# Comparative Study of Fucose, Protein, Protein Bound Fucose and Protein Bound Hexose in Sera of Hyperprolactinemia Human Female and Healthy Female

**Receivd in : 11 November 2008**
**Accepted in : 30 June 2010**

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## Abstract

This study was conducted in the specialized center of endocrinology and diabetic (Al-Kindy hospital from June 2004 to April 2005).

Sera of 80 women (include 40 diagnosed Hyperprolactinemia (HPro) and 40 healthy women as control) were used to estimate some biochemical parameters which include prolactin (PRL),total fucose (TF), total protein (TP) and protein bound fucose (PBF), and protein bound hexose (PBHex), also $\frac{TF}{TP}$, $\frac{PBF}{TP}$ and $\frac{PBHex}{TP}$ ratios. A significant elevation in TP and PBHex, in sera of HPro patients compared to control was found while PBHex/TP ratio showed a slight non significant increase in sera of patients compared to control.

On the other hand a significant decrease in TF, TF/TP and PBF/TP in sera of HPro compared to control was found. Total fucose level (TF) in sera of HPro compared to control are (6.54 ± 1.52), (10.66 ±4.00) mg/dL respectively.

Protein bound fucose level (PBF) in sera of HPro compared to control are (3.88 ± 1.25), (4.48± 1.63) mg/dL respectively, while protein bound hexose (PBHex) level in sera of HPro compared to control are (131.11±1.43) (123.64±0.497) mg/dL respectively.

The significant increase in PRL levels in sera of patients was correlated with the significant reduced level of fucose in sera of patients compared to control.

The low TF levels in patients could be due to the requirements of PRL variants to different ratios of monosaccharides including fucose and manose for glycosylation of PRL which may alter the biological activity in different species and variety of mammalian.

**Key words: Fucose, Fucose Bound Protein , Hyperprolactinemia**

## Introduction

Prolactin (PRL) is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary lobe the lactotrophs [1].

The most relevant actions of prolactin in the mammalian body are [2] Lactation, Luteal function, Female receptivity [3] and Parental behavior [4]. PRL-R are also present in a wide range of peripheral organs like the pituitary gland, heart, lung, thymus, spleen, liver, pancreas, kidney, adrenal gland, uterus, skeletal muscle, and skin[5].

Markedly raised blood PRL levels (>30ng/mL), might interfere with both endometrial proliferation and follicle growth and development and thereby reduce the likelihood of a successful pregnancy occurring [6].

Fucose (6-deoxy-L-galactose), a mono saccharide is present in low concentrations in normal circulation [7]. Fucose is found in a wide variety of natural substances from many different sources and occurs in abundance in glycoproteins and glycolipids in animals and humans [8].

Glycoconjugates are present in synaptic junction areas where nerve cells meet, implying a role in synaptic membrane involvement in nerve impulse transmissions. Human testes germ cells are rich in fucose and glucose glycoconjugates, which are altered during germ cell differentiation [9].

Glycosylated PRL has been found in the pituitary glands of a wide variety of mammalian, amphibian, and avian species. The degree of glycosylation varies from 1 to 60% among species and may also vary between reproductive states within species [10].

The aim of the present study is to measure TF, PBF, PBHex, TP levels and verify the data obtained to each other by estimation the ratio of TF/TP, PBF/TP, PBHex/TP , also try to find correlations between(Prol. and TF), (Prol. and PBF), (Prol. and PBHex), (TF and PBF).

**Sampling (Subjects)**

In a plane tube (no anti coagulant),10 mL of venous blood was placed, which was taken from the groups, left for (15 min) at room temperature, then centrifuged (at 2500 rpm for 10min) to get the serum, which is stored at (-20$^{o}$C) unless used immediately .

**Collection of blood**

The samples were collected from women patients with HPro at the diagnosis time at (Specialized center of endocrinology and diabetes, Al-Kindy Hospital).

They were classified into two groups as follows:

1) Control group: includes (40) healthy women whose ages ranged between (15-43) years old, with no previous diseases, which may interfere with the parameters analyzed in this study.
2) Hyperprolactinemia women group: includes (40) women whose ages ranged between (16-53) years old suffering from HPro.

