Estimation of Orexin Level and Some Biochemical Indicators in Pregnant Women with Normal Pregnancy and Pregnant Women After Taking Steroids

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Abstract
The study measures the level of orexin and a number of biochemical variables, including cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very low density lipoproteins. In addition, it explores the total antioxidant index. It also measures the level of Malondialdehyde in pregnant women with normal pregnancy, as well as pregnant women after taking steroids and non-pregnant women after taking steroids. The samples were divided into 40 pregnant women with natural pregnancy as a control group, (30) samples for pregnant women after taking steroids, and (30) samples for non-pregnant women after taking steroids and in different age groups (15-45) years. Blood samples were collected from women visiting Azadi Teaching Hospital as well as outpatient medical gynecological clinics for the period from 12/25/2021 to 15/5/2022. The study showed:* There was an increase with no statistical differences at the probability level (p> 0.05) in the level of orexin for the age group (26-35) years in pregnant women with normal pregnancy and pregnant women after taking steroids compared with the two age groups (15-25), (36-45). Yet, it increased with no statistical differences in the age group (36-45) years in non-pregnant women after taking steroids compared with the two age groups (15-25) years (26-35) year. * There was an increase no statistical differences at the level of probability (P> 0.05) in the MDA for the three age groups respectively for pregnant women with steroids and non-pregnant women after taking steroids compared with pregnant women with natural pregnancy. The highest percentage was in the age group (36-45) years for both pregnant women with natural pregnancy and pregnant with steroids and non-pregnant women after taking steroids compared with the age group (15-25) and (26-35) years. * There was a decrease with high statistical differences at the probability level (P≤0.01) in the level of TAC in the age group (36-45) years for pregnant women with a normal pregnancy more than the two age groups (15-25) and (26-35) years. The same happened with the two groups (pregnant women after taking steroids and non-pregnant women after taking steroids). * There were no statistically significant differences at the probability level (p>0.05) in the TC of the three age groups in blood serum of pregnant and non-pregnant women and pregnant women with steroids. * There was an increase with high statistical differences in the level of TG, VLDL at the probability level (p ≤ 0.01) for the three age groups in the blood sera of pregnant women with normal pregnancy and steroids compared with the age groups of the non-pregnant group. * There was a decrease but no statistical differences appeared at the probability level (p > 0.05) in the level of HDL in the serums of pregnant women with normal pregnancy and pregnant women after taking steroids and non-pregnant women after taking steroids for the three age groups. Here, the highest percentage decrease was recorded in the age group (G3 = 36-45).* There was an increase with no statistical differences at the probability level (p> 0.05) in the level of (LDL) in the serums of pregnant women with normal pregnancy and pregnant women after taking steroids compared with non-pregnant women after taking steroids for the three age groups. The highest percentage of increase was recorded in the age group (G3 = 36-45).

Keywords: Pregnancy, Steroids, Natural pregnancy and Infertility

1. Introduction

Infertility is one of the medical and even societal problems that face the husbands and wives. The gradual decline in fertility has started since 1955. Many factors have affected women's fertility, and this decline has several reasons, including medical and non-medical. One of the factor is the age of the woman, where the older the woman, the lesser the chances of pregnancy. Women are at the peak of their fertility at the age between 30 - 18 years and gradually declines after the age of 35 years(Mustafa, Sharifa, Hadi, IIlzam, & Aliya, 2019).

There are many reasons responsible for reducing pregnancy rates in women, including:

1- Obstruction of the fallopian tubes prevents the sperm from reaching the ovum.

2- Incompetent eggs.

