been carefully released after the require SPRs have been removed .
- It was mixed 100µl from calibrator control and samples using a vortex type mixer .

- T4 SPRs and T4 strips were inserted into VIDAS instrument , then , the color of labels with assay code on the SPRs reagent strip be matched . all assay steps were done automatically by VIDAS instrument .
- The assay was completed within approximately 40 min ., then , the SPRS and strips were removed from the VIDAS instrument and disposed into the appropriate recipient .

2.2.4.3 Content of Tetra-iodothyroxine (T4) Kit :

Content	Composition
60 T4 strips(STR)	Ready to use
60 T4 (SPRs)(solid phase receptacle) 2× 3	Ready to use. Interior of SPRs coated with anti T4 monoclonal antibodies (sheep)
T4 control (C1) 1×2 ml (liquid)	Ready to use . Human serum + L thyroxine + 1g/l sodium azide . The confidence interval in nmol / l is indicated on the MLE card after the following mention : control C1 Dose Value Range
T4 calibrator (S1) 1× 2 ml (liquid)	Ready to use . Human serum + L thyroxine + 1 g/l sodium azide . The concentration in nmol /l is indicated on the MLE card after the following mention : Calibrator (s1) RFV Range
1 MLE card (Master Lot Entry)	Specifications for the factory master data required to calibrate the test : to read the MLE data

2.2.4.4 Interpretation of results

As the assay was completed , results were analyzed automatically by the computer . Fluorescence was measured twice in the reagent strip's reading cuvette for each sample tested . The first reading was a background reading of the substrate cuvette before SPRs was introduced into substrate . The second reading was taken after incubating the substrate with enzyme remaining on the interior of the SPRs , then , RFV (Relative Fluorescence Value) was calculated by subtracting the background reading from the final result .

This calculation usually appears on the result sheet . Results were calculated automatically by the VIDAS instrument in relation to the calibration curve stored in its memory (4 -parameter logistic model) and expressed in nmol /l . Samples with a concentration greater than 9 nmol /l , may will be diluted by ½ in C1 control or normal serum .

The results will be calculated taking into account the dilution factor and the concentration of C1 or normal serum used . Results of the T4 assay must be interpreted as a part of complete clinical profile and in association with thyroid function tests including at least a TSH assay .

2.2.5 Measurement of Thyroid Stimulating Hormone (TSH) by Enzyme Linked Fluorescent Assay (ELFA)(Wondisford *et al.,*1996).

2.2.5.1 Principle

The assay principle combines an enzyme immune assay competition method with a final fluorescent detection (ELFA).The solid phase receptacle (SPR) serves as the solid phase and the pipetting device for the assay . The TSH kit contained ready- to- use reagents which predispensed in the sealed

strips . All assay steps were performed automatically by the Vitek Immuno Diagnostic Assay System (VIDAS) instrument . Thereaction medium was cycled in and out of the SPR several times .The sample was,then,transferred into the wells containing the TSH antigens labeled with alkaline phosphatase (conjugate).

Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti- TSH antibodies(sheep) coated on the interior of the SPR .Unbound components were eliminated during washing steps.

During the final detection step, the substrate (4-Methyl umbelliferyl phosphate) was cycled in and out of the SPR .The conjugated enzyme catalyze hydrolysis of this substrate into fluorescent product (4-Methylmbelliferone) the fluorescence of which was measured at 450 nm .

The intensity of the fluorescence was inversely proportional to the concentration of antigen present in the sample . At the end of the assay , results were automatically calculated by the VIDAS instrument in relation to the calibration curve stored in its memory and then printed out .

2.2.5.2 Test procedure

- The required regents were removed from the refrigerator and allowed to come to the room temperature for at least 30 min .
- For each sample to be tested ,one TSH strip and one TSHSPR were used . The storage pouch has been carefully released after the require SPRs have been removed .
- It was mixed 100 µl from calibrator control and samples using a vortex type mixer .

- TSH SPRs and TSHs trips were inserted into VIDAS instrument , then the color labels with assay code on the SPRs and the reagent strips be matched. All assay steps were done automatically by VIDAS instrument. The assay was completed within approximately40 min, then, the SPRs and strips were removed from the VIDAS instrument and disposed into the appropriate recipient .

2.2.5.3 Interpretation of results

As the assay was completed, results were analyzed automatically by the computer . Fluorescence was measured twice in the reagent strip's reading cuvette for each sample tested . The first reading was a background reading of the substrate cuvette before the SPRs was introduced into substrate. The second reading was taken after incubating the substrate with enzyme remaining on the interior of the SPRs , then , RFV (Relative Fluorescence Value) was calculated by subtracting the background reading from the final result .

This calculation usually appears on the results sheet . The TSH results were calculated automatically by the instrument using calibration curves which were stored by the instrument memory (4-parameter logistic model) the concentration were expressed in µIU /ml. Samples with TSH concentration greater than 60 µIU/ml , must be re-assayed after dilution in the TSH diluents (R1) .Multiply the result by dilution factor to obtain the sample concentration. Interpretation of test results should be made taking into consideration the patients history and the result of any other tests performed .

2.2.5.4 Content of TSH (Thyroid Stimulating Hormone) Kit:

Content	Composition
60 TSH strips(STR)	Ready to use
60 TSH SPRs (solid phase receptacle) 2× 3	Ready to use.
TSH control(C1) 1×3 ml (lyophilized)	Reconstitute with 3 ml of distilled water . Wait for 5 to 10 minutes. Mix . Stable after reconstitution for 14 days at (2-8) ^{0}C or until the expiration date on the kit at -25± 6 ^{0}C . 5 freeze thaw cycles are possible . Human serum + human TSH + preservatives . Range in μIU/ml (micro – international unit per milliliter) is indicate in MLE card after the following mention Control C1 Dose Value Range .
TSH calibrator(S1) 1× 2 ml (lyophilized)	Reconstitute with 2ml of distilled water . Wait for 5 to 10 minutes . Mix. Stable after reconstitution for 14 days at (2- 8) ^{0}C or until the expiration date on the kit at - 25 ± 6 ^{0}C . 5 freeze thaw cycles are possible . Calf serum + human TSH + preservatives . The concentration in μIU/ cmi is indicated on the MLE card after the following mention calibrator (S1) Dose Value . The confidence interval in Relative Fluorescence Value is indicated on the MLE card after the following mention Calibrator (S1) RFV Range .
TSH diluents(R1) 1×3 ml (liquid)	Ready to use . Calf serum + 0.9 g/l sodium azide
1MLEcard (Master Lot Entry)	Specification for the factory master data required to calibrate the test : to read MLE data .

2.2.6 Anti – *Helicobacter pylori* IgG Antibody ELISA method (Stolte1993).

2.2.6.1 Principle :

The quantitative immune- enzymatic determination of IgG - class antibodies against *Helicobacter pylori* is based on the ELISA (Enzyme Linked Immuno Sorbent Assay) technique . Microtiter strip wells were pre-coated with *H. pylori* antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labeled anti -human IgG conjugate was added . This conjugate bind to the captured *Helicobacter* specific antibodies . The immune complex formed by the bound conjugate was visualized by adding tetra methyl benzidine (TMB) substrate which gave a blue reaction product .

The intensity of this product was proportional to the amount of *Helicobacter* specific IgG antibodies in the specimen . Sulphuric acid was added to stop the reaction . This produced a yellow endpoint color . Absorbance at 450 nm was read using an ELISA micro well plate reader .

2.2.6.2 Reagents supplies

- *Helicobacter pylori* coated wells IgG : 12 break apart 8 well snap off strips coated with *Helicobacter pylori* antigen in re-sealable aluminium foil.
- IgG sample diluents (contains 0.1 % cathone) : 1 bottle containing100 ml of buffer for sample dilution ; pH 7.2 ± 0.2 coloured yellow ready to use .
- Stop solution:1 bottle containing15 ml sulphuric acid , 0.2 mol/lreadyto use .

- Washing solution (20 x conc. Contains 0.1 % Bronidox L after dilution) : 1 bottle containing 50 ml of a 20 - fold concentrated buffer for washing the wells ; pH 7.2 ± 0.2 .
- *Helicobacter pylori* anti – IgG conjugate (contains 0.2 % Bronidox L) :1 bottle containing 20 ml of peroxidase labeled antibodies to human IgG ;coloured blue ; ready to use .
- TMB substrate solution : 1 bottle containing 15 ml 3,3',5,5'- tetramethyl benzidine (TMB) ready to use .
- *Helicobacter pylori* IgG standers (contains 0.1 % cathone): 4 vials each containing 2 ml ; ready to use .

  Standard A : 0 NTU / ml

  Standard B : 15 NTU/ml

  Standard C : 75 NTU /ml

  Standard D : 150 NTU /ml

2.2.6.3 Assay procedure

- Dispense 100µl of each standards (A,B,Cand D) and samples were diluted into respective wells .well A1 was leaved for the substrate blank .
- wells were covered with the foil supplied in the kit .
- Incubated for 1 hour ± 5 minutes at 37 ± 1^0 C .
- When incubation has been completed ,the foil removed, the content of the wells aspirated and washed each well three times with 300 µl of washing solution .

Overflows from the reaction wells was avoided . The soak time between each wash cycle should be > 5sec. At the end the remaining fluid carefully removed by tapping strip on tissue paper prior to the next step . Washing is critical .Insufficient washing result in poor precision and falsely elevated absorbance values .

- Dispense 100µl of *Helicobacter pylori* anti – IgG conjugate into all wells except for the blank well (e. g. A 1) Covered with foil.
- Incubated for 30min. at room temperature(20 to 25) ^{0}C and not exposed to direct sunlight .
- Washing step was repeated .
- Dispense 100 µl TMB substrate solution into all wells .
- Incubated for exactly 15 min. at room temperature (20 to 25) ^{0} C in the dark .
- dispense 100 µl of the stop solution into all wells in the same order and at the same rate as for the TMB substrate solution .
- The absorbance of the specimen was measured at 450/ 620 nm within 30 min. after addition of the stop solution .

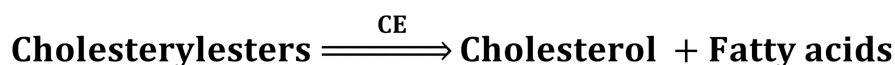
2.2.6.4 Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own patient populations in the geographical areas serviced . The following values should be considers as guide line : Reactive: > 20 NTU /ml, Grey Zone (equivocal) : 15 – 20 NTU / ml and None reactive: < 15 NTU / ml

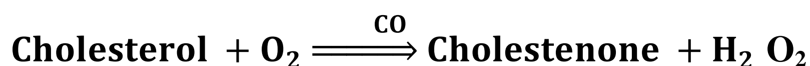
2.2.7 Cholesterol Estimation(Young *et al.,*2000).

2.2.7.1 Principle

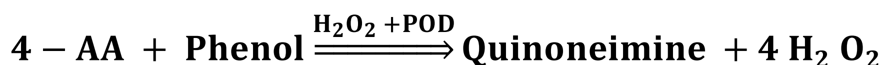
This method for the measurement of total cholesterol in serum involve use of three enzymes cholesterol esterase (CE) , cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4 -aminoantipyrine (4- AA) are condensed by hydrogen peroxide to form aquinoneimine dye proportional to the concentration of cholesterol in the sample.



$$\textbf{Cholesterylesters} \xrightarrow{\textbf{CE}} \textbf{Cholesterol} + \textbf{Fatty acids}$$



$$\textbf{Cholesterol} + O_2 \xrightarrow{\textbf{CO}} \textbf{Cholestenone} + H_2\,O_2$$



$$4 - \textbf{AA} + \textbf{Phenol} \xrightarrow{H_2O_2 + \textbf{POD}} \textbf{Quinoneimine} + 4\,H_2\,O_2$$

2.2.7.2 Reagent Composition

R1 : Monoragent p H 7.0
Sodium cholate1 mmol /l **Cholesterol esterase > 250 U/L** **Cholestero oxidase> 250 U/L** **Peroxidase> 1KU/L** **4 –aminoantipyrine0.33 mmol /L** **Phenol 4 mmol/L** **None – ionic tensioactives2 g/l (w/v)**
CAL: Cholesterol standard
Cholesterol 200 mg/ dl (5.18 mmol / L)

2.2.7.3 Procedure

- Reagents and samples were brought to room temperature .
- Pipette into labeled tubes :

Tubes	Blank	Sample	CAL standard
R1 .Monoreagent	1.0 mL	1.0 mL	1 .0 mL
Sample	—	10µL	—
CAL. Standard	—	—	10µL

- The tubes were mixed and incubated for 10 minutes at room temperature or 5minutes 37 ^{0} C .
- The absorbance (A) of the samples and the standard was read at 500 nm against the reagent blank .

2.2.7.4 Calculation of the Results

$$\frac{A\ (500\ nm)\ of\ sample}{A\ (500\ nm)\ of\ standard} \times C_{standard} = mg\ /dL\ \ total\ cholesterol$$

A= absorbance

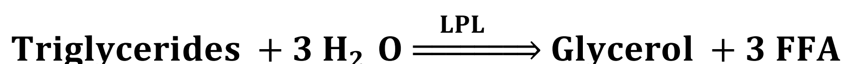
C= Cholesterol standard (CAL)

Samples with concentrations higher than 600 mg/dL should be diluted 1:2 with saline and assayed again multiply the results by 2 . If results are to be expressed as SI units apply : mg/dL × 0.0259 =mmol / L

2.2.8 Triglycerides Estimation (Young,2000).

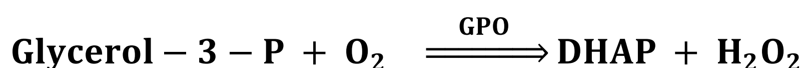
2.2.8.1 Principle

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL).The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol - 3- phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidize by glycerolphosphate oxidase (GPO) to form dihydroxy acetone phosphate (DHAP) and hydrogen peroxide .A red chromogen is produced by the peroxides(POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2) , proportional to the concentration of triglyceride in the sample .

