International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 4 Issue 11, November-2015

Comparison of Serum Prolactin level during pregnancy determined by Square Wave Voltammetric and Minividas Methods

Eman A. M. Al-Jawadi

Chemistry Department, College of Science, University of Mosul, IRAQ

ABSTRACT

This paper describes the determination of Prolactin (PRL.) by using new method depended on the Squer wave Voltammetry (S.W.V.) at HMDE. , start by selecting the optimum conditions at the beginning , a well defined peak was observed at (-0.469) V vs. (Ag/AgCl, Sat KCl) , the calibration curve of PRL. in the phosphate buffer (pH=6.5) has been studied, it was linear relationship within the correlation coefficient is (R = 0.9921). Another method it was the Minvidas method was used to comparison the results of PRL concentration during pregnancy. The aim of the study was to estimate the relationship between two methods by followed a progression rise of serum prolactin concentration from the first trimester to the second and third trimester with respect of non pregnant women as control. Serum Prolactin concentration was measured using S.W.V. and Minividas methods. As a result, it was used S.W.V. is better than the classical method by Minividas.

Keywords: Prolactin, Pregnancy, Female, S.W.V., Minividas.

INTRODUCTION

Prolactin (PRL) is one of several hormones secreted by special cells of the adenohypophysis (anterior pituitary gland), Prolactin is a polypeptide hormone involved in many biological functions in women. PRL has many different roles throughout the body, perhaps the most important classical role of prolactin is controls the initiation and maintenance of lactation to stimulate milk production in women after the delivery of a baby, so an appropriate expression of PRL. action, breast tissue requires priming by estrogens, progestins, corticosteroids, thyroid hormone, and insulin. PRL induces ductal growth, development of the breast lobular alveolar system, and the synthesis of specific milk proteins.
Usually most of the papers whose studied the case of PRL. hormone during pregnancy by Elisa or Minividas, in this article describes PRL. hormone during pregnancy using new method ,it was Squer wave voltammetry (S.W.V.) and compared the results of it with the classical method (minividas) , the visualized immuno flourescent-detecting assay(Vidas) and miniVIDAS instruments are multiparametric immunoassay systems designed to help provide better care and the most accurate laboratory results. The assay principle combines a one –step enzyme immunoassay sandwich method with that fluorescent detection(ELFA-Enzyme Linked Fluorescent Assay).

Pregnancy is a period marked by profound changes in a woman's hormonal status and metabolism. It is known that normal human pregnancy lasts for about 40 weeks as measured from the day of the last menstrual cycle (LMP). Pregnancy is usually divided into three intervals called trimesters with each one beginning a little longer than 13 weeks. The first trimester ends at the beginning of the 13th week, which is the three completed months of pregnancy. The second trimester usually ends at the end of the27 week. The third trimester can end anywhere between the 38 and 42 week.

It circulates in low levels in the bloodstream of nonpregnant women. During pregnancy, prolactin levels rises progressively from 8 weeks of gestation reaching maximum values at 40 weeks of pregnancy. Women who are not pregnant and are not breastfeeding should have lower levels of PRL (typically 2.3-23 ng/ml in women and 0.1-20 ng/ml in men are defined as "normal levels") , On the other hand, a non-pregnant woman has abnormally high levels of PRL, it may cause her difficulty in becoming pregnant or may cause directly or indirectly infertility, although spontaneous pregnancy may occur occasionally and if serum prolactin levels greater than100-300 ng/ml in non pregnant females may indicate a pituitary adenoma(tumor).

EXPERIMENTAL

Apparatus:

Voltammetric measurements were carried out using a Metrohm instrument, model 797 VA computrace (Metrohm AG,CH-9101 Herisav ,Switzeraland.), with stand three-electrodes containing a HMDE as a working electrode . Ag/AgCl, Sat. KCl as reference electrode and a platinum wire as an auxiliary electrode. Minividas measurement were carried out by Minividas reader (69280-marcy-letoile) from biomerieuxitaliy company /Italy. The pH of the solutions was controlled with a HANA pH meter.

REAGENTS:

All the chemicals used were of analytical reagents grade. Phosphate buffer was used as a supporting electrolyte for all experiments which was prepared by mixing certain amounts of 0.2 M of each of K2HPO4 and KH2PO4. Prolactin hormone and the standard of it from Prolactin hormone kit (biomerieux SA / vidas PRL.), the standard Prolactin hormone (18.0 ng/ml) reconstitute each vial of lyophilized with 3ml of distilled water ,wait 5-10 minutes then mix it and stored at (-25±6 °C).

