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STUDY THE EFFECT OF MALONDIALDEHYDE AND SOME IMPORTANT HORMONES (INSULIN, CORTISOL, ADRENOCORTICOTROPIC, TESTOSTERONE & ESTROGEN) IN (FACE & SHOULDER) ACNE PATIENTS IN BASRAH GOVERN

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Abstract
Background: Acne is associated with lifestyle and environmental changes, as it is considered a source of anxiety due to the high rate of its occurrence and its impact on the basis of social life and psychological state of the patient. Acne vulgaris is an inflammatory disease that affects the oily hair follicles. It appears frequently in adolescents on the forehead in the form of white or black heads. It can also become more complicated with atrophic or enlarged scars. Aim: of this study is to assessing the effect of oxidative free radicals through the malondialdehyde biomarker, and investigate the role of hormones, as their levels imbalance contributes to acne. Results: In the Face acne patients: (MDA, insulin & testosterone) showed highly significant increase comparing to the control group. The probability ($P < 0.05$), was considered statistically significant. Comparing statistical results between males and females in the face acne shows: (MDA & estrogen) showed highly significant increase. While (ACTH & testosterone) showed highly significant decrease. Using (ROC) curve for patients with face acne, females show highly significant statistical behavior for (MDA & ACTH), with high percentage of the area under the curve and good characteristic of validity for all tests. While males show highly significant statistical behavior for (MDA), with high percentage of the area under the curve and good characteristic of validity for all tests. In the Shoulder acne patients: (MDA) showed highly significant increase comparing to the control group, while cortisol showed highly significant decrease comparing to the control group. Comparing statistical results between males and females in the shoulder acne, (ACTH & estrogen) showed highly significant increase. While testosterone showed highly significant decrease. Spearman's nonparametric statistical correlation coefficient for biomarkers, in all patients with (face & shoulder) acne, shows different correlations as showed in the tables. Conclusion: The study correlate between biochemical markers with gender and acne site in a group of participants. The results show statistically significant behavior between them, and reflected the body physiology, skin type, and hormonal predominance on acne. Abbreviations used: MDA: Malondialdehyde, ACTH, Adrenocorticotropic and ROC, receiver operating characteristic.
***Keywords*:** Face and shoulder acne, Hormones, biomarkers & Malondialdehyde.

1. Introduction

Skin is made up of several components, including water, protein, minerals, fats, and various chemicals. It renews approximately every 27 days, at a rate (14 days for age 20 years, 37 days for

age 50 years). The skin contains, 650 sweat glands, more than 1,000 nerve terminals, and 20 of blood vessels. A person secretes every day about 500 million skin cells and gets rid of (approximately 1 gram) dead cell per day (Fulton et al., 2019) (Picardo et al., 2015).

Acne is a disease that is unique for humans, as it is linked to the sebaceous glands that are found in high density on the face, forehead and scalp. Acne affects the hair follicles and is considered one of the most common skin diseases. This is attributed to multiple factors, such as increased sebum secretion, inflammatory processes, change in lipid quality, hormonal irregularities, follicular hyperkeratosis in addition to the intrafollicular Propionibacterium acnes proliferation (Paniagua Gonzalez et al., 2018). It was found recently that, increasing androgen activity, interaction with neuropeptides, fluctuations in hormone levels, genetic factors, stress, vitamin deficiencies, obesity and follicular hyperkeratosis, and exposure to the external environment are additional conditions for doubling the chances of infection.

The sebaceous glands play the essential role in the initiation of the disease because they have all the mechanisms, such as the environmental response, the response to emotional anxiety and the response to the production of hormones and cytokines (Li et al., 2017) Also, sebaceous glands are one of the places where hormones are biosynthesized, especially androgens such as testosterone, which is the primary hormone that stimulates sebum secretion. In the case of acne, the sebaceous glands overreact to the abnormal levels of these hormones (increasing the sensitivity of the target organ) (Berth-Jones J et al., 2021).