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3-Problems related to the uterus.
4- A very long menstrual cycle (lasting 35 days or more), too short, irregular or absent can mean that there is no ovulation.
5- Irregular ovulation causes the menstrual cycle to be irregular(Campbell, 2019).
Orexins, also known as hypocretin, are a neuropeptide produced primarily by neurons located in the lateral hypothalamic area of the lateral hypothalamic area and in the perifornical area of the pericorneal region (Chen et al., 2019; Mäkelä et al., 2018).
Orexin neurons consist of the union of a small part of the neurons found in the mammary vertebral including the human(Barson & Leibowitz, 2017). They are found in different organs of the human body, including the reproductive system(Joshi & Singh, 2018).
Function: It regulates the cognitive actions, wakefulness and sleep, arousal, energy balance and appetite. Also, it controls the fat cells and the function of cardiac vessels in addition to thermoregulation, activity or motor diligence, emotional control, stimulation and others. It has an effective role in controlling the autonomic nervous system and controlling reproduction. The large number of orexin functions and diversity makes it a promising therapeutic target for the treatment of many diseases, including mental disorders, insomnia, heart disease, infertility and cognitive disorder(Mavanji et al., 2017; Sieminski, Szypenbejl, & Partinen, 2018).
Clinical importance: When observing the decrease in the proportion of orexin in most diseases, its clinical importance became clear, as it decreases in people with narcolepsy narvosa and anorexia nervosa (Almeneessier et al., 2018). It is also confirmed(Rani, Kumar, & Krishan, 2019) that the proportion of Orexin decreases in people with chronic obstructive pulmonary, and people with diabetes. Also, metabolic syndrome has proven(Wang et al., 2018) that the proportion decreases in people with Alzheimer's Disease early dementia while it increases in those with Insomnia and depression. In a study(Hayakawa et al., 2017), proportion of Orexin in people with Obstructive sleep apnea (OSA) increases during breathing stop in sleep . In women, it is clinically important and (Li et al., 2019)has been proven as a drug for polycystic ovary syndrome PCOS.
Malondialdehyde is an organic compound. The final product is final product of the process of lipid peroxidation(Wall et al., 2020). In cases of oxidative stress, oxidative stress increases the process of lipid peroxidation, a process that occurs spontaneously within the cells in the body. This process occurs when the production of free radicals increases and exceeds the absorption of antioxidant defense systems to destroy them or release and eliminate products(Sinha & Gupta, 2018).
Lipid peroxidation happens in large quantities in the membrane of sex cells because it includes large amounts of Unsaturated Fatty Acid unsaturated fatty acids. These are more susceptible to free radicals(Panti et al., 2018). The toxic products of these peroxides cause abnormalities in the sex cells(Sinha & Gupta, 2018).
Antioxidants are of great importance in the body of the organism, their function is to protect the body from multiple diseases, including aging, Atherosclerosis, various heart diseases, cancers, and the darkening of the lens of the eye. Antioxidants reduce the risk of free radicals and other functions(Panti et al., 2018). Antioxidants can work by several mechanisms, including:
1- Preventing free radicals from crossing and entering into the cellular contents(Terrón-Camero, Molina-Moya, Sanz-Fernández, Sandalio, & Romero-Puertas, 2018).
2- Detention of supporting factors such as transition metals (Mn, Cu, Fe, Sc) that contribute to the formation of free radicals in addition to disrupting the chain reaction that forms free radicals(Verma et al., 2013). They also remove types of nitrogen and oxygen because they weaken the effect of oxidation. Further, the antioxidants before they interact with other molecules have the ability to interact with free radicals and this protects against the oxidation process(Cross, van der Vliet, O'Neill, & Eiserich, 1994).
The transport of different lipoproteins between tissues plays an important role in fertility and the only lipoprotein whose amount is observed to change in the follicular fluid around the egg developing in the ovary is HDL. It prepares the lipid nutrients into the follicular fluid and into the egg for the manufacture of the cell membrane and for the production of topical steroid hormone or any of the basic processes of normal egg maturation(Andreoli, Carpenter, & Griggs, 2001). The cholesterol is also necessary for the processing of the basic material for the manufacture of steroids and supports the maturation of the follicle, ovulation and luteinization phase(Stein & Myers, 1995).