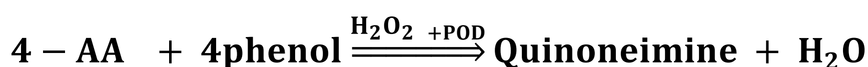


$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\,\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol} - 3 - \text{P} + \text{ADP}$$



$$\text{Glycerol} - 3 - \text{P} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$



$$4 - \text{AA} + 4\text{phenol} \xrightarrow{H_2O_2\ +\text{POD}} \text{Quinoneimine} + H_2O$$

2.2.8.2 Procedure

- Reagents and samples were brought to room temperature .
- Pipette into labeled tubes

Tubes	Blank	Sample	CAL standard
R1 .Monoreagent	1.0 mL	1.0 mL	1 .0 mL
Sample	—	10μL	—
CAL. Standard	—	—	10μL

- The tubes were mixed and incubated for15 min. at room temperature (16 -25) ^{0}C or 5 minutes at 37 ^{0} C .
- The absorbance (A) of the samples and the standard was read at 500 nm against the reagent blank

2.2.8.3 Reagent Composition

R1 : Monoragent p H 6.8	
Composition	**concentration**
LPL (lipo protein lipase)	**≥ 12 KU /L**
GK (glycerol kinase)	**≥ 1KU/L**
GPO (glycerol phosphate oxidase)	**≥ 10 KU/L**
ATP (adenosine tri phosphate)	**2.0 mmol /L**
Mg	**40 mmol /L**
POD (peroxidase)	**≥ 2.5 KU/L**
4-AA (4- amino anti pyrine)	**0.5 mmol / L**
Phenol	**3 mmol/L**
None – ionic tension actives	**2 g/ L (w/v)**
CAL: Triglycerides standard	
Glycerol 200 mg/ dl (2.26 mmol / L)	

2.2.8.4 Calculation of the Results

$$\frac{\textbf{A (500 nm) of sample}}{\textbf{A (500 nm)of standard}} \times \mathbf{C_{standard}} = \textbf{mg /dLTriglycerides}$$

A= absorbance

C= Cholesterol standard (CAL)

Samples with concentrations higher than 800 mg/dL should be diluted 1:2 with saline and assayed again . Multiply the results by 2 . If results are to be expressed as SI units apply : mg/dL × 0.0113 = mmol / L

2.2.9 HDL-Cholesterol Estimation (Tietz *et al.,*1995)

2.2.9.1 Principle

This technique use a separation method based on the selective precipitation of apoliprotien B-containing lipoproteins(VLDL,LDL and(a) Lpa) by phosphotungstic acid / $MgCl_2$, sedimentation of the precipitant by centrifugation , and subsequent enzymatic analysis of high density lipoproteins(HDL) as residual cholesterol remaining in the clear supernatant .

2.2.9.2 Reagent Composition

R1 : Precipitating Reagent
Phosphotungstic acid 0.63 mmol /L **Magnesium Chloride 25 mmol /L**
CAL: Cholestrol standard
Cholesterol 50 mg/ dl (1.3mmol / L)
R2 : Cholesterol MR

2.2.9.3 Procedure

2.2.9.3.1Precipitation

- Reagents and samples were brought to room temperature .
- Pipetted into labeled centrifuge tubes :Sample standard 0.2 m L , Precipitating reagent 0.4 m L .

$$\textbf{Ratio}\ \frac{\textbf{Sample}}{\textbf{Reagent}} = \frac{1}{2} \qquad \textbf{Dilution factor} = 3$$

- Vortex and allowed to stand for 10 minutes at room temperature .
- Centrifuged for 10 minutes at 4000 rpm or 2 minutes at 12000 rpm .
- Separated the clear supernatant within two hours .

In case of turbid supernatants caused by elevated triglycerides (> 350 g/dL) the sample should be diluted 1:2 with saline and steps 2,3,4 and 5 repeated . Multiply the result of the colorimetry by 2 .

2.2.9.3.2 Colorimetry

- Cholesterol MR mono reagent and the cholesterol standard (50 mg / dL) of the kit were brought to room temperature .
- Pipette into labeled tubes :

Tubes	Blank	Sample Supernat	Standard Supernat
Monoreagent	1.0 mL	1.0 mL	1 .0 mL
Supernate	—	50µL	—
Standard	—	—	50µL

- Mixed and allowed the tubes stand for 10 minutes at room temperatureor 5 minutes at 37 ^{0}C .
- The absorbance (A) of the supernatant and the standard was read at 500 nm against the reagent blank .

The color is stable for at least 30 minutes protected from light .

2.2.9.4 Calculation of the results

$$\frac{\mathbf{A(500\ nm)_{Supernatant}}}{\mathbf{A(500\ nm)_{standard}}} \times \mathbf{C_{standard}} = \mathbf{mg/dLHDL - Cholesterol}$$

A = Absorbance
C= Cholesterol standard

If result are to be expressed as SI units apply : mg /dL × 0.0259 = mmol / L

2.2.10 Low density lipoprotein (LDL) Estimation(Friedewald *et al* ., 1972)

LDL-cholesterol could be calculated mathematically from total cholesterol , triglycerides and HDL - cholesterol using the formula :

LDL -cholesterol = Total Cholesterol - (tri /5 - HDL) mg/ dL

2.2.11 Very Low density lipoprotein (VLDL) Estimation (Willson *et al* .,1981)

Very low density lipoprotein concentration was calculated as one fifth of the serum triglyceride .

VLDL mg/dl = Triglycerides /5 (mg / dl).

2.2.12 Body Mass Index(B.M.I) calculation

Body Mass Index (B.M.I) is a simple index of weight-for-height that is commonly used to classify under weight, over weight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2).

2.2.13 Statistical Analysis [1] :

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (10.0)(Landau and Everitt, 2004) :

- **Descriptive data analysis**:

---

[1] **All the Statistical Analysis and Findings results were Supervised by Bio-Statistician Prof. (Dr.) Abdulkhaliq Al-Naqeeb, College of Health and Medical Technology, Baghdad – Iraq.**

a- Tables (Frequencies and Percentages).
b- Descriptive Statistics (Mean, 95% Confidence Interval for mean, Standard deviation, Standard error, two extreme values Min. and Max.).
c- Contingency Coefficients for the association tables.
d- Person's correlation coefficients.
e- Graphical presentation by using :
  - Bar Charts.
  - Cluster Bar Charts.

- **Inferential data analysis:**

These were used to accept or reject the statistical hypotheses, which included the following :

a- Levene test for equality of variances and Analysis of variance test for equality of mean values as well as the Least significant difference(LSD) test.
b- T-test for testing two independent samples.
c- Contingency Coefficients test for the causes correlation ship of the association tables.
d- T-test, for testing the meaningful of the Person' correlation coefficients.
e- Odds Ratio coefficient for represents the number of times that the target Related Rates (Sapmle-1/ Sapmle-2) at the association tables with 95% Confidence interval.
f- Tests the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship. likelihood-ratio chi-square, are Applied.

For the abbreviations of the comparison significant (C.S.), we used the followings:

- NS : Non significant at P>0.05
- S : Significant at P<0.05
- HS : Highly significant at P<0.01

A

- **Aboud,R.S.**(2011). Evaluation of Anti-*Helicobacter pylori* IgGlevel in the serum of patients with autoimmune thyroid disease.*Iraqi Journal of Science.***52(4):**440-444.
- **Aguilar,G.R., Ayala,G. andFierros-Zárate,G.**(2001).*Helicobacter pylori:* Recent advances in the study of its pathogenicity and prevention. *Salud PublicaMex.* **43(3)**:237-247.
- **Ahmed,Z.,Ahmedkhan,M.,Aminulhaq,Abdullah,S.andRehman,J**.(2009). Effect of gender and age on the thyroid and thyroid stimulating hormone level in north west frontier proven Pakistan . *Ayub Med .coll Abbottabad.* **21(3) :**21-24.
- **Ansari,M.H.K.,Omrani,M.,Sayyah,B. and Ansari, S.K.**(2010).Effect of *Helicobacter pylori* infection on the lipid lipoproteins in patients with gastritis. *African J. of Microbiology Research.***4(1)**:1084-1087.

B

- **Bassi,V., Marino, G., Iengo, A., Fattoruso, O. and Santinelli , S.** (2012). Autoimmune thyroid diseases and *Helicobacter pylori* : The correlation is present only in Graves's disease. *World J .Gastroenterol.***18(10):** 1093-1097.
- **Bassi, V.,Santinelli ,C. ,Iengo, A., and Romano, C** .(2010). Identification of a correlation between *Helicobacter pylori* Infection and Graves'Disease. *Blackwell Publishing Ltd Helicobacter.***15**: 558–562.
- **Biond,B.**(2012).Natural history diagnosis and management of subclinical thyroid dysfunction.*Best practice and research clinical endocrinology and metabolism.***26**:431-446.

- **Blaser,M.J. and Parsonnet,J**. (1994). Parasitism by the slow bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. *The journal of Clinical Investigation.***94**:4-9.
- **Boelaert, K. and Franklyn A.** (2005).Thyroid hormone in health and disease. *J. of Endo.***187**:1-15.
- **Bornschein, J**. **and Malfertheiner, P.** (2011). Gastriccarcinogenesis. *Langenbecks Arch. Surg.* **396**:729–742.
- **Brown**,**R. and Francis,G.L.(**2011).Autoimmune thyroid disorders .*Journal of thyroid research.***10**:1-2.
- **Brand,O.J.andGough,S.C.(**2011).Immunogenetic mechanisms leading to thyroid autoimmunity;recent advances in identifying susceptibility genes and region.*Current genomics.***12(8)**:526-541.
- **Brent,G.A.**(2010).Environmental exposures and autoimmune thyroid disease.*Thyroid.***20(7):**755-761.
- **Bryer-Ash,M.**(2001).Evaluation of the Patient with a Suspected Thyroid Disorder.*Obst. and Gyn. Clinics.***28 (2):**421-438.
- **Bugdaci,M.S.,Zuhur,S.S.,Sokmen,M.,Toksoy,B.,Albayrak,B.,andAltuntas,Y.K**.(2011). The Role of *Helicobacter pylori* in Patients with Hypothyroidismin Whom Could Not be Achieved Normal Thyrotropin levels despite treatment with high Doses of Thyroxine. *Blackwell Publishing Ltd.***16**: 124–130.
- **Bures,J.,Kopacova,M., Koupli,L.,Vorisek,V., Rejchrt,S., Beranek, M., Seifert,B.,Pozler,O.,Zivny,P.,Douda,T.,Kolesarova,M.,Pinter,M.,Palicka,V. and Holcik.J.** (2006). Epidemiology of *Helicobacter pylori* infection in the Czech republic. *Blackwell publishing Helicobacter.***11:**56-65.

C

- **Cai,W.,Zhou, L.,Ren, W.,Deng, L., and Yu,M.(**2009). Variables Influencing Outcome of *Helicobacter pylori* Eradication Therapy in South China. *Blackwell Publishing Ltd.***14**: 443–448.
- **Canaris, G.J., Manowitz, N.R., Mayor, G.M. and Ridgway, E.C.(**2000)The colorado thyroid disease prevalence studies. *Arch. Int. Med.* **160**: 526-534.
- **Cappa,M.,Bizzarri,C., and Crea,F.** (2011). Autoimmune Thyroid Diseases in Children. *Journal of Thyroid Research.**10***:13.
- **Carayon ,P., Niccoli-Sire, P. and Lejeune, P.J**. (2002). Recommendation consensus diagnostic surveillance des maladies de la gland thyroid . *Biol. Clin. mai-Juin.* **60(3):**27-32.
- **Cardaropoli, S., Rolfo, A., Piazzese, A., Ponzetto, A. and Todros, T.** (2011). *Helicobacter pylori's* virulence and infection persistence define pre-eclampsia complicated by fetal growth retardation. *World J . Gastroenterol.* **17(47):** 5156-5165.
- **Cerbone,M.,Agretti,P.,DeMarco,G.,Improda,N.,Pignata,C.,Santamaria, F.,Tonacchera, M. and Salerno, M**. (2013). Non-autoimmune subclinical hypothyroidism due to mutation in TSH receptor.*Italian Journal of Pediatrics.***39:**5
- **Choi,H.S.Chun,H.J.,Park,S.H.,Keum,B.,Seo,Y.S.,Kim,Y.S.,Jeen,Y.,Um, S.H.,Lee,H.S.,Kim,C.D.,and Ryu,H.S.(**2012).Comparison of sequential and triple therapy for *Helicobacter pylori* infection**.***World J. Gastroenterol.* **18 (19):** 2377-2382.

- **Clyne,M., Dolan, B. and Reeves, E.P.**(2007). Bacterial factors that mediate colonization of the stomach and virulence of *Helicobacter pylori. FEMS Microbiol .Lett.* **268** : 135–143.
- **Collison, K.S., Banga, J.P. and Barnett, P.S.** (1991). Autoantibody stimulation of the human thyrotropin receptor: Regulation of adenlyatecyclase activity ,thyroglobulin and thyroid peroxidase mRNA levels in primary cultures of Graves' thyroidtissue. *Clin. Exp. Immunol.* **86(6):**1-5.
- **Cover,T. and Blaser, M.** (2009). *Helicobacter pylori* in health and disease. *Gastrology.***136:**1863-1873.