**Laboratory work.**

-An automated quantitative test was used on the VIDAS instruments, for the enzyme immunoassay determination of PRL in human serum or plasma (lithium heparinate) using enzyme linked fluorescent assay (ELFA). The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) [11,12].

-Total protein (TP) was determined according to Biuret methods[13] which depends on the reaction of peptide bond of the protein with copper ion ($Cu^{++}$) in alkaline medium to form colored products, whose absorbance is measured by (UV-Vis) at (540nm).

-Total fucose (TF) was determined by using Dische and Shettles methods [14] in a direct reaction of concentrated sulphuric acid with serum components. The reactants were combined with cysteine, and the colored product was measured at (390 and 430nm). The differences in absorbance were directly proportional to $\alpha$-L-fucose content of the solutions.

-Protein bound fucose (PBF) was determined according to Dische and Shettles methods [14] based on colored product (chromophor) was formed by fucose in strong acid medium, which

combined with color developer (Cystein hydrochloride). The colored product with cysteine measured at 390 nm, and 430nm respectively.

-The orcinol reaction [15] is used to determine protein bound hexose (PBHex), by precipitating the hexose moiety of protein-carbohydrate conjugate with ethanol (95%) at room temperature, which measured at 520 nm.

**Statistical analysis**

Data presented were the means and standard deviations; student-t-test was used to compare the significance of the difference in the mean values of any two groups. ($P \leq 0.05$) was considered statistically significant [16].

The overall predictive values for the results in all studied groups were performed according to program of Office XP 2002.

## Results and Discussion

The results of serum prolactin in Hyperprolactinemia females and control are shown in the table (1).

Many factors control, the secretion of PRL physiologically, PRL levels are controlled by the hypothalamus [17].

Hyperprolactinemia (HPro) has been recognized as a cause of infertility problems in men and women [18]. The three etiological forms of HPro are iatrogenic HPro associated with the use of certain medications (e.g. anti-depressants, tranquilizers), primary HPro associated with pituitary tumors[19], and secondary HPro (hyperthyroidism, renal insufficiency)[20]. The release of PRL is assessed at the single-cell level, the pattern of PRL secretion of individual lactotrophs shows sexual dimorphism.

In general, slightly more than half, the lactotrophs of female rats secrete PRL in a continuous pattern[21]. Several investigations have been concluded that, calcitonin-like peptides are effective in inhibiting PRL secretion when administered in vivo [22] .

From the table(2) there was a significant increase in TP level between HPro group and control with P value equals 0.003.

A significant decrease in total fucose was found between HPro and control, also a significant decrease in TF/TP ratio. For HPro compared to control was found. The plasma levels of proteins depend on the balance between their synthesis and their degradation[23].

Since PRL is a protein hormone of the anterior pituitary gland and can be synthesized and secreted which is not restricted to the anterior pituitary gland, but other organs and tissues in the body have this capability[24] .Some stimulators of pituitary PRL secretion also affect hypothalamic PRL production, for example, ovarian steroids modulate hypothalamic synthesis and release of PRL[25,26].

Fucosylated glycans have been amplicated in the pathogenesis of several human diseases[27]. High levels of fucose were reported in sera of hepato cellular carcinoma patients and diabetes mellitus[28,29], and in leukemic patients[30]. The reduction in TF for HPro patients could be due to the high biological activity of the anterior pituitary which required higher glycosylation. Where the carbohydrate residues contain varying ratios of sialic acid,

fucose, manose and galactose which differ considerably between species, physiological and pathological and pathological states[31,32,10].

The disturbance in the level of PBF and PBHex in patients could be due to the fucoligands in the cells of interest[10].