It was also found that, the family history patient's family history is a strong influence factor for acne, as it is linked to the incidence and severity of infection, (30-90) % of those infected have a history of infection. Family history factor led to the initiate and appearance of more acne early, as well as more spread in the body and more scarring (Anaba & Oaku, 2021). In addition, researchers point out that the skin is particularly sensitive to psychological stress, knowing that the exact mechanisms of triggering and exacerbating acne are not yet understood, but some believe that glucocorticosteroids and adrenal androgens released during stress exacerbate acne (Jović et al., 2017).

Excessive generation of reactive oxygen radicals (ROS) contributes to the damage of proteins, nucleic acids, and lipids. Lipid peroxides from the oxidation of polyunsaturated fatty acids by ROS lead to the formation of highly reactive aldehydes, such as malondialdehyde (MDA). It has been observed that the skin is constantly exposed to oxidative damage by reactive oxygen species generated by internal and external sources. As a result, free radical damage leads to acne (Al-Shobaili et al., 2013). Significant contributions from other hormones such as insulin, adrenocorticotropic hormone and cortisol have been found to increase the cause of acne. It is suggested that IGF-1 may be involved in the pathogenesis of acne by increasing the expression of inflammatory biomarkers as well as sebum production in fat cells (Kim et al., 2017).

Cortisol also has significant contribution in causing acne, in cases of chronic stress, the adrenal glands are stressed and the cortisol level is drops, which leads to an increase in the level of testosterone. This will lead to increased sebum secretion and the possibility of pore clogging (Alvarez-Payares et al., n.d.).

Aim of the study: Clarifying the role of some important hormones on the acne, i addition to Discuss the danger of oxidative free radicals through the biomarker malondialdehyde.

2. Research design and methods:

2.1 Research design

The biochemical study was conducted on a representative sample of (240) participants, of whom (85) had acne in the face and (79) had acne in the back and shoulder with (76) as a control group. The ages of the participants ranged between (17-35) years. The acne patients in the face and shoulder were also divided into subgroups (males & females). Age and gender were matched for all participants. The protocols for this study were approved by the College of Healthy and Medical Technologies in Basra and by the Ethical committee in the directorate of Health in Al-Basrah.

2.2 Sample Collection

Venous blood samples were collected from each participant in standardized vacuum gel tube, under standardized conditions. Serum was obtained and then stored at -20ºC until the lab testing.

2.3 Methods:

Parameters were measured according to the following procedures:

1 - (Insulin, ACTH, cortisol, testosterone and estrogen) by Cobas e 411 and Cobas c 311 automated analysers (fully automated quantitative testing system) using electroluminescence technology. The work steps were carried out according to the batch manufacturing procedures.

2- Malondialdehyde levels were measured using ELISA technique, (Sandwich-ELISA as a method). Using spectrophotometer at a wavelength of 450 nm.

3. Results and Discussion

Acne is the most discussed disease و and it is a common skin disorder related to the sebaceous glands, with inflammatory or non-inflammatory effects. In which the sebaceous gland plays a prominent role during puberty (Zhang et al., 2022). this study reviews the evaluation of the role of some hormones on acne, highlighting the important role of oxidative reactive free radicals through the Malondialdehyde biomarker.

Statistical evaluation of data was achieved by using SPSS version 25 (IBM, Chicago, Illinois, USA), using the independent test to compare the data of two different groups. ANOVA test were used when, the comparing within the same category. The P value ≤ 0.05 was considered statistically significant.

3.1 Presenting statistical comparison of biochemical markers for patients with acne in the (face & shoulder) compared to the control group as a total number.

The results of the statistical analysis listed in table (1) shows the change in these biomarkers with the two types of acne as:

For patients with acne in the Face:

The biochemical markers (MDA & insulin) showed highly significant increase comparing to the control group.

For patients with acne in the shoulder:

Malondialdehyde showed highly significant increase comparing to the control group, while cortisol showed highly significant decrease comparing to the control group.