D

- **Darwish,A.H., Al Sindi,K.A. and El Kafsi,J.**(2006). Pattern of Thyroid Diseases - A Histopathological Study. *Bahrain Medical Bulletin.***28(4).**
- **DeLuca,F., Santucci, S., Corica, D., Pitrolo, E., Romeo, M. and Aversa,T.** (2013). Hashimoto's thyroiditis in childhood presentation modes and evaluation over time. *Italian Journal of pediatrics* .**38:**8
- **De Luis, DA.,Varela, C., De la calle, H., Cantón ,R., De arguila, CM. and San román, AL.**(1998). *Helicobacter pylori* infection is markedly increased in patients with autoimmune thyroiditis. *J. Clin. Gastroenterol.* **26**:259-63.
- **Diane, K. and Kenneth, D.**(2002).Subclinical Hyperthyroidism: controversies in management. *Am. Fam .Phys*.**65**: 431-38.
- **Dubois,A.**(1995). Spiral Bacteria in the Human Stomach and the Gastric *Helicobacters. Emerging Infection Diseases*.**1(3):**79-85.

- **Davies,T.F.,Yin,X. and Latif,R.**(2010).The genetics of the thyroid stimulating hormone receptor.*Thyroid.***20(7):**727-733.

E

- **El-Eshmawy, M. M**., **El-Hawary, A. K., AbdelGawad, S. S. and El-Baiomy, A. A.**(2011).*Helicobacter pylori* infection might be responsible for the interconnection between type 1 diabetesand autoimmune thyroiditis. *Diabetology& Metabolic Syndrome*.**3**:28.
- **Eschler,D**.**C.,Hasham,A.and Tomer,Y.**(2011).The etiology of autoimmune thyroid diseases.*Clin. Rev. Allergy Immunol.***41(2)**:190-197.

F

- **Friedewald ,W**.**T.,Levy,R.I. and Fredrickso,D.S.**(1972).Estimation of the concentration of Low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge .*Clinical Chemistry.***18(6):**499-502.

G

- **Gerard,A.,Boucquey,M.,Vandehove,M. and Colin,I.M.**(2006).Expression of TPO and ThOXs in human thyrocytes is down regulated by IL-1 effect partially mediated by nitric oxide.*Am. J .Physiol. Endocrinol. Metab.***291:**242-253.

- **Gesing,A.,Lewinski,A. and Karbowink,M.**(2012).The thyroid gland and the process of aging. *Thyroid research.***5:**16.
- **Gold,B., D., Colletti ,R. B., Abbott, M., Czinn, S. J., Elitsur, Y., Hassall, E., Macarthur, C., Snyder, J. and Sherman, P. M.** (2000). *Journal of Pediatric Gastroenterology and Nutrition.***31(5)**:490–497.
- **Goodman,H.M.**(2003).Basic Medical Endocrinology.3rd ed. USA:Elsilver.
- **Guo,C.,Liao,Y.,Li,Y.,Duan,J.,Guo,Y.,WU,Y.,Cui,Y.,Sun,H.,Zhaang,J.,Chen,B.,Zou,Q. and Guo,G.**(2011).Genotyping analysis of *Helicobacter pylori* using multiple locus variable number tandem repeats analysis in five region of Chin and Japan.*BMC Microbiology.***11:**197.

H

- **Hammad,F.K.,Hassan,Z.E., Abaza,D.M.,Mosua,S.G.,Saad,A.A. and Abou El-Soud,H.M.**(2011). Association between *Helicobacter Pylori* infection and autoimmune hypothyroidism in Egyptian population. *The Egyptian Journal of Hospital Medicine***(45):** 570 – 584.
- **Hardin,F.J.and Wright,R.A.**(2002). *Helicobacter pylori*: Review and Update. www.turner-white.com.
- **Hoogendoorn, H**., **Heijer M., Jvan D. and Hermus R.**(2004). Subclinical hyperthyroidism. *Postgrade Med. Jour*.**80**:394-398.

I

- **Iglesias,P.,Devora,O.,Garcia,J.,Tajada,P.,Gracia-Irvalo,C. and Diez,J.J.** (2010). Sever hyperthyroidism etiology features and treatment outcome. *Clinical endocrinology.***72:**551-557.
- **Isomoto, H., Furusu, H., Shin, M., Ohnita, K., Miyazaki, M., Omagari, K., Mizuta, Y., Murase,K., Inoue, K., Murata, I., Koji, T. and Kohno, S.** **(**2002).Enhanced Expression of Transcription Factor E2F in *Helicobacter pylori* infected Gastric Mucosa *.Blackwell Science LtdHelicobacter.***7(3):** 152–162.
- **Isomoto, H., Ueno, H., Nishi, Y., Wen, C.Y., Nakazato,M. and Kohno, S.** (2005). Impact of *Helicobacter pylori* infection on ghrelin and various neuroendocrine hormones in plasma. *World J. Gastroenterol.***11(11):**1644-1648.

J

- **Jueckstock,J. and Mylonas, I**.(2010).Managing hyperthyroidism.*BMC Microbiology.* **8:**46.

K

- **Kao,J.Y.,Rathinavelu,S.,Eaton,K.A.,Bai,L.,Zavros,Y.,Takami,M.,Pierzchala,A. and Merchant, J.L**.(2006). *Helicobacter pylori*-secreted factors inhibit dendritic cell IL-12 secretion:a mechanism of ineffective host defense. *Am .J .Physiol.Gastrointest. Liver Physiol.***291**: 73–81.
- **Karaca, A.C., Guler,N.,Yazar,A.,Çamlica,H.,Demir,K. and Yildirim,G.** **(**2004).Is lower socio-economic status a risk factor for *Helicobacter pylori* infection in pregnant women with hyperemesis gravidarum?. *Turk J. Gastroenterol.***15 (2)**: 86-89.
- **Karnath,B.M. and Hussain, N. (**2006). Sings and symptoms of thyroid dysfunction *.Review of clinical sings*.43-48.
- **Khuroo,M.S**.(2002). *Helicobacter pylori* : The unique organism. *Annals of Saudi Medicine*.**22***:*3 -4.

- **Kim,C.S., Kang, J.G., Lee, S.J., Ihm, S.H., Yoo,H.J., Nam, J.S.,Ahn,C.W. and Kim, K. R. (**2009). Relationship of low-density lipoprotein (LDL) particle size to thyroid function status in Koreans.*Clinical Endocrinology*.**71(1):**130–136.
- **Kim, K.K. and Kim, H.B .(**2009). Protein interaction network related to *Helicobacter pylori* infection response. *World Journal of Gastroenterology.* **15(36):** 4518-4528.
- **Kim,J.,Chung,W.C.,Lee,KM.,Paik,C.N.,Lee,K.S.,Kim,H.J.,Kim,Y.W.,Jung,J.H.,Noh,S.J. and Lee,Y.K.**(2012).Association between genetic instability and *Helicobacter pylori* infection in gastric epithelial dysplasia.*Gastroenterology Research Practice.***10:**1-7.

- **Koritschoner,N.P., Al –Varezdolado, M., Kurz, S.M., Heikenwaqlder, M.F., Hacker, C., Vogel, F., Munoz, A. and Zenke, M.** (2001).Thyroid hormone regulates the obesity gene tub. *Europ.Molecul.Biolo*.**21(61)**:499-504 .
- **Kusters, J.G., Vliet, A.H. and Kuipers, E. J.**(2006).Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology review*. **19(3):**449-490.

L

- **Larizza, D., Calcaterra, V., Martinetti, M., Negrini, R., De silvestri, A. and Cisternino, M**.(2006). *Helicobacter pylori* infectionand autoimmune thyroid disease in young patients: the disadvantage of carrying the human leukocyteantigen-DRB1*0301 allele. *J. Clin .Endocrinol .Metab*. **91**:176-9.
- **Lauritano,E.,C.,Bilotta,A.,L.,Gabrielli,M.S.,carpellini,E.,Lupascu,A.,La ginestra,A.,Novi,M.,Sottili,S.,Serricchio,M.,Cammarota,G.,Gasbarrini, G.,Pontecorvi,A., and Gasbarrini,** A.(2007).Association between Hypothyroidism and Small Intestinal Bacterial Overgrowth**.** *The Journal of Clinical Endocrinology & Metabolism* .**92(11):**4180–4184.
- **Lazurova,I. and Benhatchi,K**.(2012).Autoimmune thyroid disease and non-organ specific autoimmunity. *Polskie Archiwum Medycyny Wewnetrzne J.***122(1).**
- **Lamfon,H.**(2008).Thyroid disorders in Makka,Saudi Arabia. *Ozean Journal of Applied Science* .**1(1):**55-58.

- **Landau,S. and Everitt, B.**(2004). A Hand Book of Statistical Analysis using SPSS. USA , New York.Chapman & Hall/CRC Press LLC.
- **Liwanpo,L. and Hershman,J.M.** (2009).Condition and drug interfere with thyroxine absorption. *Best Practice and Research Clinical Endocrinology and Metabolism.***23:**781-792.
- **Lorini, R. and Gastaldi, R.** (2003). Hashimoto`s thyroiditis. *Pediatric Endocrinol .Rev.***2**: 205-211.
- **Luther,J., Davis, M., Higgins, P.D. and Kac, J.Y.**(2010).Association between *Helicobacter pylori* infection and inflammatory bowel disease *.Inflame bowel Dis.***16(6):**1077-1085.
- **Lynch,R.G**.(2005).*Helicobacter pylori* and Ulcers. *American Society for Investigative Pathology*.**8(1):**15-16.

M

- **Mahadevan,S**.(2010).Graves' diseases. In Text Book of Endocrinology Dharmalingam :83-96. India. Jaypee Brothers Medical Publishers Ltd.
- **Makola, D., Peura,D.A.and Crowe,S.E.(**2007). *Helicobacter pylori* Infection and Related Gastrointestinal Diseases. *J. Clin. Gastroenterol.* **41 (6)**:548–558.
- **Mansoor,R., Rizvi, S.S.R., Kausar, W., Aslam, F. and Tulhuda, S.** (2011). Comparison of TSH, T4 and T3 Levelsin Primary Hypothyroidism in relationto Gender and age in a Tertiary Carehospital. *Ann.Pak. Inst. Med. Sci.***7(4):** 186-190.
- **Marcocci, C. and Chiovato, L.** (2000). Thyroid directed antibodies. In The Thyroid Fundamental and Clinical Text. 8th ed. Eds. Braverman LE, Utiger RD, Lippincott Williams & Wilkins, Philadelphia.414-31.

- **Marlink,k.L.,Bacon,k.D.,Sheppard,B.C.,Ashktorab,H.,Smoot,D.T.,Cover,T.L.,Deveney,C.W. and Rutten, M. J.**(2003).Effects of *Helicobacter pylori* on intracellular Ca2_signaling in normal human gastric mucous epithelial cells. *Am. J. Physiol.Gastrointest. Liver Physiol.***285**: 163–176.

- **Mcdermott,M.**(2002).Lipiddisorders.In Endocrine Secret.3rd ed .Mcdermott, M. Philadephia- Hanley and belfus Inc.
- **McLachian, S. M. and Rapport, B.**(1992).The molecular biology of thyroid peroxidase: Cloning, expression and role as autoantigen in autoimmune thyroid disease. *Endo. Rev*.**13:**192-206.
- **Minov,J.B., Karadzinska-Bislimovska, J., Vasilevska, K., Risteska-Kuc, S. , Stoleski, S. and Mijakoski, D**.(2011).The Impact of *Helicobacter Pylori. Americam Journal of Immunology.***7(4)**:62-67.
- **Misra,R.N.,Bhagat,M.andAhmed,N.** (2006). *Helicobacter pylori* in Dyspepsia – Antibiotic Sensitivity and Virulence Patterns.*MJAFI***62(1)** : 22-26.
- **Mittal,A., Sathian, B., Kumar, A., Chandrasekharan, N. and Dwedi, S.**(2010). The clinical implications of thyroid hormones and its association with lipid profile.*Nepal Journal of epidemiology***1(11):**11-16.
- **Miwa,H.,Go,M.F. and Sato,N.**(2002). *H. pylori* and Gastric Cancer: The Asian Enigma. *The American Journal Ogastroenterology.***97(5):**1106-1112.

- **Muller,A. and Solnick, J. V.** (2011). Inflammation, Immunity, and Vaccine Development for *Helicobacter pylori*. *Blackwell Publishing Ltd Helicobacter*.**16 (1):** 26–32.

N

- **Nedrud,J.G. and Czinn ,S .J.** (1999). Host heredity and *Helicobacter. Gut* .**45**: 323–332.
- **Nilsson,T., Lapidus,L.,Lindstedt,G,Nystrom,E. and Eggertsen, R.** (2000). Relations between *Helicobacter pylori* ,thyroid disease and cardiovasicular risk factor in a 56-65 year old population.*Scand J. Prim Health Care* .**18:**110-112.

O

- **Ortiz,L.(2010).**Enfermed edtiro idea autoimmune .*Revista Medicadela* .**4(1) :**1-12.

P

- **Pacifico, L., Anania, C., Osborn,J.F., Ferraro,F. and Chiesa,C**. (2010). Consequences of *Helicobacter pylori* infection in children**.** *World J. Gastroenterol.***16(41):** 5181-5194.
- **Palframan,S.L.,Kwok,T. and Gabriel,K.**(2012). Vacuolating cytotoxin A(VacA) a key toxin for *Helicobacter pylori* pathogenesis. *Cellular and Infection Microbiology***.2(92):**1-8.
- **Papamichael,K.X., Papaioannou, G., Karga, H., Roussos, A. and Mantzaris, G.J.** (2009). *Helicobacter pylori* infection and endocrine disorders. *Word Gastroenterol.* **15(22):**2701-2707.
- **Pedersen,I.B., Knudsen, N., Jorgensen, T., Perrild, H., Ovesen ,L., and Laurberg,P .**(2002). Large Differences in Incidences of Overt Hyper- and

Hypothyroidism Associated with a Small Difference inIodine Intake: A Prospective Comparative Register-Based Population Survey.*The Journal of Clinical Endocrinology & Metabolism.***87(10):**4462–4469.