Glycosylation of the asparagyl residues ($Asn^{35}$, $Asn^{80}$, $Asn^{108}$) of the extracellular domain of the PRL-R is crucial, although not absolute requirement for PRL-R activation. Although PRL-R is mainly a cell-surface receptor, deglycosylated forms of PRL-R can be accumulated in the Golgi apparatus[33]. Nitric oxide activates N-acetylglucosamine transferase, which is responsible for glycosylation of these intracellular receptors and promotes migration of these newly glycosylated receptors to the cell surface[34].

**Correlation relation**

The correlation coefficient (r) test is used to describe the association between the different studied parameters, $P<0.01$ was considered statistically significant.

1- Correlation relation between prolactin PRL and total fucose TF

Figures (1-A) and (1-B) showed a high significant positive correlation between PRL and TF marker with P value 0.0001 for hyperprolactinemia (HPro) and control groups with correlation coefficient (r) values (0.775), (0.789) respectively.

2. Correlation relation between PRL and protein bounded fucose PBF.

Figures (2-A) and (II-B) showed a high significant positive correlation between PRL and PBF marker with P value 0.0001 for HPro and control groups with correlation coefficient (r) value (0.923), (0.769) respectively.

3. Correlation relation between TF and PBF

Figures (3-A) and (III-B) showed a high significant positive correlation between TF and PBF marker with P value 0.0001 for HPro and control groups with correlation coefficient (r) value (0.893), (0.862) respectively.

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**Table (1):Levels of PRL in sera of Hyperprolactinemia patients and control**

| Group description | No. | Age | PRL. M.I.U/mL mean±SD | P |
|---|---|---|---|---|
| Control | 40 | 15-43 | 14.20 ± 5.75 | |
| Hyperprolactinemia | 40 | 16-53 | 45.74 ± 36.88 | < 0.05 |

**Table (2):Levels of TP, TF and TF/TP ratio in sera of Hyperprolactinemia patients and control**

| Group description | No. | TP g/dL mean±SD | P | TF mg/dL mean±SD | P | TF/TP mg/g mean±SD | P |
|---|---|---|---|---|---|---|---|
| Control | 40 | 6.44 ± 0.20 | | 10.66 ± 4.00 | | 1.65 ± 0.61 | |
| Hyperprolactinemia | 40 | 6.71 ± 0.54 | 0.003 | 6.54 ± 1.52 | 0.05 | 0.98 ± 0.22 | < 0.05 |

**Table (3):Levels of PBF, PBHex and PBF/TP, PBHex/TP ratio in sera of Hyperprolactinemia patients and control**

| Group description | No. | PBF mg/dL mean±SD | P | PBF/TP mg/g mean±SD | P | PBHex mg/dL mean ±SD | P | PBHex/TP mg/g mean±SD | P |
|---|---|---|---|---|---|---|---|---|---|
| Control | 40 | 4.48 ± 1.63 | | 0.70 ± 0.25 | | 123.64 ± 0.49 | | 19.22 ± 0.59 | |
| Hyperprolactinemia | 40 | 3.88 ± 1.25 | 0.07 | 0.58 ± 0.18 | 0.02 | 131.11 ± 1.43 | 0.00 | 19.66 ± 1.67 | 0.12 |

