

How to Cite:

Farman, H. A., & Abuhinna, K. A. (2022). Evaluation of apelin level in women with polycystic ovary syndrome at Al-Najaf Province. *International Journal of Health Sciences*, 6(S2), 10489–10497. <https://doi.org/10.53730/ijhs.v6nS2.7752>

Evaluation of apelin level in women with polycystic ovary syndrome at Al-Najaf Province

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Abstract---Hyperinsulinaemia is one of the most common clinical manifestations in women with PCOS. Here, hyperinsulinaemia may increase IGF- 1 expression and aggravate the effect of IGF1 by reducing the synthesis of IGF binding protein 1. IGF1 increases the expression of APLNR, and further promotes oestrogen synthesis and secretion via binding of APLN/APLNR. Patients with PCOS have high ovarian IGF1 levels. Therefore, IGF-1 and APLN may exhibit a compensatory increase in PCOS. Recently study found a high APLN/APLNR level in blood and ovary in PCOS (Liu et al., 2020). One of the first effects of APLN observed was its ability to lower glucose levels in both in fasting conditions and during a glucose tolerance test. Increased glucose uptake in target tissues such as skeletal muscle and adipose tissue was responsible for the decreased glycaemia (Yue et al., 2010).

Keywords---polycystic ovary syndrome, evaluation, apelin level, women.

Introduction

Polycystic ovary syndrome (PCOS) is the most common gynecological disease in women at reproductive age, which is characterized by metabolic and endocrine abnormalities, as well as chronic inflammation (Seyyed Abootorabi *et al.*, 2018). The most common risk factors for the progress of PCOS include family history of PCOS, fast food diet habits, lack of physical exercise, body mass index and waist circumference (Begum et al., 2017). Apelin is a fat hormone that comes from fat cells in many organs. Studies have shown that serum apelin is important in glucose regulation, and it also influences insulin secretion. We discovered that T1DM children had elevated serum apelin levels, a finding that concurs with past research (Habchi, *et al.*, 2014). May be this is because apelin acts on glucose metabolism in a way that is both complementary to insulin, increasing glucose

absorption and transport in tissues (Attane, *et al.*, 2011).

The cleavage of the 77 amino acid preproapelin gives rise to many other peptides that may interact with the Apelin receptor, the most important of which is the 12 amino acid active peptide, apelin-12. In the central tract, as well as in other tissues, apelin may be discovered. Binding to the Apelin receptors results in increased glucose metabolism and insulin production in pancreatic islet cells because of Apelin's high expression there (Chen, *et al.*, 2017). Apelin and its receptor APJ are widely expressed in several tissues (stomach, heart, lung, skeletal muscle, etc.) and in different regions of the brain, including the hypothalamus (O'Carroll, *et al.*, 2013). Moreover, Apelin is an essential adipocytokine that promotes skeletal muscle glucose absorption and augments insulin sensitivity in the heart and the brain (Jaganathan, *et al.*, 2018).

There is evidence that APLN is also involved in the development and progression of different pathologies including diabetes, obesity, cardiovascular disease and cancer. APLN is widely expressed in different organs and play an important role in glucose and lipid metabolism (lipolysis). PCOS is an endocrine disease with abnormal glucose and lipid metabolism, so it is imperative to understand the role of APLN in PCOS(Liu, *et al.*, 2020). One of the first apelin effects observed on glucose metabolism. Since the muscles represent the main entry of glucose, apelin effect was studied in isolated soleus muscle. Apelin stimulated glucose transport and its effect was additive to that of insulin(Bertrand, *et al.*, 2015).