- **Peppa,M., Betsi, G. and Dimitriadis,G.**(2011). Lipid Abnormalities and Cardio metabolic Risk in Patients withOvert and Subclinical Thyroid Disease.*Journal of lipid.***10:**1-9.

R

- **Rapoport, B. and McLachlan, M**. (2001).Thyroid autoimmunity. *Clin. Invest.* **108**:1253–1259.
- **Raven,P.H. and Johnson,G.B.**(1995).In Biology.3rd ed:701.USA:WMC Brown communications ,Inc.
- **Riis,A.L.D.,Jorgensen,J.O.L.,Gjedde,S.,Norrelund,H.,Jurik,A.G.,Nair,K.S.,Ivarsen,P.,Weeke,J. and Moller,N.**(2005).Whole body and forearm substrate metabolism in hyperthyroidism evidence of increased basal muscle protein breakdown. *Am .J .Physiol. Endocrinol. Metab.***288:**1067-1073.
- **Richard,W.H.** (2004).Endocrine and Neuroendocrine Physiology.In Animal Physiology.Richard,W.H.,Gordon,A.W. and Margaret, A.:389.USA.Sinaure Association .Inc.
- **Roesler,B.M., Costa,S.C.B., and Zeitune,M.R.**(2012).Eradication treatment of *Helicobacter pylori* infection its important and possible relationship in preventing the development of gastric cancer.*International Scholarly Research Network.***10:**1-9.

S

- **Sachs,G. and Scott, D., R.**(2012). *Helicobacter pylori*: Eradication or Preservation. *F1000 Medicine Reports.***4**:7.
- **Saini, V., Yadav, A., Arora, S., Singh, R. and Bhattacharjee, J.**(2012). Association between different degrees of hypothyroidism and serum lipids. *Internet Journal of Medical Update***7(2):**3-8**.**
- **Shiota,S. and Yamaoka,Y**.(2010).Management of *Helicobacter pylori.F1000 Medicine Reports.***2:**20.
- **Shi,W.,Liu,W.,Zhou,X.,Ye,F. and Zhang,G**.(2013).Association of *Helicobacter pylori* infection and cytotoxine associated gene A status with autoimmuno thyroid diseases.*Thyroid J.***9(5):**495-501.
- **Silva,L.M.,Chavej,J.,Canalli,M.H.and Zanetti, C.R**.(2003).Determination of IgG sub classes and avidity of antithyroid peroxidase antibodies in patients with Subclinical hypothyroidism a comparison with patient with overt hypothyroidism . *Horm. Res.***59(3):**118-24.
- **Soveid,M.,Asi,K.H. and Omrani,G.R.(**2012).Infection by cag A positive strains of *Helicobacter pylori* is associated with autoimmune thyroid disease in Iranian patients. *IranJ.Immunol.***9(1):**48-53.
- **Šterzl,**I., **Hrda, P., Matuchp, P., Čerovska, J. andZamrazil, V.** (2008). Anti-*Helicobacter Pylori*, Anti-Thyroid Peroxidase, Anti-Thyroglobulinand Anti-Gastric Parietal Cells antibodies in Czech Population. *Physiol. Res*. **57 (1):** 135-141.
- **Stole,M.**(1993). Healing gastric MAL T lymphomas eradicating *H. pylori* Lancet **342:**568.

- **Suerbaum, S. and Michetti, P**.(2002). *Helicobacter pylori* infection. *The New England Journal of Medicine*.**347(15):**1175-1185.

- **Suzuki, H., Nishizawa, T., Masaoka, T. and Mori, M.** (2005).*Helicobacter pylori* and Gastric Cancer.*JMAJ.**48(10):**497-504.*
- **Suzuki,R.,Shiota,S. and Yamaoka,Y.(**2012).Molecular epidemiology, population genetics, and pathogenic role of *Helicobacter pylori*. *Infection Genetics and Evolution.***12**: 203–213.
- **Swain, M**., **Swian, T. and Mohanty, B.K.**(2005). Autoimmune thyroid disorders. *Indian Journal of Clinical biochemistry.***20(1)**:9-17.

T

- **Tietz,N.W**.(1995).Clinical Guideto Laboratory Tests,3rd edition. W. B. Saunders Co .Philadelphia, PA..
- **Toft, A.** (2001).Clinical Practice. Subclinical hyperthyroidism. *N. Engl. J. Med.***345**:512- 16.
- **Tomasi, P.A., Dore, M.P., Fanciulli, G., Sanciu, F., Realdi, G. and Delitala,G. (**2005). Is There Anything to the reported association between *Helicobacter pylori* Infection and Autoimmune Thyroiditis?. *Digestive Diseases and Sciences.***50(2):** *385–38.*
- **Tomer,Y**.(2010). Interferon induced thyroiditis. *J. Autoimmun*. **34(3):** 322–326.
- **Tsang,K. and Lam,S**.(1999).Extragastro duodenal conditions associated with *Helicobacter pylori* infection. *HKM J.* **5(2)**:*169-74.*

U

- **Uemura,N.,Okamoto,S.,Yamamoto,S.,Matsumura,N.,Yamaguchi,S.,Yamakido,M., Taniyama, K., Sasaki ,N. and Schlemper, R.J.(**2001).*Helicobacter pylori* infection and the development of gastric cancer. *N. Engl. J. Med.***345(11)**:784-789.

Unnikrishnan,A., Jayakumar, R. Hypothyroidism. In Text Book of Endocrinology Dharmalingam :73-81. India. Jaypee Brothers Medical Publishers Ltd.

V

- **Vadiveloo, T., Donnan, P., Murphy, M. and Leese, G**.(2013).Age and gender specific TSH reference intervals in people with no obvious thyroid disease in Tayside,Scotland :the thyroid epidemiology and research study. *J. Cli. Endocrinol. Metab*. **98(3):**1147-1153.
- **Vahab,F.**(2001).Adverse effects of subclinical hyperthyroidism. The Lancet. **358**:856-57.
- **Vandenplas,Y**.(2000).*Helicobacter pylori* infection .*World Journal of Gastroenterology.* **6(1):**20-31.
- **Vander,M.P**.(2013).The epidemiology of thyroid disease. *British Medical Bulletin.***99**: 39–51.

W

- **Wei,J**.(2009).Risk factor in autoimmune thyroid disease :*Helicobacter pylori .J. of Chinese clinical medicine*.**4(6):**318-321.
- **William, S. and Messer J.** (2000).Thyroid hormones. *Endocrinology & Metabolism.***11(4):**123-128.
- **Wilson, P.W., Abbott, R.D., Garrison, R.J. and Castelli, W.P.** (1981). Estimation of Very –Low Density Lipoprotein Cholesterol from data on triglycerides concentration in plasma .*Clin. Chem.***(12)**:2008-2010.
- **Wondeisford, F.E., Magner, J.A. and Weintraub, B.D.**(1996).Chemistry and biosynthesis of thyrotropin in brave man .7th ed. Philadelphia Lippincott-Raven. 190-207.
- **Wroblewski,L.E.,Peek,R.M. and Wilson,K.L.(**2010). *Helicobacter pylori* and Gastric Cancer: factors that Modulate Disease Risk. *Clinical Microbiology Reviews*.**23(4):** 713–739.

X

- **Xie,L.D., Gao, Y.,Li,M.R., Lu,G.Z. and Guo,X.H.** (2008). Distribution of Immunoglobulin G subclass of anti-thyroid peroxidase antibody in sera from Patients with Hashimoto's thyroiditis with different thyroid function.*Clinical and experimental Immunology.***154:**172-176.

Y

- **Yamaoka,Y., Kwon, D.H. and Graham,D.,Y.(**2000). Pro-inflammatory outer membrane eprotein (oipA) of *Helicobacter pylori*. *PNAS.***97(13):** 7533–7538.

- **Yaylali,O., Kirac,S., Yilmaz,M., Akin,F., Yuksel,D., Demirkan,N. and Akdag,B.**(2009).Dose hypothyroidism affect gastro intestinal motility .*Gastroenterology Research and Practice* .**10:**1-7.
- **Young,D.S.**(2000).Effects of Drugs on Clinical Laboratory Tests ,5thed. AACCPress.

Z

- **Zdraveska,N. and Kcocava, M.(**2012).Hshimoto thyroiditis in childhood. Review of the epidemiology and genetics susceptibility and clinical aspect of disease .*Mecedonian Journal of Medical Science* .**5(3)**:336-345.

3. Results and Discussion

3.1 Distribution of healthy and thyroid disorder patients according to gender.

A total of 122 samples belonged to patients suffering from thyroid disorders, and 60 others were considered as healthy individuals . Thyroid disorder was found to be more abundant in females (100, 81.96 %) than in males (22 , 18.03%) as shown in table (3.1) . Moreover, females constructed the vast majority of groups of hypothyroidism (52, 42.62%) , hyperthyroidism (22 , 18.03%) and controlled (26, 21.31%) groups . Adversely, males showed lowest incidences of hypothyroid(5, 4.09%)hyperthyroid(3 ,2.45%) and controlled (14 , 11.47%) groups .

According to gender of healthy and patients included in the study ,statistical analysis showed that were significant differences at $p< 0.01$ between (healthy and hypothyroidism) ,(healthy and hyperthyroidism),(controlled and hypothyroidism) and (controlled and hyperthyroidism). [see appendix III]

These results closely related to a study performed by Hamad *et al.,* (2011) who found that thyroid disorders of patients infected with *Helicobacter pylori* were more common in females than males . In another studies by Vander (2013) and Darwish *et al.,* (2006) thyroid disorders were found to be higher in females than in males with ratios of 10:1 and 3:1 ,respectively . While these results were disagreed with a study performed by Mansoor *et al.,*(2011) who found that thyroid disorders were more pronounced greater in males than in females .

Mahadevan (2010) stated that thyroid disorders are more prevalent in females (with an incidence of roughly 8 to 10 times) more than in males

,which may be due to that female reproductive activity stresses the thyroid gland .

Table (3.1) Distribution of healthy and thyroid disorder patients according to gender .

Gender	Healthy (60)		Patients Group (122)						Total	
			Controlled		Hypothyroid		Hyperthyroid			
	No.	%	No.	%	No.	%	No.	%	N o.	%
Females	31	51.7	26	21.31	52	42.62	22	18.03	100	81.96
Males	29	48.3	14	11.47	5	4.09	3	2.45	22	18.03

3.2 Distribution of healthy and thyroid disorders patients according to age .

It can be observed from table (3.2) that opportunity of thyroid disorders was increased with the age. Highest occurrence of thyroid disorders were recorded in the ages between (40- 49)yrs with a total number of 30 (24.59%) which are distributed as; 11 (9.01%), 14 (11.47%) and 5 (4.09%) in the controlled, hypothyroid and hyperthyroid groups, respectively. Followed by ages between (30- 39) yrs with a total number of 24 (19.67%) distributed as; 4 (3.27%), 14 (11.47%) and 6 (4.91%) in the controlled, hypothyroid and hyperthyroid groups, respectively. While the lowest percentages of thyroid disorders were recorded in ages of less than 5 yrs and those between (5 – 9) yrs with a total number of 4 (3.27%) for each. However, no hypothyroid disorder was recorded in any patient of less than 5 yrs old.

Table(3.2):Distribution of healthy and thyroid disorders patients group according to age

Age group (year)	Healthy (60)		Patients Group (122)						Total	
			Controlled		Hypothyroid		Hyperthyroid			
	No.	%	No.	%	No.	%	No.	%	N o.	%
< 5	7	11.7	3	2.45	0	0.00	1	0.81	4	3.27
5 – 9	4	6.70	2	1.63	2	1.63	0	0.00	4	3.27
10 – 19	15	25.0	6	4.91	8	6.55	3	2.45	17	13.93
20 -29	11	18.3	3	2.45	6	4.91	6	4.91	15	12.29
30- 39	5	8.30	4	3.27	14	11.47	6	4.91	24	19.67
40-49	10	16.7	11	9.01	14	11.47	5	4.09	30	24.59
50 – 59	7	11.7	7	5.73	11	9.01	4	3.27	22	18.03
60-70	1	1.70	4	3.27	2	1.63	0	0.00	6	4.91

According to the age of healthy and patients included in the study, statistical analysis showed there were significant differences at $p < (0.01)$ between each of the (healthy and controlled) group and the (healthy and hypothyroid) group. [see appendix II]

These results were closely related to a study performed by Aboud (2011) who was found high significant differences among age groups of thyroid patients with peptic ulcer caused by *Helicobacter pylori* ,and studies performed by Pedersen *et al.,*(2002) and Vadiveloo *et al.,*(2013) who found that thyroid

disorders incidence rates increased with patients ages. On the other side Ahmed *et al.,*(2009) in their study they found that thyroid hormones levels increased in the first decade of patients life and decreased in the second and third decades , while remained unaffected beyond the fourth decade of life .

3.3 Distribution of healthy and thyroid patients according to Body Mass Index

From the body weight and square of the height, body mass index (BMI) was calculated for each of the healthy individuals and thyroid disorders patients. Results declared that thyroid disorder patients can be classified to: underweight (16.00 – 17.00 kg/m^2) , normal weight (18.50 – 25 kg/m^2) , over weight (25 – 30 kg/m^2) and obese (>30 kg/m^2) .[see appendix XV]

It can be observed from table (3.3) that highest percentages of overweight patients were recorded in the controlled (13, 10.65%) and hypothyroidism (16,13.11%) groups , while in the obese patients , the highest occurrences were in both controlled (13,10.65%) and hypothyroidism (22,18.03%) groups.