Aim of the study

The main objectives of the research were:
1. Studying the effect of levels of orexin hormone.
2.Studying the effect of TC cholesterol, TG triglycerides, HDL , LDL and VLDL .
3. Studying the effect of MDA levels and total antioxidant TAC .

2. Materials and working methods

Sample of the study

Blood samples were collected from women visiting the outpatient clinic (gynecology) and Azadi Teaching Hospital from 12/25/2021 to 5/15/2022, and the required information was taken. The study sampled (40) blood samples from pregnant married women in a natural way without taking steroids. The group of patients in the samples in the current study consisted of (30) blood samples from pregnant

women with steroids and (30) blood samples from non-pregnant women after taking steroids and their ages ranged between (15- 45) years. 5 (ml) of intravenous blood was withdrawn for patients and healthy patients by medical syringes that are used for one time only. They were put the blood in glass tubes (Gel tube) clean and sterile with a tight lid and free of anticoagulants, leave at room temperature for (10) minutes until coagulant.

To obtain the blood serum, the tubes are placed in a centrifuge for a period of time (10 min) and at a speed of (4000cycles / minute). Then the serum is extracted by micropipette to be placed in clean, sterile plastic tubes with a cap.

Determination of serum orexin level

The principle of operation of ELISA technology was adopted as a device of the type (BIO-TEK Instruments) in estimating the level of concentration of Orexin by a kit prepared by (Mybiosource – USA) and according to the leaflet attached to the kit prepared by the company.

Determination of serum cholesterol level

Measurment of serum Total Cholesterol concentration

The enzymatic method adopted color (Schettler & Nussel, 1975)using ready-made analysis kit (Kits) from the French company (Biolabo), where the enzyme cholesterol esterase in the analysis kit activates the decomposition of cholesterol esterified. Cholesterol is present in the blood serum to cholesterol and fatty acids. Then cholesterol is oxidized by the enzyme cholesterol oxidase and the presence of oxygen to produce hydrogen peroxide. Hydrogen peroxide reacts with phenol and 4 - Amino antipyrene by the enzyme peroxidase to form pink quinone amine in color, which is absorbed at wavelength (500nm). The intensity of the color is proportional to the concentration of total cholesterol in the blood serum.

Determination of serum triglycerides

Measurment of serum Triglyceride concentration

Serum triglycerides concentration was estimated using BioLAPO diagnostic kit (D. Labbé al.; Fabiny & Ertingshausen, 1971).

Determination of serum cholesterol LDL

Measurment of serum Low density lipoproteins-cholesterol (LDL-C)

The value of (LDL-C) was estimated according to the following equation according to the method of Andreoli and his group(Andreoli et al., 2001) according to the following equation:

LDL-C(mg/dl)= Total cholesterol –(HDL+ VLDL)

Determination of serum cholesterol HDL

Measurment of serum High density lipoprotein-Cholestrol (HDL-C)

The HDL percentage in the blood serum was calculated by adopting the ready-made work kit (cut) of the French company Biolaba(Stein & Myers, 1995). Depending on the enzymatic method, the principle of action depends on the enzymatic method, in which the chylomicrons and lipoproteins of LDL and VLDL are deposited. By adding Phosphotugstic acid, eventually HDL-c remains in the blood serum after the centrifugation process.

Determination of the level of very low-density lipoproteins in the serum

Measurement of very low-Density Lipoprotein – Cholesterol(VLDL-C) Concentration in serum

VLDL is extracted from the equation (Friedwalds):

VLDL = triglyceride/5(Friedewald, Levy, & Fredrickson, 1972)

Determination of Malone Dialdehyde Level in Serum

Measurment of malondialdehyde in serum (MDA)

The calculation of malondidehyde is a measurement of lipid peroxidation through the reaction of thiobarbituric-TBA with MDA(Sochor et al., 2012). TBA reacts with MDA under conditions of temperature and low pH, as the reaction occurs in an acidic medium to form the pink color of the complex [TBA] 2-malondialdehyde. The absorbency is measured at the wavelength 532nm. This is consistent with the percentage of lipid peroxide in the sample.