Malondialdehyde

(MDA) is a highly toxic molecule, whose interactions with proteins and acid are often cited as mutagenic and atherosclerotic. Its levels are greatly increased through the process of lipid peroxidation caused by oxidative stress (Williams et al., 2014). Oxidative stress occurs upon exposure to a high concentration of ROS or when antioxidants cannot set in against ROS.

It has been found that some medications used in the treatment of acne are made by reducing reactive oxygen species (Soleymani et al., 2020).

In a study aimed to determine the effects of oxidative stress on acne, an elevated level of (MDA) in blood was recorded with a positive statistical correlation with superoxide dismutase (SOD) (Mahmoud et al., 2014).

Insulin

Growth hormone (GH) is produced in the anterior pituitary gland, where it is released into the bloodstream to the liver.

This will stimulate the production of insulin-like growth hormone IGF-1, which plays a key role in promoting the growth of body cells and helps maintain skin balance.

It was found that hyperinsulinemia contributes to increased proliferation and dysfunction of keratinocytes by stimulating IGF-1 receptors. Overexpression of IGF-1 also causes adipocyte proliferation and abnormal fat production (Napolitano et al., 2015).

Evidence has also been found to support the interaction between insulin-like growth factor-1 (IGF-1) and insulin during puberty that may have a major role in the pathogenesis of acne by affecting androgen metabolism in the gonads and adrenal glands (IGF-1) (Kumari and Thapa, 2013).

Cortisol

Cortisol is known as the "stress hormone, besides regulating the body's response to external stress. It helps the body control the use of fats, carbohydrates, and proteins and the metabolism process, in addition to regulating blood pressure and sugar (Oakley & Cidlowski, 2013). The relationship between acne and stress is represented by the hypothalamic-pituitary-adrenal (HPA) axis, which in turn is reflected on the function of the sebaceous gland.

The sebaceous glands in the skin have receptors for the hormone (CRH), so the increase of this hormone in stressful situations stimulates the formation of sebum responsible for blocking the sebaceous ducts and acne production. (Jusuf et al., 2021).

Almost all tissues of the body contain glucocorticoid receptors. Therefore, cortisol can affect almost every organ (Al-Shobaili et al., 2013).

Face acne group versus shoulder acne group as a total number of participants

Acne can affect hair follicles in other places of the body such as the back, chest and shoulders. where there is a higher density of hair follicles with a density of the glands that produce sweat and oil with which secrete sebum, but if it is produced in a large amount it will contributes to acne formation (Mahto, 2017).

The biochemical markers statistical comparison showed: highly significant decrease in (insulin, cortisol & ACTH) for the face acne group to their shoulder acne peers.

3.2 Display statistical comparison for the biochemical markers in acne patients according to the gender for (face & shoulder) acne group.

The result of statistical comparison is showed in the table (2).

In table (2), the type of disease was fixed and the gender was changed, in order to obtain conclusions that show the reflection of body physiology, skin type and the dominance of hormones for both sexes on the appearance, severity and persistence of acne. The statistical results for females relative to males, shows the following:

In Face acne: (MDA & estrogen) showed highly significant increase. While (ACTH & testosterone) showed highly significant decrease.

In shoulder acne: (ACTH & estrogen) showed highly significant.

Malondialdehyde: One study found no differences associated with (MDA) according to gender and age (Moreto et al., 2014).

Other studies have also found a significant relationship of MDA levels in patients with severe acne compared to those with moderate and mild acne (Abdel-Fattah et al., 2008).

Hormones: It was noted that most adults who suffer from acne are women, due to the presence of many hormonal differences during the month, in addition to the imbalance in the level of hormones due to pregnancy or the use of contraceptives or menopause (Rahrovan et al., 2018).

For men, testosterone levels are usually the main cause of acne. Men produce this hormone more than women, and this results in thick and oily skin. In addition, their sebaceous glands are more active (from testosterone), which can lead to clogged pores (Palmer, A., 2020). Finally, men's facial hair can trap oil and make the skin a breeding ground for bacteria.