SAMPLES COLLECTION & GROUPING PROTOCOL

The study included collection the samples of femal serum from Mosul Hospitals, the samples from 160 women divided to four groups :non pregnant women as the Control case, 1st trimester gestation , 2nd trimester gestation, 3rd trimester gestation (with 40 samples for each case).

PROCEDURE

The square wave voltammetric was used the optimum conditions was find during the study with: Deposition potential (-0.700V), Deposition time (90s), Equilibration time (5s), Voltage step (0.008 V), Amplitude (0.050 V), Frequency (70.000Hz). These experiments carried out in Phosphate buffer solution (P.B.S) (0.2 M) pH=6.5 was used in cell as the media to study the optimum conditions of PRL. , the buffer solution was deareated by passing a slow stream of purified N2 gas through it for (15) min. to remove dissolved oxygen, the back current was recorded, appropriate amount of PRL.were added to this solution to reached the desired concentration and the cell solution was deareated (every new addition) by passing a slow stream of purified N2 gas through it for (15) min,,the square wave voltamogram was recorded again ,finally the calibration curve was constructed. The same above procedure was used in the presence of (160) female serum samples to constructed four calibration curves for (control ,1st trimester, 2nd trimester ,3rd trimester cases). Vidas method prolactin hormonal assay carried out for the same samples of the four cases using a commercially prepared Vidas kit, under the assay conditions in the kit.

RESULT AND DISCUSSION

Square wave voltamogram of (0.06278) ng/ml stand.PRL. in phosphate buffer at (pH=6.5) (fig.1) showed a well applied peak at -0.469 V vs Ag/AgCl /3M KCl.

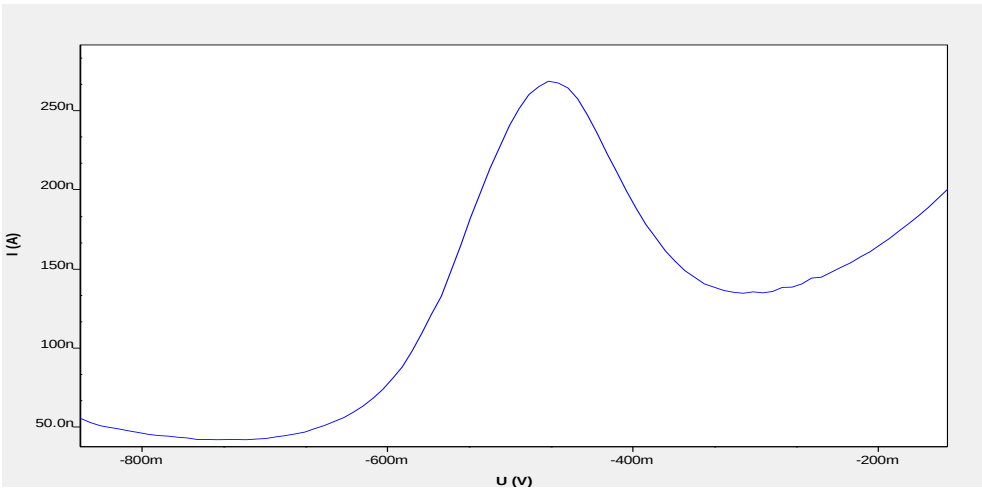

Fig. (1) : Square wave voltamogram of (0.06278) ng/ml stand. PRL in phosphate buffer (pH=6.5).

Optimum condition: The square wave voltammogram of (1.1624) ng/ml stand .PRL. was investigated in phosphate buffer (pH=6.5) variation all the parameters that it depend on the measurement in Table (1).

Table (1) : The optimum values obtained which give either the highest peak current and the best resolution of the peak

Condition	Value
Start potential (V)	-0.850
End potential (V)	-0.150
Deposition potential (V)	-0.700
Deposition time (s)	90
Equilibration time (s)	5
Voltage step (V)	0.008
Voltage step (V)	0.008
Amplitude (V)	0.050
Frequency (Hz)	70.000

EFFECT OF PH:

Square wave voltamogram of (1.1624) ng/ml stand.PRL. were investigated at different pH values (2-9) using the optimum condition in phosphate buffer show in table (1).The Ep value for stand PRL. exhibits a linear dependence on pH(Fig.2).