Measurment the level of Total antioxidant capacity (TAC) in the blood serum

The FRAP method is an easy way to calculate the capacity of antioxidant power that reduces the complex [Fe(III)-TPTZ] ferric tripyridyltriazine to TPTZ] ferrous tripyridyltriazin -[Fe (II) with intense blue color absorbed at wavelength (593nm). It is used to obtain the FRAP values by comparing the absorption change in the reaction mix test with those containing Fe (II) ions in known concentrations(Benzie & Strain, 1996).

3. Results and discussion

Orex Concentrations Values

Table (1) OXA Level in Pregnant Women with Normal Pregnancy, Pregnant Women after Taking Steroids and Non-Pregnant Women After Taking Steroids by Age Groups

Age Group	OXA Normal Pregnancy (Mean± SD)	Non-pregnant OXAafter taking steroids (Mean± SD)	Pregnant OXA after taking steroids (Mean± SD)
15-25years	± 15.12 80.37a	70.15a ± 12.60	70.20a ± 12.50
26-35years	114.90a ±13.20	78.73a± 13.11	78.39a± 13.00
36-45years	±15.3 a 83.90	112.0a± 13.10	73.17a±14.10
P-value = (0.271)ns			

The results in Table (1) show that there is an increase with no statistical differences at the level of probability (p> 0.05) for the age group (26-35) years

in pregnant women with a normal pregnancy compared to the two age groups (15-25) years (26-35) years. Yet, it increased and no statistical differences appeared in the age group (36-45) years in non-pregnant women after taking steroids compared with the two age groups (15-25) years (26-35) years. In pregnant women after taking steroids, the percentage of orexin increased in the age group (26-35) years compared to the two age groups (15-25) years (36-45) years.

Orexin in normal pregnancy is more than non-pregnant women after taking steroids and pregnant women with steroids. This in turn affects the E2, as E2 is high in normal pregnancy. Pregnant women after taking steroids and non-pregnant women after taking steroids have the same way with estradiol and these results correspond to Kiezun et al. (2017)(Marta Kiezun). Orexin works to reduce estradiol(E2) in the middle and end of pregnancy. It regulates reproductive function by affecting the axis of the ovaries under the lateral thalamus and pituitary gland. It is produced in the uterus and placenta, and changes in orexin values during pregnancy indicate that orexin activity can be regulated by intrauterine factors(Dobrzyn et al., 2018).

Values of malondialdehyde concentrations

Table (2) MDA Level of Pregnant Women with Natural Pregnancy, Pregnant Women After Taking Steroids and Non-Pregnant Women After Taking Steroids by Age Groups

Age Group	MDA Normal Pregnancy (Mean± SD)	MDA Non-pregnant After Taking Steroids	MDA pregnant women after taking steroids
15-25years	5.37a ± 0.43	6.03a± 1.14	7.93a±1.48
26-35 years	4.74a ± 1.32	5.59a± 1.19	6.27a± 1.08
36-45 years	7.88a ± 1.47	.18a± 0.39 8	9.25a±1.06
P-value = (0.121)ns			

Table (2) showed that the level of MDA rises and no statistical differences at the level of probability ($P > 0.05$) for the three age groups respectively for pregnant women with steroids and non-pregnant women after taking steroids compared with pregnant women with natural pregnancy. Here, the highest percentage was in the age group (36-45) years for both pregnant women with natural pregnancy and pregnant with steroids and non-pregnant women after taking steroids compared with the age group (15-25) and (26-35) years. This is consistent with the results of the study reached by (Castro et al., 2012; Hussein, Hassan, & Zeki, 1996) which showed an increase in the percentage of malondidehyde with age for pregnant women. The increase in the percentage of MDA with age is due to the high production of free radicals leading to an increase in the process of lipid peroxidation. It leads to a loss of equilibrium between the proportions of anti-oxidants and active oxygen classes, and this results in oxidative stress(Abou-Seif & Youssef, 2001).