3.3 Display Receiver-operating characteristic (ROC) curve analysis for all face acne patients as a (males & females).

The future operating characteristics curve (ROC) shows a clear assessment of the biomarkers in expressing the severity of the disease with the diagnostic suitability (Nahm, 2022).

When the biochemical marker shows, high statistically significant behavior with a high percentage of the area under the curve, and a good validity characteristic for all tests (% sensitivity, % specificity, PPV & NPV). This biochemical marker will be confirming the disease. Whereas, if the biochemical marker shows, non-significant statistical behavior with high percentage of the area under the curve or good validity to some tests. That means, auxiliary evidence is needed to prove the presence of the disease. Table (3) show the results and as follows:

Malondialdehyde: Show highly significant statistical behavior in both gender with high percentage of the area under the curve rang (92% - 100%). In addition to a good characteristic of validity for all of the test (Sensitivity %, specificity %, PPV & NPV).

Insulin & ACTH: These biomarkers show highly significant statistical behavior in females' group with high percentage of the area under the curve range (70-85%). In addition to a very good characteristic of validity for all of the test. Except (Sensitivity % & NPV) for insulin. The two biomarkers also show good characteristic of the validity of some test in males' group.

Cortisol, Testosterone & Estrogen hormones: These biomarker shows non-significant statistical behavior in both genders, but at the same time have a good characteristic of the validity of some test.

The results indicate an imbalance in the hormones level for both sexes. The reason can be attributed to the large difference in the body physiology of the groups, which depends on several axes ranging from environmental conditions, genetics, lifestyle and ending with diet (Zhang et al., 2022).

3.4 Display Spearman's Statistical Correlations for Biomarkers With Each Other in patients having acne in (face & shoulder).

Spearman's statistical correlation was conducted to clarify the relationship between biochemical indicators, in addition to evaluating the extent of these correlations.

3.4.1 Presentation of Spearman's nonparametric statistical correlation coefficient for biomarkers in all patients with facial acne.

The associations between biomarkers of facial acne patients were studied, as shown in table (4).

\ **ACTH,** showed highly significant difference, and low negative correlation with (MDA), while it showed moderate positive correlation with cortisol.

τ Stress is one of the factors that cause acne, and it occurs as a result of a stimulation reactions in the brain. The relationship between stress and acne centres around the hypothalamic-pituitary-adrenal (HPA) axis. This axis affects the function of the sebaceous gland, which possesses the corticotropin-releasing hormone (CRH) receptors in the skin. Therefore, in case of stress, an increase in (CRH & ACTH) stimulates the secretion of sebum is formed, which blocks the ducts of the sebaceous glands (Jusuf et al., 2021).

The negative correlation was found between (ACTH & MDA), because these biochemical markers refer to acne in opposite way.

\ **Testosterone,** hormones stimulate acne by stimulating the sebaceous glands and increasing the amount of oil production by the skin. These hormones may alter the skin cells lining the hair follicles, making them stickier and more prone to clogged pores (Zouboulis et al., 2005).

τ Testosterone, showed highly significant difference, and low negative correlation with (MDA)

τ Testosterone, showed highly significant difference, and low positive correlation with insulin.

Hyperinsulinemia is a reason of increasing proliferation and dysfunction of keratinocytes through stimulation of IGF-1 receptors. Where it was found that the excessive secretion of IGF-1 leads to an excessive increase in the production of fat by the fat cells. Insulin growth factor also enhances the synthesis of androgen, which is responsible for the excessive sebum production (Napolitano et al., 2015).

τ Testosterone, also showed highly significant difference and moderately positive correlation with (ACTH). Continuing stress makes the receptors of the fat-producing cells more sensitive, which in turn stimulates the production of more fat and causing acne (Elsaie, 2016).

\ **Estrogen,** Estrogen helps reduce oil production on the skin, which is exactly the opposite of the role of testosterone. Basically, estrogen balances testosterone and its effects. If estrogen levels are low, testosterone levels may be normal, but not balanced by estrogen. It was found in acne patients

that the difference in the ratio of androgens to estrogen may lead to an increase in the degree of acne in patients (Zhang et al., 2022).