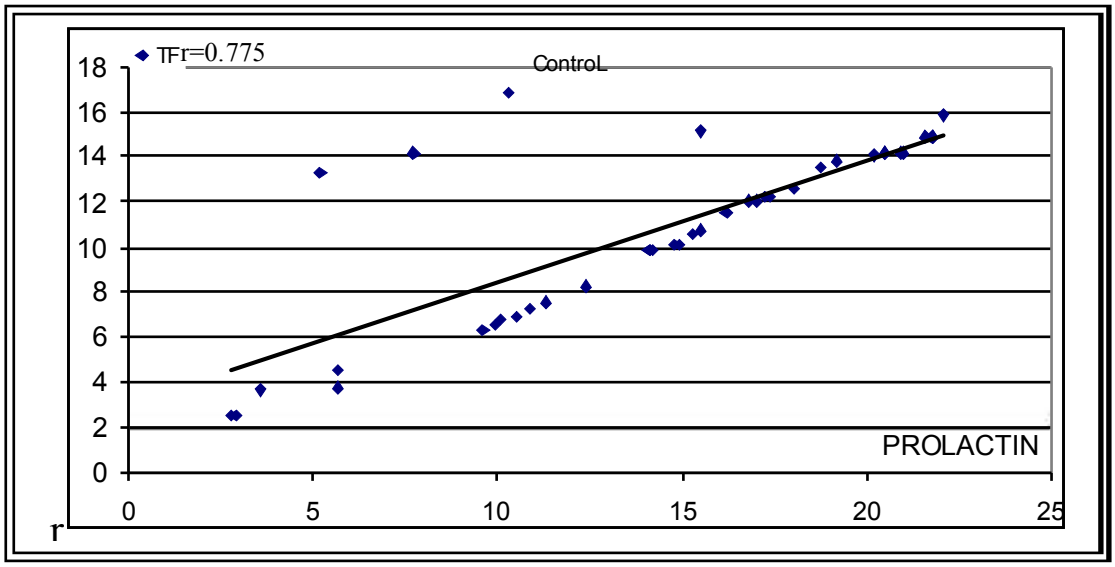


**Fig. (1-A): Correlation relation between PRL and TF in control group**

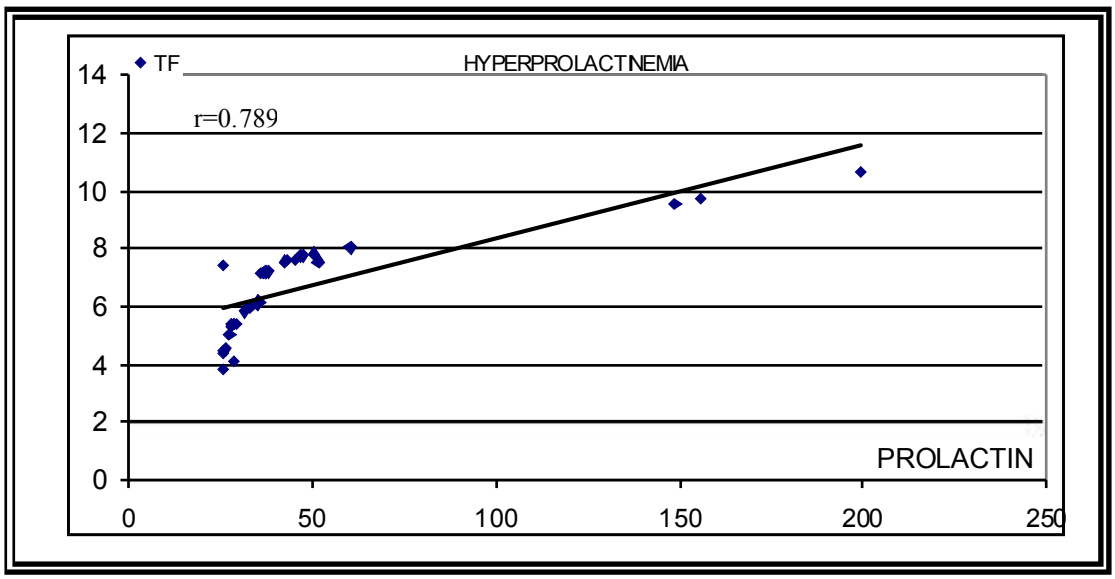


**Fig. (1-B): Correlation relation between PRL and TF in Hyperprolactinemia patients group**

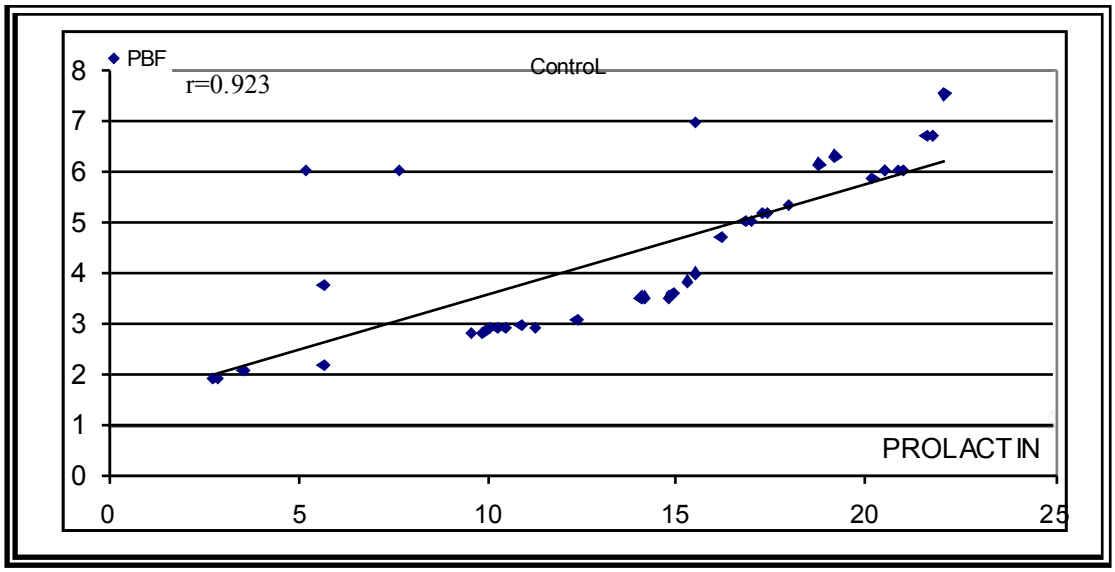