According to BMI of healthy and patients included in the study ,statistical analysis showed there were no significant differences at $p > 0.05$ between each of healthy and patients groups . [see appendix IV]

Koritschon *et al.,*(2011) declared that most important causes of obesity are unhealthy life style and hypothyroidism . Thyroid hormones are the major regulators of energy metabolism, so that any change in the thyroid status is associated with body weight change . Adversely, Mittal *et al.,* (2010) pointed out that there was no correlation between thyroid hormones status and body weight .

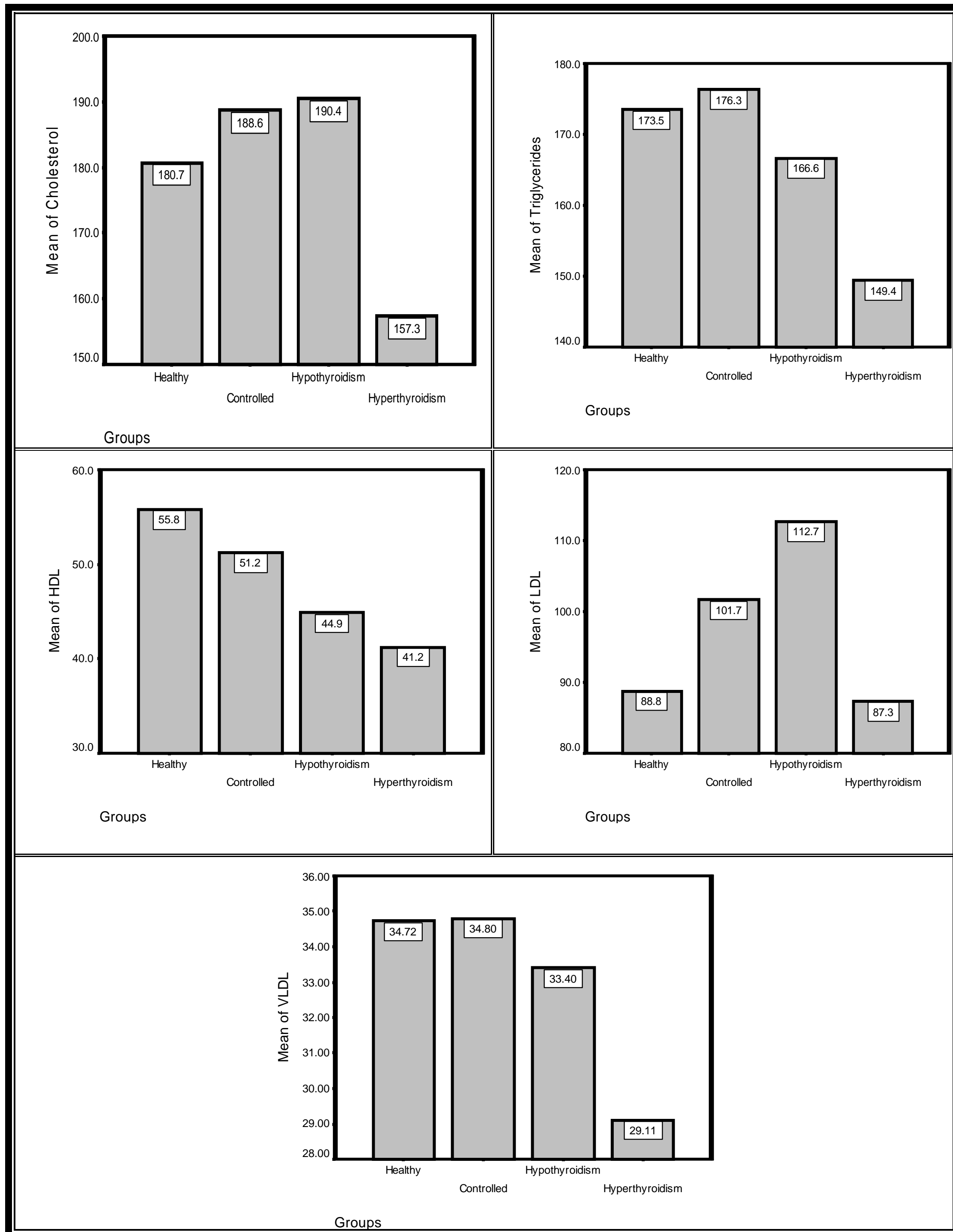
Table (3.3) :Distribution of healthy and thyroid patients according to Body Mass Index (BMI) .

B.M.I	Healthy (60)		Patient Group (122)						Total	
			Controlled		Hypothyroid		Hyperthyroid			
	No.	%	No.	%	No.	%	No.	%	N o.	%
Under weight	5	8.30	4	3.27	1	0.81	1	0.81	6	4.91
Normal weight	19	31.7	10	8.19	18	14.75	8	6.55	22	18.03
Over weight	16	26.7	13	10.65	16	13.11	8	6.55	37	30.32
Obese	20	33.3	13	10.65	22	18.03	8	6.55	43	35.24

3.4 Lipid profile in healthy and thyroid patients.

The results in figure (3.1) declared that normal levels of cholesterol were recorded in healthy , controlled and hypothyroidism groups mean value (180.7 mg/dl) , (188.7 mg/dl) and (190.4 mg/dl) respectively, while lower cholesterol levels were recorded in hyperthyroidism group mean value (157.3 mg/dl).

Normal triglycerides levels were recorded in both healthy and thyroid patients with mean value (173.5 mg/dl) in healthy group , (176.3 mg/dl) in controlled group , (166.6 mg/ dl) in hypothyroidism group and (149.4 mg/dl) in hyperthyroidism group.



Figure(3.1):Mean value of lipid profile among healthy and thyroid patients

[see appendix VII]

High density lipoprotein (HDL) results showed that the normal mean values were recorded in healthy (55.8 mg/dl) and controlled (51.2 mg/dl) groups higher than that were recorded in hypothyroidism (44.9 mg/dl) and (41.2 mg/dl) in hyperthyroid groups .

Low density lipoprotein (LDL) results recorded normal value in both healthy (88.8 mg/dl) group and thyroid patients groups : (101.7 mg/dl) in controlled , (112.7 mg/dl) in hypothyroidism and (87.3 mg/dl) in hyperthyroidism .

Very low density lipoprotein (VLDL) results were recorded normal values in healthy (34.72 mg/dl) group and in thyroid patients groups : (34.80 mg/dl) in controlled ,(33.40 mg/dl) in hypothyroidism and (29.11 mg/dl) in hyperthyroidism.

Cholesterol and triglycerides are the major circulating lipids which are water insoluble, so that they can not be transferred throughout blood stream as individuals molecules; a large spherical particles called lipoproteins package them into a core surrounded by a shell of water -soluble proteins and phospholipids so that , lipoproteins serve as vehicles to transport cholesterol and triglycerides from one part to another in human body (Mcdermott 2002).

According to the lipid profile of healthy and patients included in the study , statistical analysis showed that were significant differences at $p < 0.01$ between (controlled and hyperthyroidism) , (hyperthyroidism and hypothyroidism) and significant differences at $p < 0.05$ between (healthy and hyperthyroidism) according to cholesterol levels . As well as significant differences at $p < 0.01$ between (healthy and hypothyroidism) , (healthy and hyperthyroidism) and significant differences at $p < 0.05$ between (controlled and hyperthyroidism) according to HDL levels and there were significant differences at $p< 0.01$ between

(healthy and hypothyroidism) , (hypothyroidism and hyperthyroidism) according to LDL levels . [see appendix IX]

Thyroid diseases are associated with various metabolic abnormalities due to the effect of thyroid hormones on the major metabolic pathways (Peppa *et al.,*2011). The explanation of thyroid hormones affected on lipid metabolism is that thyroid hormone regulates the activity of some key enzymes in lipoproteins transport and ,therefore, alter the lipoprotein levels in hypothyroid patients (Saini *et al,* 2012). As well as there was an association between *Helicobacter pylori* and lipid abnormalities so, these results were closely related to Ansari *et al .,*(2010) when they found that there was an association between *H. pylori* infection and increased level of cholesterol and decreased level of HDL thus they suggested that *H. pylori* infection can be caused a lipid metabolism disorders.

Results of the present study were closed to those of Peppa *et al.,* (2011) and Kim *et al.,* (2009) who recorded significant differences among the low density lipoprotein (LDL) levels in both hyperthyroidism and hypothyroidism groups of patients .

3.5 Thyroid status in healthy and thyroid patients

Regarding to the thyroid hormones (tri-iodothyronine T3 and tetra-iodothyroxine T4) and thyroid stimulating hormone (TSH), results as shown in figure (3.2), high level of T3 and T4 hormones and TSH hormone were recorded in hyperthyroidism group ,while low level of T3 and T4 hormones and high level of TSH hormone were recorded in hypothyroidism group when compared with healthy and controlled groups. [see appendix VI]

Tomer (2010) mentioned that many genetic and environmental factors played role in development of thyroid diseases(hypothyroidism or hyperthyroidism),

while Cappa *et al* (2011) listed age, gender, pregnancy, bacterial infection and socioeconomic level as the most affected factors on thyroid diseases .

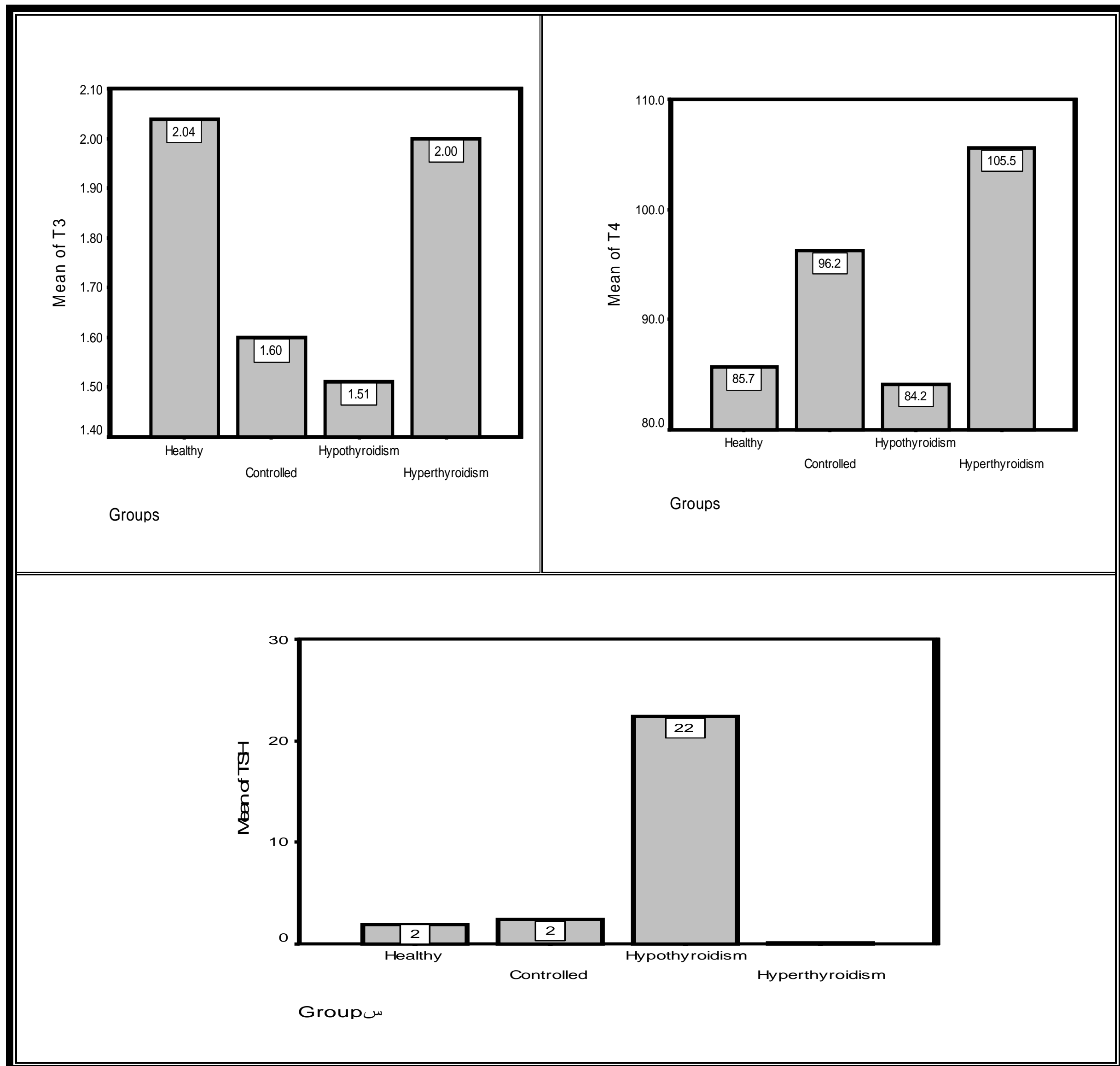


Figure (3.2): Mean value of thyroid hormones (T3 and T4) and TSH hormone among healthy and thyroid patient groups.

3.6 Distribution of *Helicobacter pylori* among healthy and thyroid patients.

Results of anti –*H. pylori* IgG antibodies (table 3.4) showed that highest percentages of *H. pylori* infections were detected in both hypothyroid (94.7%) and hyperthyroid (72 %) groups, while the lowest percentage was recorded in the healthy group; this could be related to the fact that *H. pylori* infection incidence increased in thyroid patients groups who have abnormal levels of T3,T4 and TSH hormones. In the controlled group, the percentage of *H. pylori* infection occurrence was (77.5%), which falls between those results of healthy individuals and the thyroid patients group .