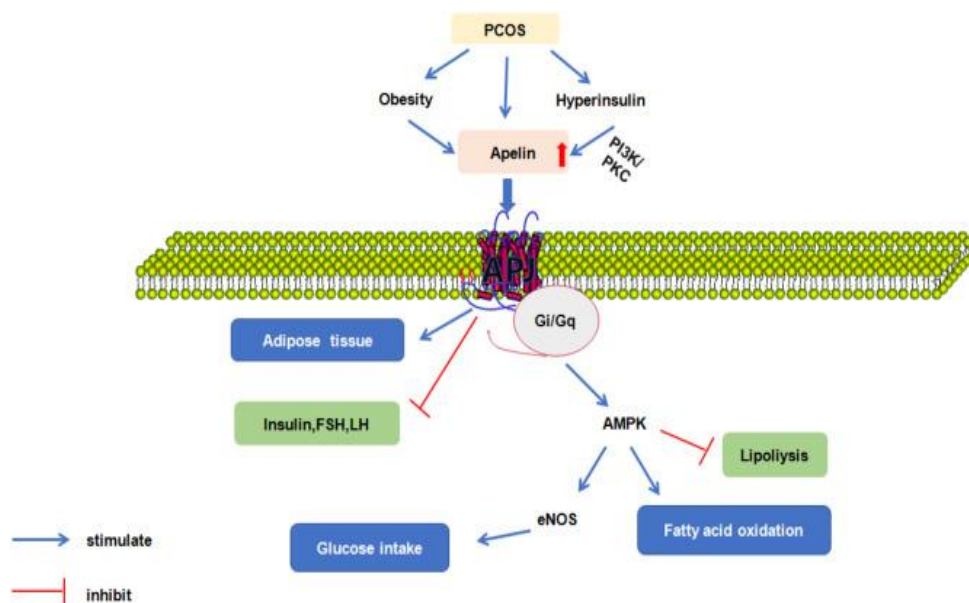


Figure (1): Apelin is involved in the regulation of glucose and lipid metabolism in Polycystic Ovary Syndrome(Liu, *et al.*, 2020).

Material and Methods

The study included 128 subjects is divided into two groups: The first group is the patient group, which consists of 83 patients with PCOS, which excludede 38

sample according to exclusion criteria, and remained 45 patients. PCOS was identified by specialized gynaecologists of the "Fertility Center in AL-Sadder Teaching Hospital in Najaf Governorate/ Ministry of Health/Iraq, during the period from the 1st Nov. 2021 to 1st March. 2022, according to the Rotterdam Consensus (2003), which requires the presence of at least two of the following characteristics: polycystic ovaries on ultrasound scan, menstrual irregularities and hyperandrogenism. The age group of the patients ranged from (17-49) years. The inclusion criteria for the patients are all patients with PCOS. The second group is the control group, which consists of 45 females with no PCOS, normal testosterone, a regular menstruation period, and normal ovulation are eligible, with normal ovaries as they were observed by the gynaecologists which do not take contraceptive, with no past of somewhat malady. The age group ranged from (17-50) years.

Exclusion criteria

Thirty eight samples were excluded from the initial sample of Eighty three due to these pathologies such as Diabetes mellitus, hypoplasia of ovary, white heifer disease and ovarian tumor.

Blood Collection

The blood samples of this study were obtained from female in 2nd of menstrual cycle through draw 5 ml of blood by using of medical sterile syringes from brachial vein, and placed in a gel tube. Then the gel tube were placed at room temperature for 30 minutes to coagulate the blood, and then samples were centrifuged (6000 rpm/min) for 5 minutes to separate the serum from other components of the blood. The serum was withdrawn by micro pipette and then placed in the Eppendorf tubes in two repeaters and kept frozen at -20 °C for the determination of Apelin, Endoglins, Inhibin B, LH, FSH, TSH, Prolactin (Lesser *et al.*, 2020).

