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# The Relationship of Betatrophin and Kisspeptin with Related Hormones in Women Newly Diagnosed with Polycystic Ovary Syndrome

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**Abstract:** Polycystic ovary syndrome (PCOS) is one of the most widespread endocrine hormone disorders that affects women of reproductive age. It's a major public health concern that leads to dysregulations in the reproductive, metabolic, and psychological systems. Anovulation, which has a significant impact on female fertility, and hyperandrogenism are the most characteristic of PCOS. Because of its endocrine role, adipose tissue secretes several proteins called adipokines that work as hormones controlling bodily functions, including resistin, betatrophin, etc. The cases involved in this study were 90 women. For comparison, 30 healthy women with regular periods suitable with age were used as a control group. 30 Obese PCOS women were diagnosed depending on the Rotterdam criteria as $group_2$, and 30 non-obese PCOS women as $group_3$. The results showed there was a significant elevate in insulin and insulin resistance and related hormones between groups compared to the control, and a significant increase at ($P \leq 0.01$) in levels of betatrophin in $group_2$ compared to the control, with a significant difference between the $group_2$ and $group_3$, and a significant increase at ($P \leq 0.01$) in levels of kisspeptin in $group_2$ compared to the control. No significant difference between $group_2$ and $group_3$ and a significant difference between $group_3$ and the control group.

## العلاقة بين البيتاتروفين والكيسبيتين مع الهرمونات ذات الصلة في النساء المشخصات حديثا بمتلازمة المبيض متعدد الأكياس

**الكلمات المفتاحية :**
البيتاتروفين . الكيسبيتين؛ متلازمة المبيض متعدد الاكياس مقاومة الانسولين.

**المستخلص :** متلازمة المبيض متعدد الأكياس واحدة من أكثر اضطرابات هرمونات الغدد الصماء انتشارا التي تصيب النساء في سن الإنجاب، وهي مصدر أساسي لها علاقة بالصحة العامة التي تؤدي إلى خلل في الأنظمة التناسلية والأيضية والنفسية، يعد انقطاع الإباضة الذي يلعب دورا أساسيا في خصوبة المرأة ، وفرط الأندروجين من أكثر خصائص المتلازمة بسبب دوره في الغدد الصماء ، يفرز النسيج الدهني عددا من البروتينات التي تسمى بالاديبوكاينز والتي تعمل بوصفها هرمونات تتحكم بوظائف الجسم ، مثل الريزستين والبيتاتروفين. هذه الدراسة شملت 90 امرأة. للمقارنة: 30 امرأة تتمتع بصحة جيدة مع فترات حيضية منتظمة ومناسبة للعمر، والوزن عرفت بمجموعة السيطرة ، 30 امرأة بدينة مصابة بمتلازمة المبيض متعدد الأكياس تم تشخيصهن بالاعتماد على معايير روتردام عرفت بالمجموعة الثانية، 30 امرأة ليست بدينة مصابة بمتلازمة المبيض متعدد الأكياس عرفت بالمجموعة الثالثة. النتائج أظهرت وجود ارتفاع كبير في الإنسولين، ومقاومة الإنسولين مع الهرمونات ذات الصلة بين المجموعات مقارنة بمجموعة السيطرة ، مع وجود زيادة معنوية في مستويات البيتاتروفين في المجموعة الثانية مقارنة بمجموعة السيطرة ، مع اختلاف معنوي بين المجموعتين الثانية والثالثة، إضافة إلى وجود زيادة معنوية في مستويات الكيسبيتين في المجموعة الثانية مقارنة بمجموعة السيطرة، مع عدم وجود اختلافات بين المجموعتين الثانية والثالثة ، مع وجود اختلافات في المجموعة الثالثة مع مجموعة السيطرة.

## INTRODUCTION

Adipose tissue secretes a variety of chemicals known as adipokines and functions as both an active endocrine organ and a passive energy store. Adipokines are a class of cytokines that control several physiological processes, including immunity, metabolism, hunger, inflammation, and cardiovascular health. Among the numerous additional adi-

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pokines are resistin, adiponectin, and leptin. Adipose tissue's production of adipokines is contingent upon the nature of the adipocytes (brown or white), as well as its size, quantity, arrangement, and interactions with other cells. White adipocytes and brown adipocytes are the two primary subtypes of adipocytes (Mancuso, 2016).