Statistical analysis has been showed that by comparison between (healthy and controlled) groups an odd ration showed that negative outcomes of *H. pylori* infection increased 1.7 times in healthy group when compared with controlled group ,that is mean positive outcomes in controlled group increased in the same ration when compared with healthy group. But when compared (healthy and hypothyroid) groups the odd ratio showed that negative outcomes increased 9 times in healthy than hypothyroid group as well as the positive outcomes increased at the same ratio in hypothyroid group when compared with healthy group. While the comparison between (healthy and hyperthyroid) groups odd ration showed that positive outcomes increased 1.3 times in hyperthyroid than healthy .

In comparison between (controlled and hypothyroidism) groups the odd ratio showed that negative outcomes of *H. pylori* infection increased 5 times in controlled than in hypothyroidism group, and vice versa the positive outcome increased at hypothyroidism group than controlled group. While when compared the (controlled and hyperthyroidism) groups the odd ratio showed that the negative outcome of *H. pylori* infection increased 0.8 times in controlled

than hyperthyroid. As well the odd ration showed that positive outcomes of *H. pylori* infection increased 6.99 times in hypothyroid when compared with hyperthyroid . [see appendix XI]

These results showed that there were a high significant correlation between *H. pylori* infection and hypothyroid disorders among each of the healthy individuals , controlled and hyperthyroidism groups . This may be referred to the fact that the thyroid hormones may influence the gut motility modulation neurology and smooth muscles function . Hypothyroidism could be associated with decreasing frequency of rhythmic colonic activity and slowing oro-cecal transit time . The pathogenic link could be that intestinal motor dysfunction associated with hypothyroidism reduces ability of the small bowel to clear luminal bacteria (Lauritano *et al* 2007 and Yaylali *et al* .,2009)

Results of the present study are close to a study performed by Hamad *et al.* (2011) who found significant correlation between *H. pylori* infection and hypothyroidism, and the study performed by Sterzl *et al.* (2008) who found an association between *H. pylori* infection and autoimmune thyroid diseases (ATD). Adversely, Tomasi *et al.*(2005) found that there was no association between *H. pylori* infection and autoimmune thyroid diseases (ATD) , as well as Bassi *et al.*(2010) who detected high significant increase in the *H. pylori* prevalence in the Graves' diseases patients (hyperthyroidism) and Bassi *et al.* (2012) found a marked correlation between the presence of *H. pylori* and Graves' disease but not in the Hashimotos' thyroiditis (hypothyroidism).

Table(3.4):distribution of *H. pylori* among healthy and thyroid patients .

Group	Freq. &Percentages	*Helicobacter pylori*		Total
		Neg.	Pos.	
Healthy	Freq.	20	40	60
	% Group	33.3%	66.7%	100%
	% *Helicobacter pylori*	51.3%	28%	33%
Controlled	Freq.	9	31	40
	% Group	22.5%	77.5%	100%
	% *Helicobacter pylori*	23.1%	21.7%	22%
Hypothyroidism	Freq.	3	54	57
	% Group	5.3%	94.7%	100%
	% *Helicobacter pylori*	7.7%	37.8%	31.3%
Hyperthyroidism	Freq.	7	18	25
	% Group	28%	72%	100%
	% *Helicobacter pylori*	17.9%	12.6%	13.7%
Total	Freq.	39	143	182
	% Group	21.4%	78.6%	100%
	% *Helicobacter pylori*	100%	100%	100%

Bugdaci *et al.*(2011)in their study found a high prevalence of *H. pylori* infection in the hypothyroidism patients, in addition the effect of *H. pylori* eradication was in adequate response to thyroxine therapy, while in a

study performed by Soveid *et al.* (2012) found significant association of *H. pylori* infection with both hypothyroidism and hyperthyroidism patients was reported.

The fact of association between thyroid patients and *H. pylori* infection was supported also by other studies; such as those performed by El-Ashmawy *et al.,*(2011) who found that a correlation between *H. pylori* infection and the presence of autoantibodies against thyroid antigens, and highly significant prevalence of *H. pylori* infection in the ATD patients when compared with healthy individuals .

Another studies were performed to improve the effect of some factors on development of autoimmune thyroid patients infected with *Helicobacter pylori* a study performed by Wei (2009) to improve the association between *H. pylori* infection and autoimmune thyroid disease in addition the influence of geographical factor on opportunity of the development of such correlation . Another study performed by Karaca *et al.* (2004) reported how the lower socioeconomic status is considered as an important risk factor for the development of *H. pylori* infection . As well as other study performed by Shi *et al.,*(2013) when they found an association between *H. pylori* infection and autoimmune thyroid diseases , they suggested that *H. pylori* may play a role in the development of autoimmune thyroid diseases.

According to the results of this study that were found highly incidence rate of *Helicobacter pylori* infection in hypothyroidism group, such correlation depending on several factors that may influence the differences between the results from one study to another.

1.2 *Helicobacter pylori* historical review

Helicobacter pylori is considered to be as one of the most common pathogenic bacteria that colonizes human stomach, varying from 70 % in developing countries and less than 40% in the developed countries (Dubois1995 ; Minov *et al.*, 2011). *H pylori* is the major causative agent of gastritis and responsible for development of adenocarcinoma by stimulating cell proliferation and induces apoptosis (kim *et al.,*2012). This bacteria has been classified as group I carcinogen by the World Health Organization WHO (Blaser and Parsonnet, 1994).

H. pylori has been discovered by Marshal and Warren in 1983 ,initially named *Campylobacter* like organism then subsequently named *Campylobacter pyloridis* ,*Campylobacter pylori* and finally *Helicobacter pylori* (Kusters *et al.*, 2006). Genus *Helicobacter* which consists of 20 species belongs to the subdivision of the *Proteobacteria* , order *Campylobacter*, family *Helocobateracae* (Vandeplans, 2000).

1.2.1 Bacterial morphology and growth requirement

Helicobacter pylori is a Gram- negative spiral bacteria measuring 2-4 µm in length, 0.5-1 µm in width and has 2-6 sheathed flagella 3 µm in length (Nedrud and Czinn ,1999 ;Guo *et al.*, 2011). Kusters *et al.*(2006) found that *H. pylori* growth at optimal rang of : temperature 34- 40 C^0, pH5.5 -8.0 but can survive a t pH 4 and the key feature of *H. pylori* its microaeropholicity. Growth at optimal level of : 2 -5 % oxygen , 5- 10 % carbon dioxide and 85 % nitrogen.

1.2.2 Pathogenecity of *Helicobacter pylori*

Helicobacter pylori colonizes the stomach and induce chronic gastritis. This bacteria survive in the stomach for long time without any symptoms in most of infected people (Pacifico *et al*.,2010). In order to colonize the stomach *H. pylori* must survive in acidic pH, its survival depending on production of urease enzyme (Lynch, 2005), in addition to this enzyme this pathogenic bacteria produce other enzymes which damage of host epithelial cell such as catalase, protease and phospholipase (Tsang and Lam, 1999). Bacterial spiral shape and flagellar motility play an essential role in stomach colonization and allow it to move easily through the mucous layer (Dubois,1995).

1.2.3 Bacterial virulence factors

1.2.3.1 Urease enzyme

Urease enzyme is a cytoplasmic enzyme that consisting of two structural subunits (UreA and UreB). It hydrolyze the urea to carbon dioxide and ammonia in order to neutralize the acidity of the stomach (Suerbaum and Michetti 2002 ; Aguilar*et al.,* 2001).

1.2.3.2 Vacuolating cytotoxine A (VacA)

VacA could be the most sever toxin secreted by all *H. pylori* strains. VacA is associated with tissue damage by induce vacuolation, cytochrome release from mitochondria leading to apoptosis and responsible for initiation of pro-inflammatory response (Palframan *et al* .,2012).VacA toxin is encoded by *vac A* gene which express in all *H. pylori* strains (Suzuki *et al*.,2012).

1.2.3.3 Cytotoxin associated gene A(cagA)

CagA toxin is directly injected into the host cells via type IV secretion system and undergo tyrosine phosphorylation to enhance its activity in promoting cellular proliferation and carcinogensis (Miwa *et al.,*2002 ;Suzuki *et al.,*2005). Cardaropoli *et al.,*(2011) were described CagA as a highly immunogenic toxin encoded by *cag A* gene which located at one end of Cag pathogenesity island (PAI) which encodes type IV secretion system through which Cag A toxin delivered to host cells.

1.2.3.4 Adhesions

Adherence of *H. pylori* to gastric epithelium facilitates initial colonization, persistence of infection and delivering of virulence factors to host cells ,also adhesions is necessary for initiation of inflammatory cascade (Makola *et al*.,2007). The best characteristics *H. pylori* adhesion molecules that have been described to date is the outer membrane protein BabA (blood group antigen binding adhesion) which encodes by *babA* genes .BabA toxin mediates strong binding of bacteria to fucosylated Le^x blood group antigen structure found on the surface of epithelial cells so that, BabA toxin involve in bacterial and host cells interaction(Yamaoka *et al*.,2000; Clyne *et al*.,2007).

Other adhesion of *H .pylori* have been identified was SabA (sialic acid binding adhesion A) bind to sialytated glycoprotein Le^x (carbohydrate structure) which expressed in inflamed gastric tissue (Wroblewski *et al*.,2010).

1.2.4 Host immune response

Helicobacter pylori has been developed a variety of mechanism to survive and interact with respective host cells (Marlink *et al*.,2003). Isomoto *et al.* (2002) have been mentioned that *H. pylori* activate several different signaling pathways within host gastric cells that eventually lead to development of peptic ulcer and gastric cancer, also induce production of interleukin, tumor necrosis factor and pro inflammatory cytokines (Kim and Kim, 2009).

Regulatory T cells have a key role in immune response to *H. pylori* (Bornschein and Malfertheiner,2011) which triggered through bacterial attachment to host epithelium cells by binding to the surface class II major histo-compatibility complex molecules leading to secretion of inflammatory chemokines (Kao *et al*.,2006). Suzuki *et al.* (2005) stated that persistence of *H. pylori* infection due to insufficient immune response which failure to eliminate infection and inhibit T cells proliferation , macrophage , nitric oxide production and eventually prevent Phagocytosis.

1.2.5 Epidemiology and transmission

Bures *et al.,*(2006) found that *Helicobacter pylori* prevalence gradually increases with age . The epidemiological studies showed that *H. pylori* infection is a worldwide phenomena (Khuroo,2002). There are many factors that associated with *H. pylori* prevalence and transmission, they include: place of residence, parents education, low socioeconomic level, crowded accommodation and unsafe water supplies all may considered as a high risk factors for spread infection (Hardin and Wright ,2002).

1.2.6 Diagnosis of *Helicobacter pylori* infection

Several invasive and non invasive tests are available to detect *H. pylori* infection. Invasive techniques indeed endoscopy and mucosal biopsy that will be later subjected to culture or rapid urease test and histological examination , endoscopy is more expensive , unpleasant for patients and there is a definite risk of complications (Misra *et al*., 2006) thus using of noninvasive tests for diagnosis of *H. pylori* infection become more frequent due to high accuracy rate, inexpensive, readily available and enable to discriminate between active or past infection with the organism (Gold *et al*., 2000).

Invasive techniques includes : histology examination , rapid urease test and bacterial culture (Uemura *et al*. ,2005).While non invasive techniques are : stool antigen test , urea breath test and immune assay test (Isomoto *et al*., 2005).

1.2.7 *Helicobacter pylori* prevention and treatment

Cover and Blaser (2009) mentioned that *Helicobacter pylori* colonized the human stomach for many decades without adverse consequence , the presence of *H. pylori* is associated with an increased risk of development of several diseases including : peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) and gastric cancer. Shiota and Yamaoka (2010) suggested that early eradication of *H. pylori* should be recommended for patients with peptic ulcer to prevent the development of gastric cancer .

For the eradication of *H. pylori* infection , some conclusions were reached among them that treatment should used at least three associated drugs (Cai *et al.,*2009) by considering some criteria of the chosen drugs which include: knowledge about the structural characteristics and the pharmacokinetics of each drug, each medication should be act directly on the bacterium, be able to

dissolve rapidly in stomach and remain stable across a wide pH range especially in acidic environment (Roesler *et al .,* 2012). Therapeutic options usually include : clarithromycin , amoxicillin and metronidazole (triple therapy) as well as proton pump inhibitor considered as the first line defense (Choi *et al.,* 2012; Sachs and Scott 2012).While Muller and Solink (2011) mentioned that development of vaccine against *H. pylori* is a desirable option and significant progress has been made in treatment of infection.

1.3Thyroid gland

Thyroid gland is a butterfly shaped in the base of the neck just below the larynx that produces important hormones tetra-iodothyroxine(T4) and tri-iodothyronine (T3).Thyroid hormones are necessary for several functions including : brain development, growth, fuel metabolism, reproduction ,regulate body temperature and blood pressure. T3 and T4 production were controlled by thyroid stimulating hormone (TSH) which is produced from pituitary gland. TSH production controlled by thyroid releasing hormone (TRH) produced by hypothalamus . That means the thyroid gland regulates its hormonal secretion with the aid of hypothalamus and the pituitary gland in a process is TRH is triggered pituitary to secrete TSH which in turn tells thyroid gland to capture iodine from the blood to synthesized and produced T4 andT3. Hypothalamus and pituitary gland reduce TRH and TSH whenT4 is reach to adequate level in circulation (Goodman, 2003).