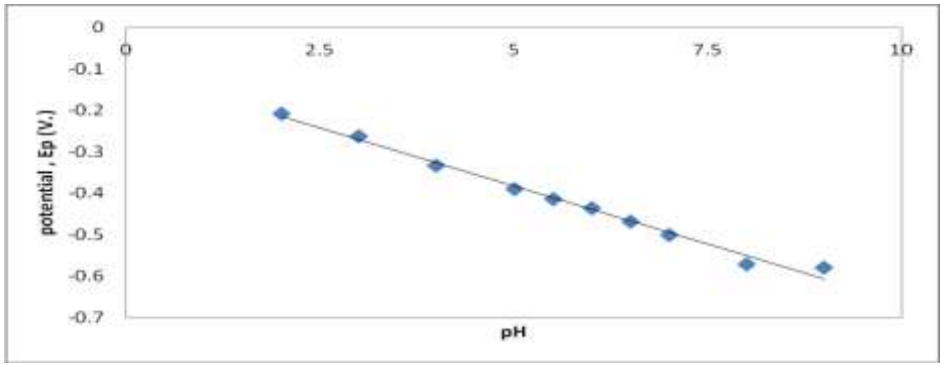

Fig.(2) : Effect of pH on S.W.V. peak of (1.1624) ng/ml stand.PRL.

From these result was showed that pH 6.5 gives the shape and highest peak current,which is then used in the subsequent experiments . On the other hand the peak potential (Ep) is found to be dependent on pH and shifted to more negative value with increasing pH , the plotof pH vs. Ep gives a straight line with correlation coefficient (R=0.989),the value of slope is very near to theortical value obtained by Hammett equation which is equal to (-0.059 VpH-1).

ANALYTICAL CONSIDERATION

Using the optimum condition showing in Table (1), the calibration curve was constructed using a serial addition **(0.08995 - 16.3636) ng/ml** of a standard PRL. in (10 ml)aqueous-phosphate buffer solution(**P.B.S**, pH=6.5). The results are shown (fig.3).

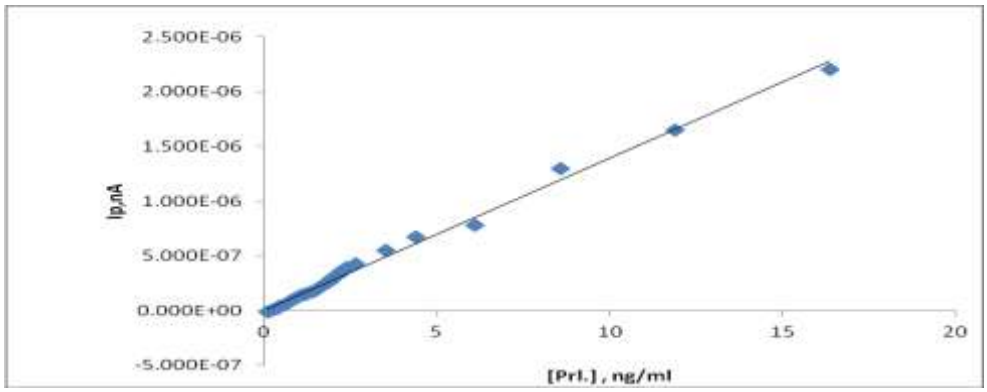

Fig. (3):The relation between peak current (Ip) and concentration of (0.08995 - 16.3636)ng/ml of a standard PRL. at (pH=6.5) in P.B.S.

The plot peak current Ip vs. concentration of stand PRL. are showing in fig. 3. It is very clear that the intended relation shows straight line, on the concentration range of (0.08995 - 16.3636)ng/ml of a standard PRL. at (pH=6.5) in P.B.S., with correlation coefficient (R=0.993).

APPLICATIONS OF PROPOSED METHOD

The present work was conducted to study the level of prolactin for pregnant women. For this purpose four groups of subjects were studied : Group one :no pregnant women's as control , pregnant women's were distributed as (Group two :1st trimester , Group three :2nd trimester , Group four :3rd trimester gestation)

The result obtained from the study carried out in two procedures (S.W.V. & Vidas) was arranged in table and interpreted using the T-test analytical tool and draw graphs of it . The data obtained consisted of 40 women in each group. From the study carried out, a comparison of the two procedures (S.W.V. & Vidas) for prolactin levels according to four groups (fig.4 -A,B,C,D. respectively as above groups) , it suggested a possible association between two method for prolactin blood levels represented at table (2) the suggested equations to improve the precision between two methods for prolactin blood levels to give the possibility to use any of this methods for the same samples by the correct factor in this equations.