τ Estrogen showed highly significant difference and low negative correlation with insulin, and moderate negative correlation with (ACTH & testosterone).

τ The adrenal glands produce both progesterone and cortisol. In case of increased stress, the adrenal glands produce less progesterone due to the need for more cortisol. This in turn leads to estrogen dominance. In situations of extreme stress cortisol becomes less available in the bloodstream and testosterone levels rise, which increases the risk of acne (Rao et al., 2021).

3.4.2 Presentation of Spearman's nonparametric statistical correlation coefficient for biomarkers in all patients with shoulder acne.

Acne, can be developed in any area of the body that contains hair follicles or sebaceous glands, except for the palms of the hand and the soles of the feet. The associations between biomarkers of shoulder acne patients were studied. This is shown in table (5).

\ **Malondialdehyde,** showed high significant difference with both (cortisol & ACTH) with different correlation.

τ MDA, shows moderate negative correlation with cortisol.

τ MDA, shows good negative correlation with (ACTH). The negative correlation is attributed to the opposite behavior of these biochemical markers to express the presence of acne.

\ **Cortisol,** showed highly significant difference and good positive correlation with (ACTH). In times of stress, the body releases cortisol after it releasing adrenaline hormone (the fight-or-flight hormones). Cortisol must also be obtained at optimal levels, and this is done by the proper functioning of the adrenal glands and pituitary glands (Megha, R., et al. 2021).

\ **Estrogen,** showed highly significant difference and low negative correlation with insulin, as well as a moderate negative correlation with testosterone. The same behavior was discussed in the facial acne group.

Conclusions:

The study linked the levels of some hormones and malondialdehyde as an indicating marker for oxidative stress with (acne site and gender) for a group of participants. According to the obtained results, we conclude the following:

1- The level of malondialdehyde is high in acne patients compared to its level in the control group. Also, all statistical treatments, shows highly statistically significant relationship between (MDA) and (acne type & gender). This confirms that, wherever acne is present there is oxidative stress in patients.

2-To obtain, conclusions showing the reflection of body physiology, skin type, and hormonal predominance for both genders on the appearance, severity and persistence of acne. Statistical study was performed for patients with face acne (comparing males with females). It was found that testosterone and estrogen showed highly significant statistically relationship when compared.

3- By using the receiver operating characteristic (ROC) curve to assess the ability and accuracy of biomarkers in expressing disease severity (in face acne patients), it was found that:

(MDA, insulin & ACTH), showed good test characteristic for females. In the same way (MDA) shows good test characteristic for males.

4- From Spearman's statistical correlations, it was observed that there were positive and negative significant correlations between these indicators, confirming their link to the mechanics of getting acne.

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Table (1): Statistical analysis of biomarkers for patients with (face & shoulder) acne compared to the control group as a total number, using Post hoc analysis of ANOVA.

Biomarker	Control (76) (mean±SD)	Face Acne (85) (mean ±SD)	Shoulder Acne (79) (mean ±SD)	*P*- value*		
				Face acne Vs control	Shoulder acne Vs control	Face Acne Vs shoulder acne
MDA	3.35 ± *0.84*	9.17 ± 3.25	9.22 ± 2.94	0.0001	0.0001	0.999
Insulin	15.91 ± 5.18	29.70 ± 25.44	19.47 ± 15.88	0.0001	0.520	0.001
Cortisol	12.70 ± 2.63	11.38 ± 4.32	9.64 ± 5.19	0.134	0.0001	0.026
ACTH	19.58 ± 4.09	25.78 ± 66.44	9.97 ± 10.33	0.695	0.356	0.036
Testosterone	2.68 ± 2.66	4.58 ± 5.18	3.45 ± 3.33	0.009	0.131	0.989
Estrogen	56.04 ± 59.71	65.89 ± 99.87	66.30 ± 93.41	0.945	0.921	0.946

The probability ($P < 0.05$), was considered statistically significant.