The process of thyroid hormones synthesis occurs in follicles (follicles are a small globular functional units that compose thyroid gland),these follicles contains proteincious material called colloid . The process begins when iodide

enter the thyroid gland via protein channel called sodium iodide symproter (NIS) which its activity is simulate by TSH .Once iodide enters the cell is converted to iodine, iodine thrown out into colloid by protein called pendrin then bound to tyrosine in a series steps to form monoiodotyrosine (MIT) and diiodotyrosin(DIT),a molecule of MIT couples with DIT to form triidotyrosine (T3) and two molecules of DIT combine to form T4. All these steps are catalyzed by thyroid peroxidase(TPO) enzyme(Fig. 1.1) .

Fig.1.1:Steps involved in thyroid hormone synthesis.MIT,DIT,T3 and T4 stand for mono-, di-, and tetra iodothyronine respectively. The steps of thyroid hormone release are detailed A:uptake of thyroiglobulin by endocytosis ,B:thyroglobulin lysae breaks down thyroglobulin,deiodinases break down thyroid hormones. Excess iodine is extruded from vesicles ans C: the exocytosis of the vesicles causes of secretion of T4 and T3 (Unnikrishnan and Jayakumar 2010).

The amount of iodine regulates the hormones secreted by thyroid gland. Iodine deficiency leads to a reduction in thyroid hormones synthesis, while the absence of iodine leads to the removing of inhibition effect for TSH production that is mean high TSH level this in turn increase thyroid gland activity affected by elevated TSH level and no sufficient iodine supplies for T3 and T4 synthesis , this known as hypothyroidism.While high amount of iodine leads to increasing of T3 and T4 synthesis rate and reduce TSH level thus lead to accumulation of T3 and T4 in follicles and cause thyrotoxicos , this is known hyperthyroidism (Raven and Johnson, 1995; Richard ,2004).

1.4 Thyroid diseases

1.4.1 Hypothyroidism

Hypothyroidism is a state of low serum level of thyroid hormones(tri-iodothyroinine T3 and tetra-iodothyroxineT4)and high level of thyroid stimulating hormone(TSH). The most common cause of this disorder is autoimmune thyroid disease. There are a genetic and several environmental factors can triggered of such disease (William ,2000). Brown and Francis (2011) mentioned that hypothyroidism during fetal development or early infancy results in cretinism (congenital hypothyroidism) which causes respiratory difficulties, bone and muscle dystrophy, and mental deficiency in children and females more susceptible than males.

1.4.1.1 Symptoms of hypothyroidism

Most common symptoms of hypothyroidism are: thyroid gland enlargement ,patient may begin to feel tired as well as skin , hair, and

fingernails also grow more slowly and became thickened, dry, and brittle. some hair loss may be noticed. In some cases of hypothyroidism tissues beneath the skin appear to a puffy characteristic , swollen appearance known as myxedema this is often particularly apparent around face and eyes (Boelaert, 2005). Zdraveska and Kocova (2012) mentioned that circulation were affected and heart rate slow down, intestinal activity slowdown, patient may become constipated. A few pounds of weight gain may occur, muscles become painful. As well as Hoogendoorn (2004) who has explained some other symptoms such as memory loss, decreased ability to think, depression. Some patients suffer loss of balance and difficulty in walking and hypothyroidism affected on females reproductive system and caused longer, heavier and more frequent menstruation .

1.4.2 Hyperthyroidism

Hyperthyroidism is a state of high level of thyroid hormones (tri-iodothyroinine T3 and tetra-iodothyroxine T4) and low level of thyroid stimulating hormone (TSH). In the early stages of hyperthyroidism, a person may have virtually no symptoms but laboratory tests may show a suppressed (below normal) TSH level. Thyroid stimulating hormone is the most sensitive test in diagnosing thyroid disorders. The most common cause of hyperthyroidism is Graves' disease, called toxic goiter. This is an autoimmune disease in which immune system over-stimulates whole gland to make too much hormone (Rapoport 2001). A patients with Graves' disease also have some involvement with their eyes in which the eyes may become inflamed and appear enlarged. This is described as thyroid eye disease or "exophthalmos". Hyperthyroidism is also caused by toxic nodular goiter, a condition in which one or more nodules of the

thyroid becomes overactive. The overactive nodules actually act as benign thyroid tumors (Brand and Gough, 2011).

1.4.2.1 Symptoms of Hyperthyroidism

The most common signs and symptoms of hyperthyroidism may include: high heart beat , diarrhea (Iglesias *et al .,* 2010) and muscles weakness as found by Riss *et al.,*(2005) that muscle protein breakdown increased in hyperthyroidism.

1.4.3 Subclinical hypothyroidism

The term subclinical hypothyroidism is used for patients who have a mildly increased levels of serum thyroid stimulating hormone (TSH) but normal thyroid hormone (thyroxineT4 and triiodiothyronine T3) levels (Lorini 2003). Subclinical hypothyroidism is a relatively common condition increased with age and characterized by a mild thyroid gland failure (Gesing *et al.,*2012). The main causes of subclinical hypothyroidism are autoimmune thyroiditis , genetic abnormalities , iodine deficiency, obesity and inherited syndrome (Cerbone *et al*., 2013).

1.4.4 Subclinical hyperthyroidism

Subclinical hyperthyroidism is defined as the combination of a suppressed TSH concentration, and normal serum free T3 and T4 concentrations (Toft 2001). Patients with subclinical hyperthyroidism are usually euthyroid. The sensitivity of the pituitary gland to respond to minor elevations in serum or tissue T3 and T4 levels is the main pathophysiological mechanism of subclinical hyperthyroidism. Abnormal TSH levels may remain for years

without clinical symptoms of overt hyperthyroidism (Diane ,2002 and Biond ,2012). According to its cause, subclinical hyperthyroidism can be classified as endogenous and exogenous.The endogenous causes of subclinical hyperthyroidism include multinodular goiter, Graves' disease (early), thyroiditis and other causes of hyperthyroidism (e.g., trophoblastic tumors). The exogenous causes of subclinical hyperthyroidism include treatment with levothyroxine, exogenous iodine exposure such as recent administration of radio contrast material(Vahab 2001).

1.5 Autoimmune thyroid disease (ATD)

Autoimmune thyroid disease (ATD) is common in the middle aged women and the prevalence rate increases with advancing age (Canaris 2000). ATD comprises a series of interrelated conditions including hyperthyroid Graves disease (GD), Hashimoto's (goitrous) thyroiditis, atrophic autoimmune hypothyroidism, postpartum thyroiditis (PPT) and thyroid associated orbitopathy (TAO). Out of all these diseases, Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the commonest types one form of the disease may change to other as the course of the immune process progresses. (De Luca *et al*.,2013). The development of auto-antibodies to thyroid peroxidase (TPO), thyroglobulin (TG) and thyroid stimulating hormone receptor (TSH-R) is the main hallmark of ATD (Marcocci, 2000).

1.5.1 Etiology

The etiology of Autoimmune Thyroid Disease(ATD) are multifactorial combination of genetic , environmental factors , infection by some bacteria

such as *Helicobacter pylori* and geographical dependency because of alimentary iodine intake that occurs in different geographical location (Brent ,2010 ; Lamfon ,2008) .

1.6 Thyroid autoantibodies

Auto-antibodies causes cellular damage and alters thyroid gland function. Cellular damage occurs when sensitized T-lymphocytes and/or auto-antibodies bind to thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking auto-antibodies on cell membrane receptors. Three principal thyroid auto-antigens are involved in ATD. These are thyroid peroxidase (TPO), thyroglobulin (TG) and the TSH receptor (Bryer-Ash, 2001).

1.6.1Thyroid peroxidase (TPO) antibodies

Thyroid peroxidase is the key enzyme catalyzing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm of thyrocytes. It was previously known as thyroid microsomal antigen (Mclachian,1992). Anti-TPO autoantibodies are found in patients with autoimmune hypothyroidism and Graves' disease. Togethe r with TG antibodies these are the predominant antibodies in TH. Anti-TPO antibodies are mainly of the IgG class with IgG1and IgG4 subclasses in excess (Silva, 2003).

1.6.2Thyroglobulin (TG) antibodies

Thyroglobulin composed of two identical subunits. It is secreted by the thyroid follicular cells into the follicular lumen and stored as colloid. Each

TG molecule has around 100 tyrosine residues. These residues were coupled to form the thyroid hormones triiodothyronine (T3) and thyroxin (T4). The sequence of human TG has been determined (Ortiz 2010). Thyroglobulin autoantibodies are found in patients with lymphocytic thyroiditis and Graves' disease patients. They are polyclonal and mainly of IgG class with all four subclasses represented. TSH regulates the cell surface expression of TPO and TG altering the mRNA transcription of these two proteins. These effects are mimicked by auto antibodies (both blocking and stimulating) in the sera of the patients with GD (Collison ,1991).

1.6.3 Thyroid stimulating hormone receptor (TSH-R) antibodies

Thyroid stimulating hormone receptor (TSHR) is expressed on the plasma membrane of thyroid epithelial cells, it is a central to the regulation of thyroid growth and functions. TSH-R is the major autoantigen in the autoimmuno hyperthyroidism of Graves' Disease, where T cells and autoantibodies are directed against the TSHR antigen. Activation of the receptor involves binding of cognate hormone to the large ectodomain of the THSR followed by interaction between receptor and trans membrane domain (TMD) leading to the initiation of multiple signaling pathways and thyroid hormones synthesis and secretion (Davies *et al.,*2010).

1.7 Pathogenesis of Autoimmune Thyroid Disease (ATD) .

Graves' disease shares many immunologic features with autoimmune hypothyroidism and though the thyroid stimulating hormone (TSH) receptor antibody (TRAB) are the ultimate cause of both goiter and hyperthyroidism in Graves' disease , the nature of immune dysfunction involves many aspects of

immune system including changes in both B cell and T cell function . In Graves' disease T lymphocytes become sensitized to antigens within the thyroid gland and stimulate B lymphocytes to synthesize antibodies to these antigens . One such antibody is directed against the TSH receptor site and binding of anti- TSHR autoantibody to the TSHR on thyroid membrane results in thyroid hormone hyper secretion (Mahadevan, 2010) .

The serum concentrations of these antibodies vary among patients and there is no direct correlation between serum concentration of TRAB and serum thyroid hormone concentrations in patients with Graves' hyperthyroidism but the presence of these antibodies is positively correlated with active disease and with relapse of the disease(Xie *et al* ., 2008) .

Hashimotos' thyroiditis(HT) is characterized by chronic destruction of the normal thyroid architecture , leading to hypothyroidism . Programmed cell death (apoptosis) has been implicated in the pathogenesis of HT normal thyrocytes express the cell surface death receptor FAS , but do not express its ligand (FASL). Graves' disease and HT thyrocytes express both FAS and FASL , but only HT thyrocytes appear to be vulnerable to apoptosis . Th1 type cytokines , which predominate in the thyroid gland of the person with HT , appear to promote FAS mediated apoptosis through the induction of a group of enzymes known as caspases . In contrast , Th2 type cytokines protect thyrocytes in Graves' disease by up regulating anti- apoptics proteins. In Grves's disease(GD) thyrocytes expressing FASL can promote apoptosis of infiltrating lymphocytes presenting FAS ,thyrocytes survive in GD cell mediated cytotoxicity and produce excess thyroid hormone in response to thyroid stimulating immune globulins Igs (Gerard *et al* .,2006).

1.8 Management of Thyroid disease

Both tri-iodothyroxine T3 and tetraiodothyroxine T4 used for the treatment of thyroid hormones deficiency (hypothyroidism) they are both absorbed well by gut so they given orally. Levothyroxine is the most commonly used synthetic thyroxine form, it is a stereoisomer of physiological thyroxine, which is metabolized more slowly and hence usually only needs once daily administration(Unnikrishnan and Jayakumar ,2010)

Hashimotos' thyroiditis (hypothyroidism) has no cure , but can controlled by thyroid hormone replacement and regular blood tests to check levels of thyroid hormones. Dose of levothyroxine depending on age and body weight . The aim of treatment of Graves' disease correction of thyrotoxicosis . current treatment of GD:

- Medication : anti-thyroid drugs , which inhibit production or conversion of the active thyroid hormone
- Radioactive iodine : iodine damages thyroid cells to shrink the thyroid gland , thus reducing hormone levels .
- Surgery : a subtotal thyroidectomy , in which a surgeon removes most of the thyroid gland and renders it incapable of over producing thyroid gland hormone (Mahadevan ,2010)

1.9 The relationship between autoimmune Thyroid Disease (ATD)and *Helicobacter pylori* infection.

As mentioned before there are many factors that affect development of autoimmune thyroid disease (ATD), which include genetics and none genetics (environmental and microbial infection) factors .

Jueckstock and Mylonas (2010) mentioned that *Helicobacter pylori* plays role in ATD pathogenesis. Genetic factors include thyroid specific genes and immune regulatory genes while none genetic factors include: smoking, stress, iodine intake, medication , pregnancy and bacterial and virus infection that have been implicated with etiology of ATD (Eschler *et al.,* 2011).

There have been controversial reports liking *Helicobacter pylori* infection to thyroid disorders including autoimmune thyroid disorders (ATD). Thus some studies have reported an increased prevalence of *Helicobacter pylori* infection in adults and children with ATD and relationship between *Helicobacter pylori* infection and the presence of high titers of thyroid autoantibodies , such as anti-thyroglobulin (anti -Tg) and antithyroperoxidas(anti -TPO) antibodies resulting in abnormalities of gastric secretion function. It has also been suggested that Cag A + *Helicobacter pylori* strains increase the risk for ATD especially in women , and that they are involved in the pathogenesis of Hashimoto's thyroiditis this is based on the detection of monoclonal antibodies against Cag A+ *Helicobacter pylori* strains which cross -react with follicular cells of the thyroid gland and also on the fact that *Helicobacter pylori* strains possessing the Cag A pathogenicity island carry a gene encoding for an endogenous peroxidase . Moreover , the strong correlation between IgG anti -*Helicobacter pylori* antibodies and thyroid auto - antibodies as well as the observation that eradication of *Helicobacter pylori* infection is followed by gradual decrease in the levels of

thyroid auto- antibodies, suggest that *Helicobacter pylori* antigens might be involved in the development of autoimmunoatrophic thyroiditis or that autoimmuno function in this disease may increase the likelihood of *Helicobacter pylori* infection(Papmichael *et al.,* 2009 ; Nilsson *et al* ., 2000)

Both malabsorptive disorders and condition that impair gastric acidity can affect the bioavailability of levothyroxine . These finding suggest that preexisting malabsorpition can reduce the bioavailability of levothyroxine this association appears to be strongest with *Helicobacter pylori* infection (Liwanpo and Hershman ,2009) .