Total Antioxidant Concentrations Values

Table (3) TAC Level in Pregnant Women with Normal Pregnancy, Pregnant Women after Taking Steroids and Non-Pregnant Women After Taking Steroids by Age Groups

Age Group	TACNormal Pregnancy (Mean± SD)	TACNon-pregnant after taking steroids (Mean± SD)	TAC pregnant women after taking steroids (Mean± SD)
15-25 years	0.3743from ± 0.03261	0.3885from ± 0.03516	0.4457a ± 0.04521
26-35 years	0.2835c ± 0.02521	0.3708from ± 0.02616	0.3572b ± 0.03315
36-45 years	0.2318bc ± 0.02631	0.3668from ± 0.02721	0.3265b± 0.03415
P-value =(0.002)**			

Table (3) reveals the total antioxidants for both pregnant women with a natural pregnancy and pregnant women after taking steroids and non-pregnant women after taking steroids. It was found that there was a decrease with high statistical differences at the level of probability ($P \leq 0.01$) of the age group (36-45) years for pregnant women with a normal pregnancy more than the two age groups (15-25) and (26-35) year. This is also the case for the two groups (pregnant women after taking steroids and non-pregnant women after taking steroids).

Antioxidant activities decrease in older women when compared to younger women because of the high percentage of free radicals with age(Carbone et al., 2003).. (Khan et al., 2010) also pointed out that the decrease in antioxidant activities is due to the accumulation of free radicals and the inhibition of antioxidant systems can lead to the aggregation of H 2 O 2 (Ghara, Ghadi, & Dhawan, 2016). The reason for this decrease can be the fact that as women age, the percentage of free radical production increases. The reason for the loss of the balance between the effectiveness of antioxidants and free radicals and thus the proportion of antioxidants in the blood will decrease(Hussein et al., 1996).

Values of fat concentrations

Table (4) shows the levels of lipids in blood serum of pregnant women with normal pregnancy, pregnant women after taking steroids and non-pregnant women after taking steroids by age groups.

Totals	Age Groups	TC Mean±SD	TG Mean± SD	HDL Mean± SD	LDL Mean± SD	VLDLMean± SD
Natural pregnancy	15- 25 years	154.30_{a}± 19.40	147_{a}±18.0	48.33_{a}± 5.75	83.1_{a}±10.31	26.02_{a}± 2.39
	26-35years	159.90_{a}± 19.1	134.90_{a}±18.1	48.24_{a} ± 6.90	85.8_{a}±11.4	26.99_{b}±3.37
	36-45 years	164.62_{a}± 18.01	141.90_{a}± 18.5	45.38_{a}±4.53	88.8_{a}± 11.51	28.39_{b}±3.54
Non-pregnant women after taking steroids	15-25 years	161.50_{a}±20.80	96.65_{c}±12.64	*47.67_{a}± 5.39*	85.5_{a}±12.5	19.33_{c}±2.33
	26-35 years	180.80_{a}±21.70	94.45_{c}± 12.21	45.36_{a}± 4.51	83.6_{a}±12.6	18.89_{c}±2.51
	36 -45 years	186.80_{a}±20.3	84.04_{c}± 10.6	44.60_{a}± 4.6	87.9_{a}±10.5	22.91_{c}±2.11
Pregnant women after taking steroids	15-25years	162.77_{a}± 20.6	109.24_{b} ±13.20	46.44_{a}±3.54	94.5_{a}± 12.8	21.85_{bc}±2.73
	26-35 years	153.77_{a}±19.18	115.93_{b}±13.5	45.82_{a}± 4.5	84.8_{a}±12.1	23.19_{bc}±3.15
	36-45 years	164.00_{a}±20.1	110.63_{b}±13.6	44.75_{a}± 4.43	97.9_{a}±12.7	24.33_{bc}±2.51
		P=0.550)^{ns}	P= 0.0002)**	P=0.339)^{ns}	P=0.127)^{ns}	P= 0.002)**