Table (2): Statistical analysis of biomarkers according to acne patient groups in (males & females), using Mann Whitney's test.

Diseased Group						
Face Acne			Biomarker	Shoulder Acne		
P value*	Gender			Gender		P value*
	Female (43) (mean±SD)	Male (42) (mean±SD)		Male (40) (mean±SD)	Female (39) (mean±SD)	
0.001	10.32 ± 1.86	7.99 ± 3.91	MDA	9.78 ± 2.97	8.64 ± 2.83	0.085
0.916	29.42 ± 27.78	30.00 ± 23.14	Insulin	21.10 ± 19.14	17.80 ± 11.68	0.359
0.826	11.28 ± 4.71	11.48 ± 3.95	Cortisol	9.11 ± 3.79	10.18 ± 6.32	0.364

0.039	11.15 ± 10.90	27.50 ± 19.52	ACTH	7.53 ± 8.07	12.47 ± 11.80	0.033
0.0001	0.68 ± 0.39	8.56 ± 4.76	Testosterone	6.99 ± 2.45	0.89 ± 0.73	0.0001
0.008	102.77 ± 130.48	28.12 ± 10.41	Estrogen	33.00 ± 11.69	100.45 ± 124.13	0.0001

Table (3): Receiver-operating characteristic (ROC) curve analysis of biomarkers for all face acne patients according to the gender

Gender (In Face Acne)												
Male (42)						Female (43)						
NPV	PPV	Spec %	Sen %	*P*-value (= 0.05)	Area under the curve	Biomarker	Area under the curve	*P*-value (= 0.05)	Sen %	Spec %	PPV	NPV
91	97	97	90	0.0001	0.924	MDA	1.000	0.0001	100	98	98	100
60	92	97	36	0.675	0.545	Insulin	0.699	0.002	30	99	97	57
50	50	99	1	0.681	0.423	Cortisol	0.400	0.120	1	99	80	51
51	80	99	4	0.651	0.530	ACTH	0.853	0.0001	67	98	97	75
49	38	92	5	0.599	0.534	Testosterone	0.440	0.357	81	41	58	67
49	14	94	1	0.562	0.538	Estrogen	0.515	0.815	10	97	77	52

Table (4): Nonparametric Spearman's statistical correlation coefficient for biomarkers in all face acne patients.

Biomarkers / Variable		Insulin	Cortisol	ACTH	Testosterone	Estrogen
MDA	*P* Value	0.026	- 0.104	- 0.298	- 0.231	0.180
	r Value	0.741	0.188	0.0001	0.003	0.022
Insulin	*P* Value		- 0.006	0.011	0.238	- 0.293
	r Value		0.935	0.886	0.002	0.0001
Cortisol	*P* Value			0.376	0.112	0.013
	r Value			0.0001	0.156	0.873
ACTH	*P* Value				0.425	- 0.381
	r Value				0.0001	0.0001
Testosterone	*P* Value					- 0.474
	r Value					0.001

Table (5): Nonparametric Spearman's statistical correlation coefficient for biomarkers in all shoulder acne patients.

Biomarkers / Variable		Insulin	Cortisol	ACTH	Testosterone	Estrogen
MDA	*P* Value	-0.120	-0.417	-0.628**	-0.016	-0.082
	r Value	0.137	0.0001	0.0001	0.847	0.312
Insulin	*P* Value		0.186	0.133	-0.080	-0.210
	r Value		0.021	0.098	0.323	0.009
Cortisol	*P* Value			0.681**	-0.038	-0.027
	r Value			0.639	0.639	0.738
ACTH	*P* Value				- 0.067	- 0.022
	r Value				0.405	0.783
Testosterone	*P* Value					- 0.432
	r Value					0.0001

The study considered the value of the correlation coefficient within the limits:

- **(0.50.-0.60) good indicator for the relationship between binary variables.**
- **(0.36-0.50) moderately indicator for the relationship between binary variables.**
- **(0.20 -0.35) low indicator for the relationship between binary variables.**

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