**Fig .(2-A): Correlation relation between PRL and PBF in control group**

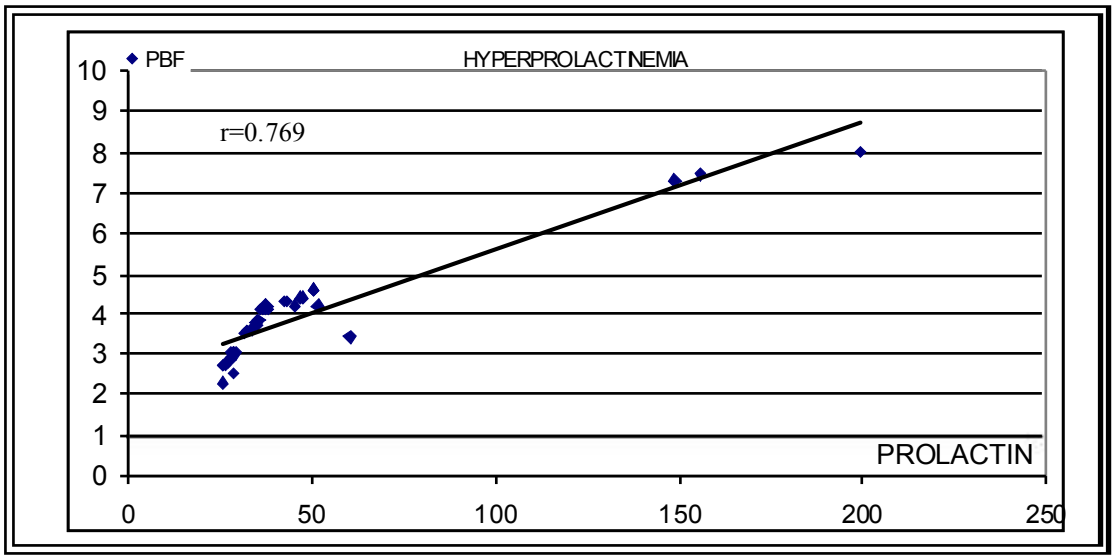


**Fig. (2-B): Correlation relation between PRL and PBF in Hyperprolactinemia patients group**

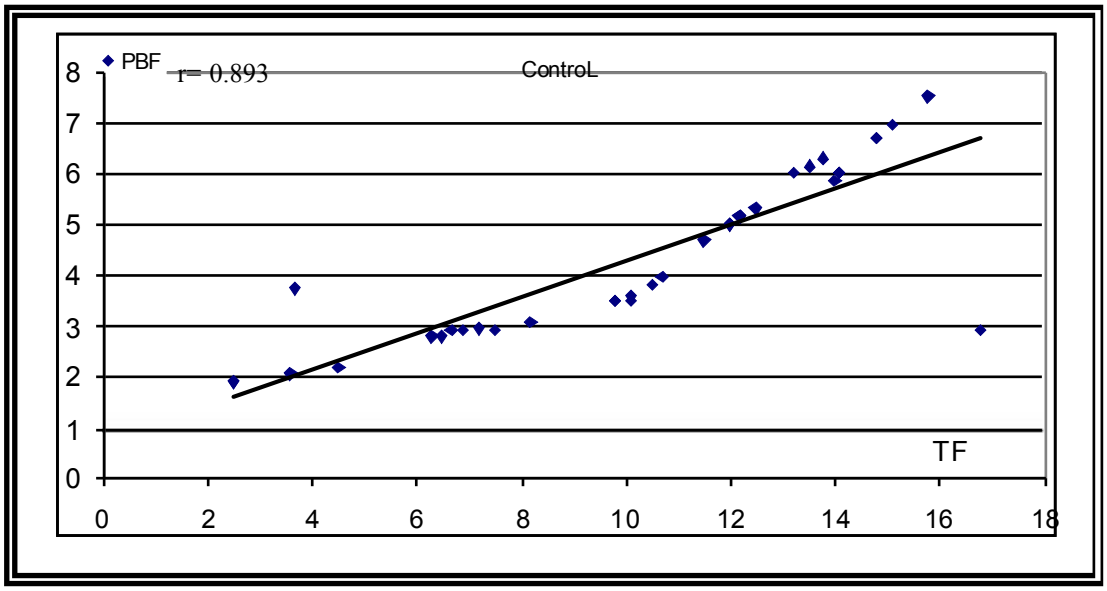


**Fig (3-A): Correlation relation between TF and PBF in control