Summary

This study was aimed to investigate the association between thyroid disorders and *Helicobacter pylori* infection in 122 patients (100 females and 22 males) referred to the Specialized Center for Endocrinology and Diabetes at Al –kindy Hospital in Baghdad . For comparison, 60 healthy individuals (31 females and 29 males),who had no thyroid disorders, were also included in the study. Blood samples were collected from both patients and the healthy individuals. Enzyme Linked Fluorescent Assay (ELFA) technique through using Vitek Immuno Diagnostic Assay System (VIDAS) was applied to measure levels of the thyroid hormones (tri-iodothyronine T3, tetra-iodothyroxine T4) and thyroid stimulating hormone (TSH).

From the results obtained, patients were classified into three groups: 40 were considered as belonging to the controlled * group (2٦ females and 14 males), 57 to the hypothyroidism group (52 females and 5 males) and 25 belonged to hyperthyroidism group (22 females and 3 males) .

Females constituted the vast majority of both healthy and thyroid disorders patients with percentages of (51.7%) and (81.96%), respectively. On the other hand, highest incidence rate of thyroid disorders were recorded in the age group of (30-39)yrs. 19.67% , followed by (40-49)yrs. with 24.59% and(50-59)yrs. with 18.03%.

The results also declared that there was a correlation between increasing the cholesterol level and decreasing level of high density lipoprotein (HDL) . When concentration and presence of anti -*Helicobacter pylori* IgG antibodies in the human blood samples were detected and measured by Enzyme Linked Immuno Sorrbent Assay (ELISA) technique , the results showed high

*Controlled group: individuals who have been under thyroid drug treatment either thyroixne or carbimazole

prevalence rates of *H. pylori* infection detected in the hypothyroidism patients (94.07%), while the lowest prevalence rates were recorded in the healthy individuals (66.7%).

Statistical analysis of anti –*Helicobacter pylori* IgG antibodies distribution among both healthy and thyroid patients showed that highly significant differences ($p < 0.01$) were found between thyroid disorders patients groups. Such results ensure the existence of the relationship between anti-*H. pylori* IgG antibodies and thyroid disorders occurrence.

بِـــــــسْمِ اللَّهِ الـــــرَّحْمَنِ الـــــرَّحِيمِ

(قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (٢٥) وَيَسِّرْ لِي أَمْرِي (٢٦) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (٢٧) يَفْقَهُوا قَوْلِي(٢٨))

صدق الله العظيم

سورة طه الاية ٢٥-٢٨

الخلاصة

تهـــد ف هذه الدراسة الـــى التحري عن الــعلاقة بين اضطراب الغدة الــدرقيـة و الاصـــابة ببكتريـــا *Helicobacter pylori* وقـــد تـــم جـــمع العـــينات من الـــمركز الـــتخصصي لامراض الـــغدد الـصم والسكري/مستشفى الكندي / بغداد .

حيث تم جمع عينات الدم من ١٢٢ مريض (١٠٠نساء ،٢٢ رجال) و مقارنتهم مع ٦٠ شخص (٣١ نساء،٢٩ رجال) من الاصحاء . وقد تم قياس مستوى هرمونات الغدة الدرقية (T٣، T٤) ومستوى الهرمون المحفز للدرقية (TSH) و ذلك باستخدام تقنية (Enzyme Linked Fluorscent Assay). و بالاعتماد على النتائج التي تم الحصول عليها تم تقسيم مجموعة المرضى الى ثلاث مجاميع فرعية : المجموعة الاولى وهي مجموعة المسيطر عليهم و التي تشمل ٤٠ مريض (٢٦ نساء ،١٤ رجال) والمجموعة الثانية التي تتضمن المرضى الذين يعانون من قلة افراز هرمونات الدرقية (Hypothyroidism) وتشمل ٥٧ مريض (٥٢ نساء ،٥ رجال) و المجموعة الثالثة التي تتضمن المرضى الذين يعانون من فرط افراز هرمونات الدرقية (Hyperthyroidism) وتشمل ٢٥ مريض (٢٢ نساء ، ٣ رجال)

وقد وجد ان اعلى نسبة من المصابين باضطراب الغدة الدرقية سجلت في الفئات العمرية التالية: (٣٩-٣٠) سنة بنسبة ١٩,٦٧ % ، (٤٩-٤٠) سنة بنسبة ٢٤,٥٩% و (٥٩- ٥٠) سنة بنسبة ١٨,٠٣% .وان النساء يشكلون الغالبية العظمى في كل من مجموعتي المرضى والاصحاء بنسبة (٥١,٠٧%) للاصحاء و (٨١,٩٦%) للمرضى .

كما اظهرت نتائج الاختبارات ان هناك علاقة بين ارتفاع مستوى الكولسترول وانخفاض مستوى الدهون عالية الكثافة (HDL) عند مقارنة مجاميع المرضى والاصحاء .

كما تم التحري عن الاجسام المضادة (IgG) لبكتريا *H . pylori* لكل من المرضى والاصحاء باستخدام تقنية (Enzyme Linked Immuno Sorbent Assay) وكانت اعلى نسبة للاجسام المضادة للـ *H. pylori* (IgG) سجلت في مجموعة المرضى الذين يعانون من قلة افراز هرمونات الدرقية (Hypothyroidism) بنسبة ٩٤,٠٧% واقل نسبة كانت عند الاصحاء بنسبة ٦٦,٠٧% .

كما اظهرت نتائج التحليل الاحصائي ان هناك فروق معنوية بين مجموعة الاصحاء ومجموعة المصابين باضطراب الغدة الدرقية بالاعتماد على وجود الاجسام المضادة للـــ *H. pylori* (IgG) .هذه النتائج توضح ان هناك علاقة بين اضطراب الغدة الدرقية ووجود الاجسام المضادة لبكتريا *H. pylori* (IgG) .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

العلاقة بين اضطراب الغدة الدرقية والاصابة ببكتريا *Helicobacter pylori*

رسالة

مقدمة الى كلية العلوم/جامعة النهرين
كجزء من متطلبات نيل درجة الماجستير علوم/ تقانة احيائية

من قبل
ساره طالب كاظم المفرجي
بكالوريوس علوم/تقانة احيائية/كلية العلوم /جامعة النهرين
(٢٠١٠-٢٠١١)

اشرف عليها

د.عبدالواحد باقر الشيباني (استاذ)

د.سند باقر الاعرجي (استاذ مساعد)

رمضان١٤٣٤ ب ٢٠١٣

List of Abbreviations

Abbreviations	Full name
BabA	Blood group antigen binding adhesion A
CagA	Cytotoxine associated gene A
CE	Cholesterol esterase
CO	Cholesterol oxidase
DHAP	Di hydroxyl acetone phosphate
DIT	Diiodo tyrosine
ELFA	Enzyme Linked Fluorescent Assay
ELISA	Enzyme Linked Immuno Sorbent assay
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
LPL	Lipoprotein lipase
MIT	Mono iodo tyrosine
NIS	Sodium iodine symproter
POD	Peroxidase enzyme
Sab A	Sialic acid binding adhesion A
SPR	Solid phase receptacle
T3	triiodothyronine
T4	Tetraiodothyroxine
TPO	Thyroid peroxidase enzyme
TSH	Thyroid stimulating hormone
Vac A	Vacuolating cytotoxine A
VIDAS	Vitek Immuno Diagnostic Assay System

List of contents

Item No.	Title	Page No.
Chapter one :Introduction and Literature Review		
1.	Introduction and Literatures Review	1
1.1	Introduction	1
1.2	*Helicobacter pylori* historical review	3
1.2.1	Bacterial morphology and growth requirement	3
1.2.2	Pathogenecity of *Helicobacter pylori*	4
1.2.3	Bacterial virulence factors	4
1.2.3.1	Urease enzyme	4
1.2.3.2	Vacuolating cytotoxin A(VacA)	4
1.2.3.3	Cytotoxin associated gene A(CagA)	5
1.2.3.4	Adhesions	5
1.2.4	Host Immune Response	6
1.2.5	Epidemiology and Transmission	6
1.2.6	Diagnosis of *Helicobacter pylori* infection	7
1.2.7	*Helicobacter pylori* prevention and treatment	7
1.3	Thyroid gland	8
1.4	Thyroid diseases	١٠

1.4.1	Hypothyroidism	١٠
1.4.1.1	Symptoms of hypothyroidism	10
1.4.2	Hyperthyroidism	١١
1.4.2.1	Symptoms of hyperthyroidism	12
1.4.3	Subclinical hypothyroidism	12
1.4.4	Subclinical hyperthyroidism	1٢
1.5	Autoimmune thyroid diseases(ATD)	13
1.5.1	Etiology of ATD	1٣
1.6	Thyroid autoantibodies	14
1.6.1	Thyroid peroxidase (TPO)antibodies	1٤
1.6.2	Thyroglobulin(TG)antibodies	1٤
1.6.3	Thyroid stimulating hormone receptor(TSH-R)antibodies	15
1.7	Pathogenesis of autoimmune thyroid disease(ATD)	1٥
1.8	Management of thyroid diseases	17
1.9	The Relationship between autoimmune thyroid diseases (ATD) and *Helicobacter pylori*	1٨
Chapter two: Materials and Methods		
2.	Materials and Methods	20
2.1	Materials	20
2.1.1	Apparatus	20
2.1.2	Kits	20

2.2	Methods	20
2.2.1	Sample collection	20
2.2.2	Sample treatment	21
2.2.3	Measurement of tri-iodothronine(T3)by Enzyme linked fluorescent assay (ELFA)	21
2.2.3.1	Principle	21
2.2.3.2	Test procedure	22
2.2.3.3	Interpretation of results	23
2.2.3.4	Content of T3 kit	24
2.2.4	Measurement of thyroxine (T4)by enzyme linked fluorescent assay(ELFA)	24
2.2.4.1	Principle	24
2.2.4.2	Test procedure	25
2.2.4.3	Content of T4 kit	26
2.2.4.4	Interpretation of results	27
2.2.5	Measurement of thyroid stimulating hormone(TSH) by enzyme linked fluorescent assay (ELFA)	27
2.2.5.1	Principle	27
2.2.5.2	Test procedure	28
2.2.5.3	Interpretation of results	29
2.2.5.4	Content of TSH kit	30
2.2.6	Anti-*Helicobacter pylori* IgG antibody ELISA method	31

2.2.6.1	Principle	31
2.2.6.2	Reagents supplies	31
2.2.6.3	Assay Procedure	32
2.2.6.4	Interpretation of results	33
2.2.7	Cholesterol estimation	34
2.2.7.1	Principle	34
2.2.7.2	Reagents composition	34
2.2.7.3	Procedure	35
2.2.7.4	Calculation of the results	35
2.2.8	Triglycerides estimation	36
2.2.8.1	Principle	36
2.2.8.2	Procedure	36
2.2.8.3	Reagents composition	37
2.2.8.4	Calculation of the results	37
2.2.9	HDL-cholesterol estimation	38
2.2.9.1	Principle	38
2.2.9.2	Reagents composition	38
2.2.9.3	Procedure	38
2.2.9.3.1	Precipitation	38
2.2.9.3.2	Colorimetry	39

2.2.9.4	Calculation of the results	39
2.2.10	Low density lipoprotein (LDL) estimation	40
2.2.11	Very low density liporotien (VLDL)calculation	40
2.2.12	Body mass index calculation	40
2.2.13	Statistical Analysis	40
Chapter three: Results and discussion		
3.	Results and discussion	43
3.1	Distribution of healthy and thyroid disorder patients according to gender	43
3.2	Distribution of healthy and thyroid disorders patients according to age .	44
3.3	Distribution of healthy and thyroid patients according to Body Mass Index	46
3.4	Lipid profile in healthy and thyroidism patients	47
3.5	Thyroid status in healthy and thyroidism patients	50
3.6	Distribution of *Helicobacter pylori* among healthy and thyroidism patients.	52
	Chapter four: Conclusions and Recommendations	
4.	Conclusions and Recommendations	56
4.1	Conclusions	56
4.2	Recommendations	57
	References	58
	Appendices	x

	Appendices	76

List of Tables

Item No.	Title	Page No.
2.2.3.4	Content of T3 (triiodothyronine)Kit	23
2.2.4.3	Content of Tetra-iodothyroxine (T4) Kit	25
2.2.5.4	Content of TSH (Thyroid Stimulating Hormone) Kit	29
3.1	Distribution of healthy and thyroid disorder patients according to gender .	43
3.2	Distribution of healthy and thyroid disorders patients group according to age	44
3.3	Distribution of healthy and thyroid patients according to Body Mass Index (BMI) .	46
3.4	Distribution of *H. pylori* among healthy and thyroid patients .	53

List of figures

Item No.	Title	Page No.
1.1	Steps involved in thyroid hormone sunthesis	9
3.1	Mean value of lipid profile among healthy and thyroid patients	47
3.2	Mean value of thyroid hormones (T3 and T4) and TSH hormone among healthy and thyroid patients groups	50