Produced by the fat cells are physiologically active compounds with properties similar to those of traditional hormones. These are signaling proteins found in cells that control or modify a range of biological functions in the target organs, such as the immune system, blood vessels, brain, liver, muscles, and heart (Fasshauer & Blüher, 2015). They have a variety of roles and can affect a wide range of processes, such as the regulation of hunger and energy, the metabolism of lipids and glucose, insulin sensitivity, endothelial cell function, inflammation, angiogenesis, blood pressure, hemostasis, the development of atherosclerosis, and metabolic syndrome. (Farkhondeh et al., 2020; Metz et al., 2020). Also, they may help to explain some pathophysiologic infertilities like polycystic ovary syndrome (PCOS) (Bongrani et al., 2019).

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine system conditions affecting women of reproductive age, also known as hyperandrogenic anovulation (HA) or Stein –Leventhal syndrome (El Hayek et al., 2016). This chronic and heterogeneous disorder manifests as menstrual dysfunction, infertility, hirsutism, acne, and obesity (Motlagh Asghari et al., 2022). It depicts a syndrome in which at least one ovary develops an estimated ten tiny cysts, with diameters ranging from 2 to 9 mm, and at least one ovary has an ovarian capacity larger than 10 ml (Balen et al., 1993). It is usually only diagnosed when complications develop that significantly reduce a patient's quality of life (e.g., hair loss, alopecia, acne, and infertility–related problems) (Azziz, Sanchez, et al., 2004). According to a systematic screening of women using the National Institutes of Health (NIH) diagnostic standards, 4–10% of reproductive-age women are predicted to have PCOS worldwide (El Hayek et al., 2016). A changed LH/FSH ratio is caused primarily by hormonal abnormalities, specifically elevated luteinizing hormone (LH), and normal or suppressed follicle-stimulating hormone (FSH). Additionally, hyperinsulinemia and insulin resistance are linked to the clinical signs of hyperandrogenism. It is unclear what factors may predispose a woman to develop PCOS, but it has been observed in some instances that the condition is genetic in nature and that obesity contributes to hyperinsulinemia, which predisposes people to the condition (Asunción et al., 2000; Barth et al., 2007).

Betatrophin (angiopoietin-like protein 8 (ANGPTL8)) is a hormone that was only recently found in the liver of humans. White adipose, brown adipose, and mammalian liver tissues have all been found to contain numerous homologous sequences. Insulin resistance, lipid metabolism, and the onset of type 2 diabetes (T2D) are all dependent on betatrophin (Guo et al., 2022). Betatrophin was identified in 2004 as a serum antigen unique to tumors (Dong et al., 2004). Cumulating evidence suggests that Betatrophin is significantly associated with adiposity, type 2 diabetes, and metabolic syndrome (Abu-Farha et al., 2016; Ghasemi et al., 2015; Yamada et al., 2015). Most women with PCOS display impaired glucose tolerance and are at higher risk for developing T2DM. Moreover, Betatrophin has a close relationship with insulin resistance and T2DM (Chen et al., 2015; Stepto et al., 2013; Yamada et al., 2015).

Kisspeptin was first discovered in 1996 as a metastasis inhibitor in melanoma cell lines (Lee et al., 1996). It is a peptide that is mostly expressed in the hypothalamic infundibular nucleus. Kisspeptin is essential for controlling reproductive processes. It is thought to be the primary factor in the regulation of the menstrual cycle and fertility, the

start of puberty, and the hypothalamic-pituitary-gonadal axis. Many processes, including follicular maturation, ovulation, steroidogenesis, and ovarian senescence, are influenced by kisspeptin activity. Studies on the function of kisspeptin in puberty have been sparked by the discovery of kisspeptin receptor mutations that result in hypogonadotropic hypogonadism. A significant factor in the development of PCOS, functional hypothalamic amenorrhea, and perimenopausal vasomotor symptoms is pathologies affecting the neurons secreting kisspeptin (Szeliga & Meczekalski, 2022).

## MATERIALS AND METHODS

A total of 90 women between 18– 40 years old were collected from Iraqi hospitals and private laboratories in Mosul and Erbil governorate from the period 15/8/2022 to 22/12/2022.