group**

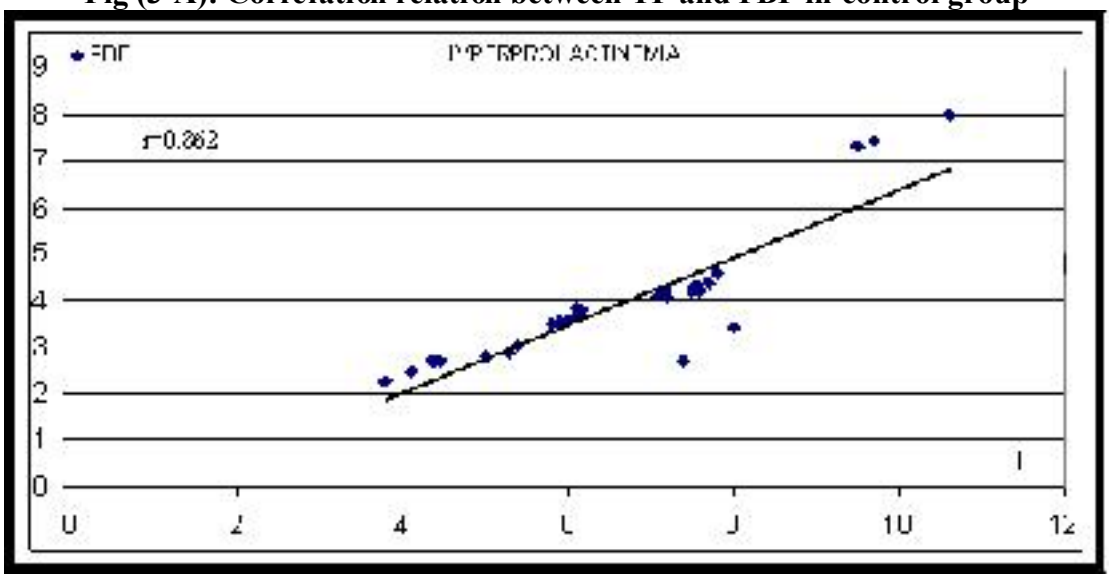


**Fig. (3-B): Correlation relation between TF and PBF in Hyperprolactinemia patients group**

# دراسة مقارنة لسكر الفوكوز، البروتين، فوكوز المرتبط بالبروتين والسكريات المرتبطة بالبروتين في مصول دم النساء المصابات بأرتفاع هرمون البرولاكتين والنساء الاصحاء