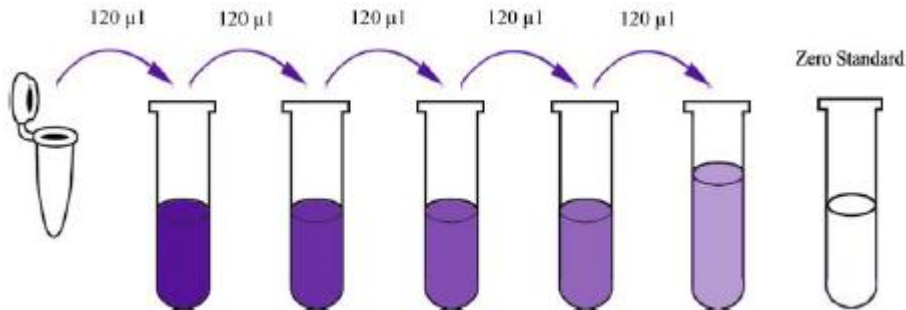
Apelin measurement method

Assay Principle

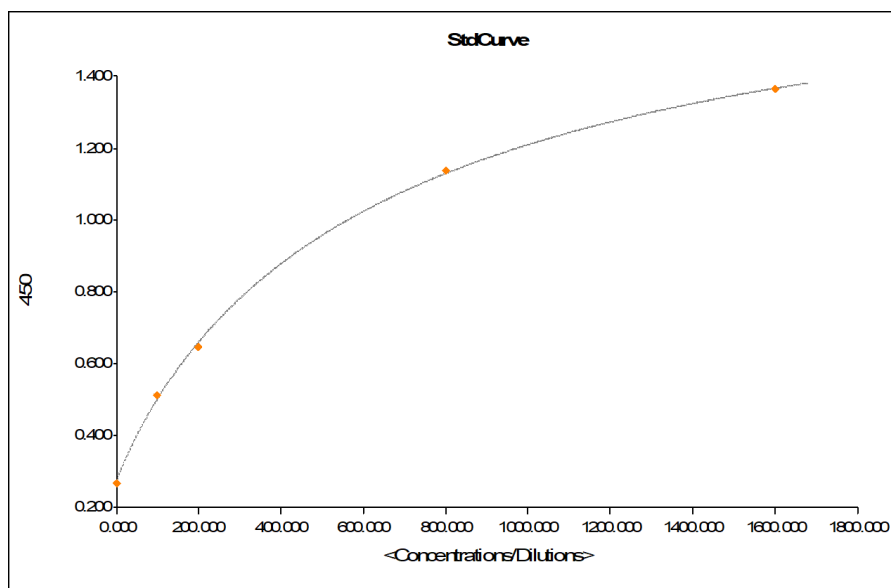
This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human APLN/AP antibody. APLN/AP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human APLN/AP Antibody is added and binds to APLN/AP in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated APLN/AP antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human APLN/AP. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Assay Procedure

1. Prepared all reagents, standard solutions and samples as instructed. Brought all reagents to room temperature before used. The assay performed at room temperature.



2. Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Added 50µl standard to standard well. Note: Didn't add antibody to standard well because the standard solution contained biotinylated antibody.
4. Added 40µl sample to sample wells and then added 10µl anti-APLN/AP antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.
5. Removed the sealer and washed the plate 5 times with washed buffer. Soaked wells with at least 0.35 ml washed buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and washed 5 times with wash buffer, overfilling wells with washed buffer. Blotted the plate onto paper towels or other absorbent material.
6. Added 50µl substrate solution A to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50µl Stop Solution to each well, the blue color would changed into yellow immediately.
8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.



Figure(2): Standard curve of Apelin

Result and Discussion

As shown in the table (1) and figure (3), the mean level of Apelin in PCOS patients women group was (218.078 ng/L) and it was high significantly higher than in control women group (NO PCOS) that mean level was (105.005 ng/L).

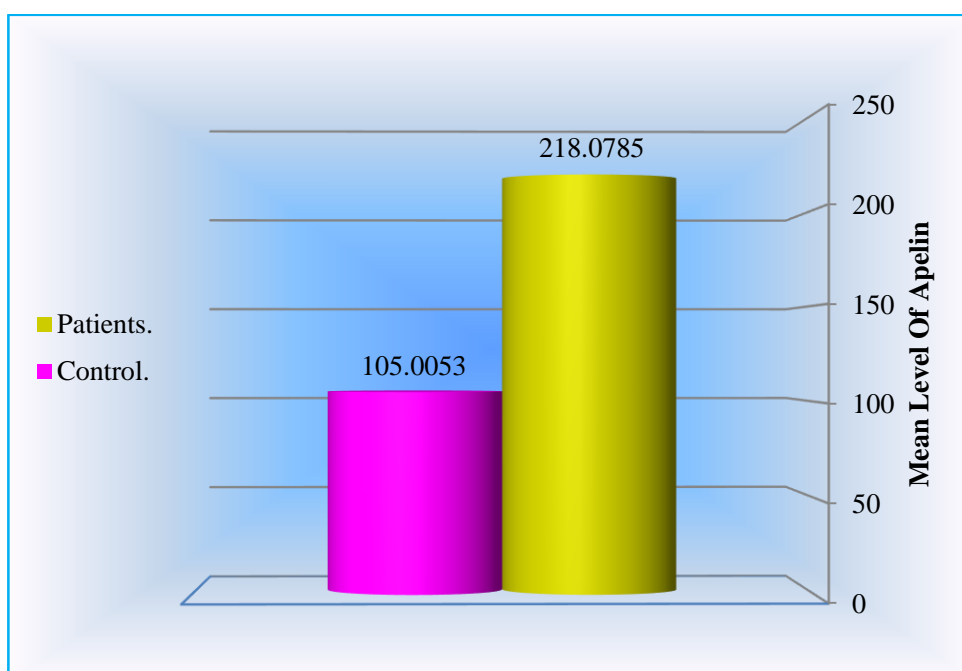


Figure (3) :Comparison the mean levels of Apelin in PCOS patients women group and control women groups (NO PCOS).