A gynecologist made a diagnosis based on the Rotterdam criteria, which includes: oligo/anovulation, hyperandrogenism, quantifying it biochemically, noting its signs such as hirsutism, acne, baldness, and examining the ovary's morphology using pelvic ultrasound (ESHRE & Group, 2004). Age, BMI, blood pressure, smoking history, T2DM, primary and secondary infertility, and symptoms of hyperandrogenism such as hirsutism, acne, and male pattern baldness were among the questions on a questionnaire created for the women participating in this study (Halawa et al., 2020), All women were divided into three groups:

**Group1**: Control, included 30 healthy women who have normal serum parameters level with no PCOS symptoms.

**Group2**: This group included 30 obese women with PCOS, and the diagnosis was relied upon by a specialized gynecologist relying on the Rotterdam criteria with BMI (31.72) Kg/m2.

**Group3**: This group included 30 normal-weight women with PCOS and BMI (21.09) Kg/m2. None of the women had used oral contraceptives, glucocorticoids, ovulation induction agents, anti-obesity drugs, or any other steroid-containing drug, but they were diagnosed for the first time with PCOS.

**Collecting Samples**: After 16 h of overnight fasting, 5 ml of venous blood was collected from the women and placed in gel tubes. The serum was separated by a centrifuge and the separated serum was placed in a sterile plastic Eppendorf tube and frozen at (– 20) C until assayed.

**Hormonal and Biochemical Tests**: Laboratory investigations included the following: BMI, (calculated as $Kg/m^2$). Betatrophin, KISSPEPTIN, Insulin, LH, and Testosterone, determined by ELISA methods. The index for insulin resistance; HOMA IR was calculated according to the equation: Fasting plasma glucose (mg/dl) × Fasting serum insulin μIU/ml/405 (Matthews et al., 1985).

**Statistical Analysis:** Data were gathered, updated, coded, and put into the IBM SPSS statistical software program. When the distribution of the quantitative data was determined to be parametric, the mean, standard deviations, and ranges were reported. The Duncun's multiple range test revealed that the different coefficients were substantially separated by various letters of the alphabet under the probability threshold of ($P \leq 0.01$). Therefore, the data were investigated using a system of simple experiments and a full random design. The variables were also correlated with one another (Antar & Al-Wakaa, 2017).

## RESULTS

Depending on the table below, the results showed a significant increase in BMI in obese women with PCOS ($Group_2$), while there was no significant difference between non-obese women with polycystic ovary

syndrome ($Group_3$). In addition, there was a significant increase in fasting glucose, insulin, and HOMA–IR in $group_2$ and $group_3$ compared to the control group, noting a significant difference between $group_2$ and $group_3$ with these parameters. A significant increase was observed in both LH and testosterone in the $group_2$ compared to the control group, with no significant difference observed between $group_2$ and $group_3$. Finally, the results showed a significant increase in Betatrophin in obese women with PCOS ($Group_2$), while there was no significant difference between non-obese women with polycystic ovary syndrome ($Group_3$). Also, the results showed of Kisspeptin that there was no significant difference between $group_2$ and $group_3$ with a significant difference between $group_2$ and $group_3$ with the control group.

**Table: (1).** The value of all study parameters in the different groups

| Group Parameters | $Group_1$ (no.=30) | $Group_2$ (no.=30) | $Group_3$ (no.=30) |
|---|---|---|---|
| BMI ($Kg/m^2$) | 21.10 ± 1.91b | 31.72 ± 2.81 a | 21.07 ±1.68 b |
| Fasting glucose(mg/dl) | 88.07 ± 6.64 c | 155.8 ± 12.1 a | 102.7 ±13.8 b |
| Insulin (µIU/ ml) | 7.42 ± 0.86 c | 8.29 ± 0.81 a | 7.91± 0.53 b |
| HOMA–IR | 1.62 ± 0.26 c | 3.18 ± 0.49 a | 2.01± 0.38 b |
| LH (µIU/ ml) | 2.8 4± 0.91 b | 13.04 ± 1.74 a | 13.28± 1.24 a |
| Testosterone(ng/ml) | 1.12 ± 0.34 b | 3.13± 0.61 a | 3.15± 0.69 a |
| Betatrophin (pg/ml) | 202.94± 23.79 b | 507.99 ± 102.45a | 193.44± 25.31 b |
| Kisspeptin (ng/ml) | 150.15±6 .97 b | 193.97±5 .75 a | 195.85±6 .51 a |