**استلم البحث في :11 تشرين الثاني 2008**
**قبل البحث في : 30 حزيران 2010**

**وفاء الطائي ،منتهى عباس العقابي**
**قسم الكيمياء، كلية التربية ابن–الهيثم ، جامعة بغداد**

## الخلاصة

إجريت هذه الدراسة في المركز التخصصي للغدد الصم والسكري التابع لمستشفى الكندي المدة من حزيران 2004 الى غاية نيسان 2005.

لقد استعمل مصل دم 80 امرأة 40 منهن مصابات بارتفاع في إفراز هرمون البرولاكتين و 40 امرأة من الأصحاء مجموعة سيطرة لتقدير بعض الدوال الكيموحيوية التي تضم هرمون مولد الحليب (PRL)، والفوكوز الكلي ،(TF) والبروتين الكلي (TP)، والفوكوز المرتبط بالبروتين (PBF) والسكريات السداسية المرتبطة بالبروتين (PBHex) كذلك حسبت نسبة الفوكوز الكلي إلى البروتين الكلي ($\frac{TF}{TP}$) ونسبة الفوكوز المرتبط بالبروتين إلى البروتين الكلي ($\frac{PBF}{TP}$) ونسبة السكريات السداسية المرتبطة بالبروتين إلى البروتين الكلي ($\frac{PBHex}{TP}$) .

لقد وجدت زيادة معنوية في مستوى البروتين الكلي والبروتين المرتبط بالسكريات السداسية والكالسيوم الكلي. البروتينات الدهنية العالية الكثافة في مصول دم النساء المصابات بارتفاع في الهرمون المولد للحليب مقارنة مع الأصحاء، بينما أظهر مستوى الكولسترول ونسبة البروتين المرتبط بالسكريات السداسية إلى البروتين الكلي ارتفاعاً غير معنوي مقارنة مع مجموعة السيطرة.

لقد وجد انخفاض معنوي في مستوى الـ TF و $\frac{TF}{TP}$ و $\frac{PBF}{TP}$ في مصول دم النساء المريضات مقارنة مع مجموعة السيطرة،كما أظهرت مستويات ال(PBF) قلة غير معنوية في مصول دم المريضات مقارنة بمجموعة السيطرة.

إن مستوى الفوكوز الكلي في مصول دم المريضات المصابات بارتفاع الهرمون المولد للحليب مقارنة مع الأصحاء هي (1.52±6.54) و (4.00±10.66) ملغم/100مل على التوالي.

إن مستوى البروتين المرتبط بالفوكوز في مصول المريضات هي (1.25±3.88) و (1.63±4.48) ملغم/100مل على التوالي والبروتين المرتبط بالسكريات السداسية (PBHex) في مصل دم المريضات المصابات بارتفاع هرمون البرولاكتين (1.43±131.11) و (0.49±123.64) ملغم/100مل على التوالي الزيادة المعنوية في مستوى البرولاكتين في

مصل دم المريضات تم ربطها مع المستويات المنخفضة معنوياً للفوكوز في مصول المريضات أنفسهن مقارنة مع مجموعة السيطرة.

وقد يعزى الانخفاض في مستوى الفوكوز الكلي في مصول دم المريضات لمتطلبات البرولاكتين بمختلف الأوزان الجزيئية إلى اختلاف نسب السكريات الأحادية والتي تشمل الفوكوز والمانوز لعملية تسكّر Glycosylation هرمون البرولاكتين الذي قد يغير من الفعالية البيولوجية له في الأصناف والأنواع المختلفة من اللبائن.

**الكلمات المفتاحية : الفوكوز، البروتين المرتبط مع الفوكوز، فرط هرمون البرولاكتين**