Table (1) :Comparison the mean, standard error and p-value of Apelin between PCOS patiens women group and control women groups (NO PCOS)

Parameter		N	Mean	Std. E	P-value
Apelin	Patients	45	218.078	33.69	0.0001**
	control	45	105.005	14.4	

P > 0.05 = Non-Significant (NS)

P < 0.05 = significant (*)

P < 0.01 = Highly significant (**)

In Gören *et al.*, 2012 study, apelin levels were found to be higher in patients with PCOS compared to the controls. In these study, however, serum apelin levels are significantly higher in PCOS women compared with controls (Ibrahim *et al.*, 2020). Only four studies have assessed apelin in patients with PCOS, and their results are inconsistent. Two previous studies (Chang *et al.*, 2011, Choi *et al.*, 2012) reported lower apelin levels in women with PCOS, similar to the data of the present study; however, one study (Cekmez *et al.*, 2011), reported higher and the other (Olszanecka-Glinianowicz *et al.*, 2013) reported similar apelin levels between women with and without PCOS.

Obesity is an important cause of lipid abnormalities. Adipocytes, as one of the major communication tools, participate in the regulation of appetite and energy balance, lipid metabolism and angiogenesis. APLN is a new adipokine secreted by adipose tissue. The study found that the expression of APLN in adipose tissue was significantly increased (Rayalam *et al.*, 2008). Many new proteins are strongly involved with PCOS physiopathology and IR such as some adipocytokines (adiponectin, visfatin, vaspin, and apelin), Many other proteins have been proposed as potential new markers of IR in PCOS (Polak *et al.*, 2017).

Apelin (APLN) is a recently discovered adipokine involved in the regulation of various metabolic functions. Its receptor, APLNR, is expressed in reproductive tissues; however, its role in human ovarian cells is unknown. PCOS patients are more vulnerable to develop diabetes, cardiovascular diseases, and metabolic syndrome (Indran *et al.*, 2018). Adipose tissue is implicated in the secretion of several hormones such as adiponectin, resistin, leptin, visfatin, apelin, and RBP4 called adipocytokines that are involved in energy homeostasis and metabolism (Masri *et al.*, 2005).

Apelin is a peptide isolated from bovine stomachs, but it is expressed in several other organs and also in visceral and subcutaneous tissues (Zhang *et al.*, 2018). APLN and APLNR were expressed in the ovary and pituitary gland. APLN and APLNR expression was detected in the CL of bovine ovaries, and APLN and APLNR expression was decreased at the late luteal phase and declined sharply during corpus luteum (CL) regression, which suggested that APLN and APLNR are involved in CL formation, function and regression during the oestrous cycle. Meanwhile, progesterone stimulated the expression of APLNR in granulosa cells, which suggested that the change in oestrogen level may be responsible for the expression levels of APLN and APLNR in follicle development and maturation (Schilffarth *et al.*, 2009).

The serum insulin-like growth factor-1 (IGF-1) level in patients with PCOS was significantly increased in comparison with that of healthy women. Meanwhile, the concentrations of IGF-1 in follicular fluid from women with PCOS were significantly higher than they were in healthy women. IGF-1 promoted the production of oestrogen by stimulating APLN and APLNR. It was also reported that APLN and APLNR had high expression in granulosa cells and follicular fluid of women with PCOS (Roche *et al.*, 2016). Hyperinsulinaemia is one of the most common clinical manifestations in women with PCOS. Here, hyperinsulinaemia may increase IGF-1 expression and aggravate the effect of IGF1 by reducing the synthesis of IGF binding protein 1. IGF1 increases the expression of APLNR, and further promotes oestrogen synthesis and secretion via binding of APLN/APLNR. Patients with PCOS have high ovarian IGF1 levels. Therefore, IGF-1 and APLN may exhibit a compensatory increase in PCOS. Recently study found a high APLN/APLNR level in blood and ovary in PCOS (Liu *et al.*, 2020). One of the first effects of APLN observed was its ability to lower glucose levels in both in fasting conditions and during a glucose tolerance test. Increased glucose uptake in target tissues such as skeletal muscle and adipose tissue was responsible for the decreased glycaemia (Yue *et al.*, 2010).

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