Similar letters mean that there are no significant differences between them
Different letters mean a significant difference

Table (4) showed that the level of cholesterol increase and did not show statistical differences at the level of probability (p>0.05) for the three age groups in the blood serum of pregnant women, non-pregnant women and pregnant with steroids and that these results were consistent with the results(J.R., 1999) which indicated an increase in the level of cholesterol stubbornly aging. The reason for high cholesterol in old age can be due to the slowdown of metabolism as it progresses with age, which has been proven the lack of parity between the demolition process and the construction process, and the tendency to high percentage of demolition, causing a high percentage of body fat and an increase in blood cholesterol(J.R., 1999).

The results showed an increase with high statistical differences in the level of triglycerides at the probability level ($p \leq 0.01$) for the three age groups in the blood sera of pregnant women with normal pregnancy and steroids compared with the age groups of the non-pregnant group as in Table (4). These results are consistent with the results of previous research, which indicated an increase in triglycerides in the serums of pregnant women. The reason for this increase is due to the breakdown of lipids and fatty acid discharge, leading to Increasing the percentage of triglycerides in serum(Al-Obeidi, 2005).

The results shown in Table (4) showed that the percentage of very low density lipoproteins (VLDL) increases with high statistical differences at the probability level ($p \leq 0.01$) for pregnant women with normal pregnancy, pregnant with steroids and non-pregnant for the three age groups. Here, the highest percentage of increase was recorded in the age group (G3=36-45). The reason for the Oxidation in the body increases the proportion of very low density lipoproteins leading to a decrease in the effectiveness of the enzyme Lipoprotein lipase, which is present in the tissue of the body. This decrease leads to an imbalance in the percentage of lipids and an increase in the proportion of triglycerides in the serum. The percentage of glycerides in very low density lipoproteins rises and this reason can be explained by the mechanism of high triglycerides (T.G) in very low density lipoproteins (VLDL) according to the mechanism of increasing triglycerides itself and this is consistent with the study(Salem, Badr, & Neamat-Allah, 2011).

It is clear from Table (4) that there is a decrease and no statistical differences appeared at the probability level ($p > 0.05$) in the level of HDL in the serums of pregnant women with normal pregnancy, pregnant women after taking steroids and non-pregnant women after taking steroids for the three age groups. Here, the highest percentage decrease was recorded in the age group (G3=36-45). The reason for its decrease is the high activity of Hepatic lipase. The hepatic enzyme analyzes the triglycerides inside the HDL molecule, leading to the rapid removal of HDL from the blood, thus contributing to the reduction of its percentage(Kim, Nian, & McIntosh, 2007).

The results in Table (4) showed an increase with no statistical differences at the probability level ($p> 0.05$) in the level of (LDL) in the serums of pregnant women with normal pregnancy and pregnant women after taking steroids compared with non-pregnant women after taking steroids for the three age groups. The highest percentage of increase was recorded in the age group (G3=36-45) due to the receptors of low density lipoproteins (LDL) on the surface of the liquefied cell which is responsible for the bodies of LDL lipoproteins. These bodies have an important role in the rise in the level of LDL in the targeted serum; this increase reduces the

performance of the pathway that has been adopted for LDL acceptors and enhances the flow of low-density lipoprotein molecules in the walls(Orekhov, Bobryshev, Sobenin, Melnichenko, & Chistiakov, 2014). It is due to the increase in cholesterol because low-density lipoproteins are the main carrier of cholesterol.

Conclusions

There is a significant increase in the level of (TC, TG, VLDL, LDL, MDA, OXA, among pregnant women with normal pregnancy and pregnant women after taking steroids compared with non-pregnant women after taking steroids. These traits were compatible with age. There is also a decrease in the level of HDL and total antioxidants in pregnant women with normal pregnancy, pregnant women after taking steroids and non-pregnant women after taking steroids. These qualities were compatible with age.

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