The no. followed by different letters means there is significant difference.
The values is means ± standard deviation SD

## DISCUSSION

Adipose tissue releases a variety of adipokines that control lipid metabolism, glucose metabolism, and insulin resistance (Coelho et al., 2013). The results showed a significant increase in BMI in $group_2$ compared with the control group. Numerous processes regulate how obesity and excess weight affect the emergence of PCOS. The metabolic impacts of insulin resistance, as well as the steroidogenic and reproductive implications of hyperinsulinemia, are significant processes. It appears that adipokines involved in metabolism, are produced by visceral and subcutaneous fat. It's important to consider any possible effects of PCOS on future weight gain, or at the very least, on attempts to reduce weight and maintain it through dietary and lifestyle changes, given the complex nature of PCOS etiology (Barber et al., 2019).

Research results indicated that in PCOS–affected women, Betatrophin concentrations had a strong association with BMI and insulin resistance (Calan et al., 2016). Insulin resistance promotes Betatrophin to be produced. In insulin-resistant mice, Betatrophin has been observed to increase the rate of beta cell division, stimulate pancreatic beta cell proliferation, and improve metabolic regulation (Yi et al., 2013).

(Sun et al., 2017) displayed that in mice who had diabetes induced by streptozotocin, the transplantation of Betatrophin –expressing adipose-derived mesenchymal stem cells caused β–cell proliferation (Sun et al., 2017). They found that Betatrophin overexpression enabled islet cell proliferation, expression of transcription factors unique to beta cells, and insulin production when glucose was stimulated (Bulmuş et al., 2020). IR and associated compensatory hyperinsulinemia are one of PCOS's causes, and it has been estimated that 70% of PCOS patients reflect IR symptoms. However, the pathogenesis of PCOS is still not fully understood (Hillman & Dale, 2018). Furthermore, HOMA-IR and fasting insulin levels were both substantially positively correlated with serum Betatrophin levels according to a Spearman rank analysis.

These results support those of older populatin-based studies that showed that insulin

resistance and higher circulating Betatrophin levels were present in T2DM patients (Chen et al., 2015). In another research, we observed that insulin stimulation more prominent Betatrophin expression in hepatocytes. Treatments with metformin and rosiglitazone reduced the expression of Betatrophin in insulin-stimulated hepatocellular (Wang et al., 2017). This study's results are in agreement with the majority of other examinations that found higher Betatrophin levels in IR individuals or PCOS women (Erol et al., 2017). In a previous study, (Adamska et al., 2017) demonstrated a connection between Betatrophin and island β-cell release and IR (Adamska et al., 2017).

As a result, IR and PCOS may be determined by excessive Betatrophin levels. We think that circulating Betatrophin has a significant clinical effect on adipose IR reflection. The mechanism through which adipose IR raises circulating Betatrophin levels is unknown. We suggest that IR may be the cause of the enhanced production and release of Betatrophin in adipose tissue as a compensatory mechanism. Additionally, in IR circumstances, macrophage-secreted inflammatory cytokines may encourage Betatrophin expression and production (Wang et al., 2017). A peptide hormone termed Betatrophin is synthesized from adipose and liver tissue (Erol et al., 2017). This protein influences lipid metabolism and glucose to a healthy equilibrium (Eksi Haydardedeoglu et al., 2019). Circulating Betatrophin levels have been observed in T1DM and PCOS (Ersahin et al., 2017). However, the pathogenic effects of Betatrophin on insulin secretion and glucose homeostasis are not fully recognized (Erbag et al., 2016).

The reproductive phenotype of PCOS and its pathogenesis. LH secretion is specifically increased by increased pulsatile GnRH release. LH facilitates the generation of testosterone in ovarian theca cells. Due to a relative FSH shortage, the surrounding granulosa cells are unable to appropriately aromatize testosterone. Additionally, the activity of several steroidogenic enzymes is constitutively elevated in polycystic ovaries, which boosts androgen synthesis. Additionally, PCOS may produce more adrenal androgen. Alopecia, hirsutism, and acne are all signs of androgen excess that are indirectly caused by testosterone. As a result of unopposed estrogen activity on the endometrium, androstenedione, and testosterone may be capable of aromatizing extragonadally to produce estradiol and estrone. Testosterone feeds back on the hypothalamus to lessen sensitivity to the effects of estradiol's typical feedback (Diamanti-Kandarakis & Dunaif, 2012). According to certain studies, the LH reacts strongly to the GnRH released by the hypothalamus in PCOS. Additionally, one of the primary features of PCOS is hyperandrogenism, particularly with regard to testosterone. This condition results in an increase in LH production without a negative feedback loop, which in turn causes an increase in androgens with a reduction in SHBG (Hřebíček et al., 2002).