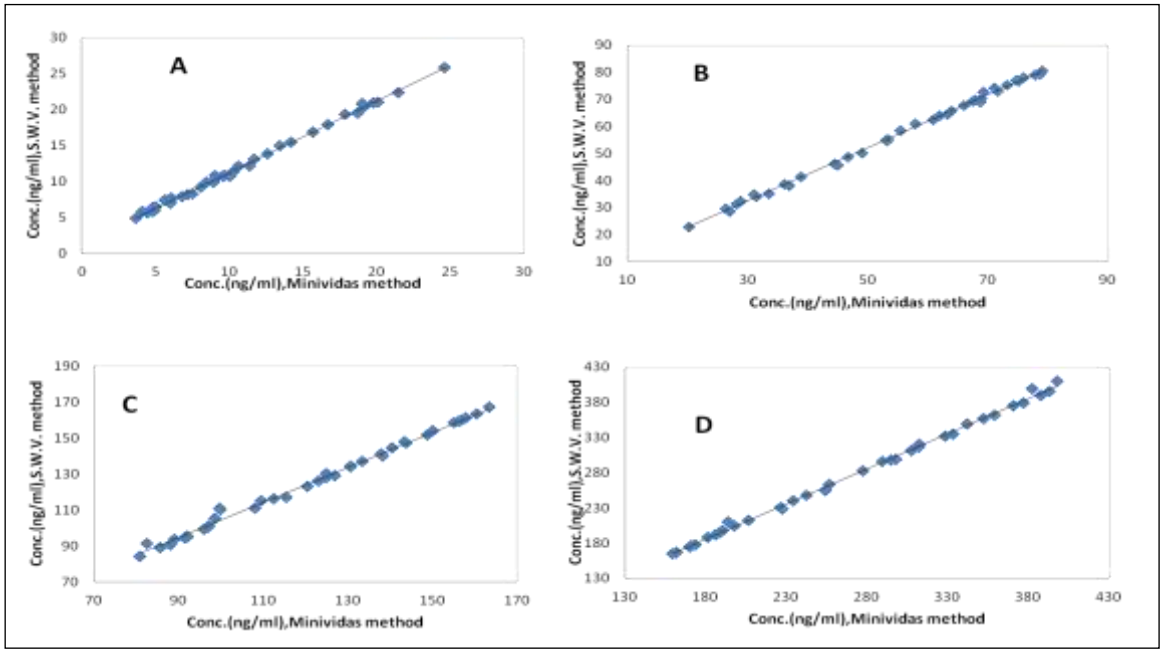

Fig .4.) Relationship between S.W.V. & minividas of Prolactin levels in pregnant and control groups: A) group1 , B) group 2, C) group 3, D) group4

Table (2): Correlation equations & R2 represented of Prolactin levels in pregnant and control groups by S.W.V. & minividas

	The correlation equations represented of PRL. (ng/ml)of SWV. Method & Minividas method	
	Correlation equations	**R^2**
Group one (n=40) Control	Vidas method=[(0.9944*S.W.V.method)+(1.3116)]	R^2 = 0.9972
Group two(n=40) 1st trimester	Vidas method =[(0.9811*S.W.V.method)+(3.1364)]	R^2 = 0.998
Group three(n=40) 2nd trimester	Vidas method=[(0.9758*S.W.V.method)+(6.7087)]	R^2 = 0.9941
Groupfour(n=40) 3rd trimester	Vidas method=[(0.9981*S.W.V.method)+(5.9495)]	R^2 = 0.9981

The women in all groups studied a mean prolactin level which represents at table(3), which was rises progressively from 8 weeks(group2) of gestation reaching maximum values at 40 weeks of pregnancy so it was greater than the mean

of the normal prolactin level for control at group one range 10 ± 2 ng/ml. The women in group 2 were greater than the mean of the normal prolactin level and raises will continue with group 3 & 4 respectively .shown (fig.5). On comparison of the prolactin levels in groups (one, two, three and four) by using two suggested methods, the student t-test was used and a p value of (0.00004690) was obtained. This value indicates that the prolactin level difference between the 2 groups was insignificant. It was observed that the higher the prolactin level, the lower the duration of infertility and vice versa.