According to several research, serum Betatrophin levels are strongly correlated with PCOS progression and are important to the IR process. Since Betatrophin may be a sensitive biomarker of PCOS development, it may also be a useful target for PCOS therapy (Gong et al., 2021). In addition, increasing androgen levels in PCOS patients also cause ovarian cells to release more Betatrophin (Eksi Haydardedeoglu et al., 2019). This might be the cause of its rise in PCOS patients and its link to LH and Testosterone, as one of the most significant symptoms of this medical condition is a rise in LH (Azziz, Woods, et al., 2004), which then causes a surge in testosterone and, subsequently, a rise in Betatrophin (Eksi Haydardedeoglu et al., 2019). According to data from the Northern Finland Birth Cohort 1966, BMI and symptoms of PCOS are significantly correlated at all ages (Ollila et al., 2016). However, it's still unknown how PCOS and BMI are related to one another. Some research suggest that Kisspeptin increases in-

sulin production and blood glucose levels, which raises BMI (Miranda et al., 2013).

In the present research, we reported that patients with higher BMIs had greater blood levels of Kisspeptin than patients with normal BMIs, which was consistent with (Rafique & Latif, 2015). Additionally, there was an association between Kisspeptin levels and serum testosterone and BMI (Umayal et al., 2019). Our findings conflict with those of Rafique and Latif (Rafique & Latif, 2015). who carried out their research on groups of female patients from Saudi Arabia and found that Kisspeptin blood levels were the same in normal and overweight individuals. As compared to individuals with overweight BMI, it has been observed that PCOS patients with normal BMI had higher amounts of Kisspeptin (Akad et al., 2022).

Additionally, in women with polycystic ovarian syndrome, Kisspeptin has been linked to measures of insulin resistance as well as body mass index (Panidis et al., 2006). Kisspeptin enhances the release of insulin from isolated islets that produce insulin in humans and mice, in addition to its effects on the hypothalamus (Hauge-Evans et al., 2006). Kisspeptin's demonstrated capacity to increase insulin secretion, its confirmed mediating role in the influence of changed nutritional status on reproductive function, and the significance of pancreatic β–cells (Bowe et al., 2009). This will clarify the way in which insulin secretion and kisspeptin are related. The methods by which Kisspeptin and many other receptor-operated agonists enhance nutrient-induced secretion while preventing the initiation of a secretory response are similar in that they promote insulin secretion in the presence of a stimulatory concentration of glucose. These experiments were conducted in vitro. Rats with normal nonfasted blood glucose levels, however, showed a substantial increase in plasma insulin when Kisspeptin was administered in vivo (Bowe et al., 2009).

Kisspeptin's effect on modulating the pulsatile release of GnRH and its associated effect on ovulation was very recently discovered. (Azziz, 2016). This discovery makes it possible to explore an additional path of the physio-pathogenesis of chronic anovulation. LH levels are more powerful in PCOS–afflicted women, which might be related to raised kisspeptin activity (Terao et al., 2004), an essential regulating element in the pulsatile release of GnRH, which, as in our study, increases LH production. It is essential to emphasize that, in addition to other variables impacting PCOS traits, such as BMI and insulin disruption, an aberrant GnRH to LH ratio may be an indicator of the physio-pathogenesis of PCOS. According to our study, kisspeptin is connected with both LH and testosterone as well as BMI (Araújo et al., 2020). LH levels are higher in PCOS–afflicted women, which may be related to a raised kisspeptin production (Terao et al., 2004). A significant regulating element in the pulsatile release of GnRH, which in turn increases LH production. It is essential to emphasize that, in addition to other variables impacting PCOS traits, such as BMI and insulin failure, an abnormal GnRH to LH ratio may be a characteristic of the physio-pathogenesis of PCOS (Araújo et al., 2020). Hypothalamic abnormalities in the irregular production of GnRH may have a role in the physiopathology of PCOS. The hypophysis secretes more LH as a result of the alterations. Kisspeptin is a neuropeptide that binds to GPR54, a G-protein-coupled transmembrane receptor that exists in GnRH neurons, causing it to become more active and raising LH levels as an outcome (Lee et al., 1996). Kisspeptin may affect ovarian function through elevated LH secretion (Azziz, 2016), causing theca interna cells of the ovarian follicles to secrete more androgen. Such hyperactivity inhibits the development of follicles, lowers the likelihood of ovulation, and, as a result, minimizes the clinical suppression of GnRH via progesterone during the luteal phase. The testosterone lined with kisspeptin