Table (3) Comparison of Mean of amount Prolactin levels in pregnant and control groups by S.W.V.& minividas

	Mean of amount of PRL. (ng/ml)by	
	Minividas method	SWV. method
Group one (n=40) Control (women non pregnant)	10.708	11.9289
Group two(n=40) 1st trimester	53.25625	55.3099
Group three(n=40) 2nd trimester	117.6988	121.3792
Groupfour(n=40) 3rd trimester	262.9925	268.3067

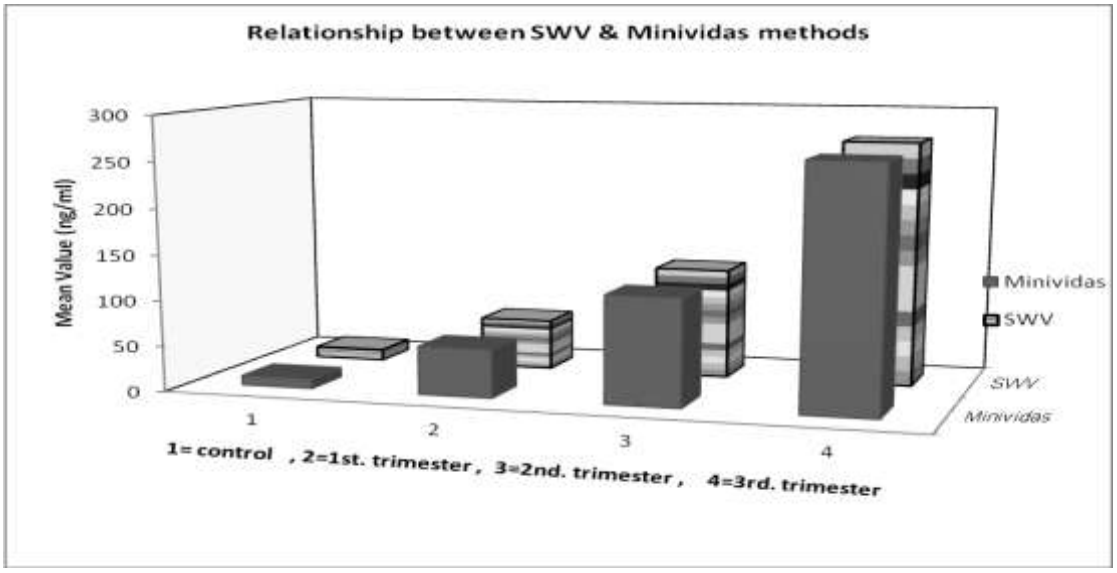

Fig.5): The mean value of Prolactin hormone concentrations in pregnant and control groups by S.W.V. &Vidas methods

CONCLUSION

Changes in Prolactin level during pregnancy have long been established, the serum values increase as the pregnancy progresses. What is novel in our study is the positive correlation observed in the values of Prolactin level in all the stages of pregnancy between the new suggested method (S.W.V.) and the classical method miniVidas which it used at routine laboratory test, they are simple, easy to use methods and that it was also some more advance ,We show it practicality , for S.W.V. related with high sensitivity and less interference due to the basic of S.W.V. which depend on the main substrate redox potential lead to lower interference effect .

REFERENCES

[1]. Crosignani PG., J Reprod Med., Dec;44(12Suppl):1116-20, 1999.
[2]. M. Ajibola, A. Oloruntoba, and O.Valeria, IOSR Journal of Dental and Medical Sciences (JDMS) ,Volume 2, Issue 2 (Nov.-Dec. 2012)., PP 38-41.
[3]. Zavalza-Go´mez AB, Anaya-Prado R, Rinco´n-Sa´ nchez, Jose´ AR, Mora-Martı´nez M., Diabetes Res ClinPract, 80:8–15, 2008.
[4]. Akinloye O., Obikoya O.M., Jegede A.I., Oparinde D.P. and Arowojolu A.O ., Int J Med Biomed Res;2(1):3-12 3, 2013.
[5]. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA , Endocr Rev ,19:225-268, 1998.
[6]. A. Darwish, M. Abdellah and M.AbdelAleem (2012). Hyperprolactinemia and Woman's Health, Some Related Issues, Prof. Atef Darwish (Ed.), ISBN: 978-953-51-0166-6, InTech, Available from: http://www.intechopen.com/books/basic-gynecology-some-relatedissues/hyperprolactinemia-and-women-s-health.