and the current study's findings show a favorable link (Yilmaz et al., 2014).

However, it was not capable of determining if the rise in kisspeptin was due to hyperandrogenism (Lopes et al., 2014). Kisspeptink's possible effects on PCOS may be mediated through neurokinin B and its receptor. In actuality, the change in LH pulsatility and redution of kisspeptin –mediated LH production were caused by the blockade of NKBNK3R signaling. Another theory is that unusual LH or its receptor is caused by susceptibility genes, as evidenced by the discovery of multiple novel risk loci and candidate genes for PCOS by genome–wide association studies (Crespo et al., 2018). Additionally, because Kisspeptin mRNA is highly abundant in both rat and human gonads, Kisspeptin may directly affect rat ovaries (Terao et al., 2004). It could be a further mechanism for Kisspeptin to affect the pathophysiogenesis of PCOS (Araújo et al., 2020).

The theory that an overactive Kisspeptins system supports greater HPG-axis activity, which in turn causes irregular menstrual cycles and excessive androgen production in PCOS women, is consistent with the overall rise in Kisspeptin levels in the PCOS population (Tang et al., 2019). It was determined through experimental research on humans that supplying patients with kisspeptin causes their LH levels to improve (Dhillo et al., 2007). Administration of GnRH possesses an effect on human beings with KISS1R mutations and hypogonadotropic hypogonadism. We can state with trust that kisspeptin is activating stimulants for the GnRH in the hypothalamic-pituitary-gonadal (HPG) axis as demonstrated by recent research (d'Anglemont de Tassigny, 2007).

Kisspeptin can raise LH levels, but its effects on the FSH take more time to appear and are less prominent. Different gonadotrophin secretory patterns or actions to kisspeptin may have less impact on FSH (McCann et al., 2002). In the 2014 data collection by (Jayasena et al., 2014), kisspeptin treatment increased LH levels by more than twofold while having little to no effect on FSH levels (Jayasena et al., 2014). Numerous investigations have shown that kisspeptin administration directly affects the upstream regulation of the depolarization process by GnRH neurons and the upregulated expression of GnRH mRNA, which raises the LH/FSH ratio (Akad et al., 2022).

## CONCLUSION

There was a significant elevation in LH, testosterone, insulin, fasting glucose (FBS), and insulin resistance (HOMA-IR) in $group_2$ and $group_3$ compared to the control group. It was also noted that results show a significant increase at ($P \leq 0.01$) in levels of Betatrophin in $group_2$ (507.99± 102.45) pg/ml compared to the control group (202.94± 23.79) pg/ml with noting that there was a significant difference between $group_2$ and $group_3$ with (193.44± 25.31) pg/ml and a significant increase at ($P \leq 0.01$) in levels of kisspeptin in $group_2$ (193.97± 5.75) ng/ml compared to the control group (150.15± 6.97) ng/ml with noting that there was no significant difference between $group_2$ and $group_3$ with (195± 5.85) ng/ml and significant difference between $group_3$ and the control group.

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## ETHICAL

The study was conducted with the approval of the concerned authorities in the Department of Biology, the Deanship of the College of Science, and the Nineveh Health Department, in an official letter dated 2022/6/13 and numbered (3142) to collect samples from hospitals

(Al-Batoul Teaching Hospital and Al-Salam Teaching Hospital) in the city of Mosul, as well as the patient's knowledge and consent to take the sample and with the required health information.

**Duality of interest:** The authors declare that they have no duality of interest associated with this manuscript.

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