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ORIGINAL STUDY

Health Effects of Cadmium and Manganese Exposure on Luteinizing Hormone Levels: A Pilot Study of Female Infertility

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Abstract

Female infertility occurs for many reasons, some unknown. Exposure to heavy metals is a risk factor that negatively impacts on female reproductive system. Therefore, this study aimed to evaluate the effect of cadmium Cd and manganese Mn on luteinizing hormone LH levels for females' infertility. This study included a healthy group (n = 20), primary infertile females (n = 40), and secondary infertile females (n = 20) collected from Al-Zahraa hospital in Najaf center, Iraq. The Atomic Absorption Spectroscopy (AAS) was used to measure the cadmium and manganese concentrations and LH levels were measured by a Maglumi 800 from Snibe (CLIA). For healthy females, Cd, Mn, and LH concentrations were 0.1307 ppm, 0.00049 ppm, and 6.769 IU/L, respectively. For females with primary and secondary infertility, high concentrations of cadmium, 0.2453 ppm and 0.2296 ppm, respectively, while, the concentrations of manganese were 0.000165 ppm and 0.000307 ppm, respectively. LH levels increased for infertile females (primary and secondary) at 14.2018 IU/L and 15.122 IU/L, respectively. Cadmium and manganese concentrations were indicator to understand why some females unexplained infertility. From the result, the higher concentration of cadmium can act as endocrine disruptors, interfering with the communication between the brain and ovaries. This disruption has been linked to higher levels of luteinizing hormone (LH) and decreased egg quality. The lower concentrations of manganese hinder the body's ability to regulate the pituitary gland, resulting in hormonal imbalances and elevated LH levels. Many women who experience unexplained infertility have underlying hormonal imbalances. The combination of high cadmium levels and low manganese levels creates a compounded effect: cadmium increases oxidative stress and disrupts the function of the pituitary gland, while low manganese limits the body's ability to combat this stress or manage the resulting hormonal fluctuations.

Keywords: Female infertility, Luteinizing hormone, Cadmium, Manganese, Logistic regression

1. Introduction

Infertility is a medical condition that can cause psychological, physical, and medical damage to the patient; this medical condition affects both the patient and their partner as a couple. The most important study indicated that 85 % of females become pregnant within 12 months. Based on the results of this study, the fertility rate is 25 % in the first three months of unprotected intercourse and then declines to 15 % during the remaining nine months. The American Society for Reproductive Medicine (ASRM) determines when a couple should undergo infertility evaluation [1]. ASRM recommends initiating infertility evaluation after failure to conceive within 12 months of unprotected intercourse, where female infertility is divided into two main types: primary infertility and secondary infertility. Primary infertility refers to the inability to conceive after trying for a year (or 6 months if the

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woman is 35 years or older). In contrast, secondary infertility is the inability to conceive again after a previous pregnancy. Infertility affects approximately 13 % of females. Infertility rates in Iraq rose after the second Gulf war in 1991 and reached an unprecedented level after 2003. Infertility is divided into psychological and physiological types, and both types are the result of the wars and calamities that the country has experienced over the past three decades. The major causes of female infertility are an ovulation, fallopian tube disease, pelvic adhesions, endometriosis, and unexplained infertility [1,2].

High concentrations of heavy metals in the human body are associated with female reproductive disorders, premature births, and miscarriages. Heavy metals, such as cadmium, can accumulate in embryos as early as the four-cell stage and, in high doses, may inhibit their development into blastocysts [3−6]. This can potentially decrease the chances of a successful pregnancy. Several essential elements are critical for female fertility, and both their deficiency and excess can lead to infertility [7−9]. Maintaining adequate levels of these elements is essential for reproductive health. Each metal has a specific impact on the body. While some metals can provide health benefits, others may be harmful. Unhealthy levels of heavy metals in the body can lead to heavy metal poisoning, which, if left untreated, can result in death or permanent bodily damage, as well as fertility issues and other serious health problems [10−13]. Due to the significance of the topic, numerous studies have been conducted on it. A study examined the effects of cadmium on the female reproductive system, highlighting its mechanisms of action and potential treatment options. Cadmium poisoning can have severe consequences, including impaired production of steroid hormones, delayed puberty and menstruation, miscarriage, imbalances in menstrual and reproductive hormones, premature birth, and low birth weight. The way cadmium acts in the body involves displacing essential metals from their native enzymes, disrupting their biological functions. Furthermore, oxidative stress plays a significant role in the toxicity linked to cadmium exposure [14]. Another study examined how changes in manganese levels may impact women's reproductive health. The researchers measured serum manganese levels in different groups: pregnant women, non-pregnant women, women who had experienced a miscarriage, and those facing primary infertility. The results revealed that pregnant women had significantly higher serum manganese levels, 40 % higher than the control group and 16 % higher than the other women studied [15]. It was revealed that even low levels of Cd exposure can affect the hormonal environment associated with the risk of PCOS [16]. Also, it has been reported that vanadium improves metabolic disturbances caused by chronic cadmium exposure [17]. Similarly, copper deficiency reduces the body's tolerance to cadmium [18]. Chronic exposure to cadmium (Cd), a heavy metal known to act as an estrogen, can have harmful effects. The impact of environmental heavy metal exposure on female fertility is well-established. Therefore, the underlying mechanisms should be explained and updated regularly [19]. Beneficial essential nutrients can help counteract the harmful effects of non-essential heavy metals, which cannot be completely avoided in our environment. For instance, incorporating copper, zinc, and manganese into our diet can reduce lead levels in the body [20]. Heavy metals significantly impact the human reproductive system, especially female fertility, and can even influence the success of artificial insemination [21]. In this study, the relationship between serum cadmium concentrations and infertility, as well as long-term amenorrhea, was examined in women aged 20 to 49 years. The analysis for infertility included 1990 women, while the long-term amenorrhea analysis involved 1919 women. The serum cadmium concentrations were significantly higher in infertile women compared to those who were pregnant. Furthermore, multiple logistic regression analyses indicated a negative association between cadmium levels and long-term amenorrhea in females [22]. Furthermore, exposure to toxic metals and synthetic phenolic antioxidants in follicular fluid increases the risk of endometriosis and reduces ovarian reserve, respectively [23]. In this comparative study, 388 women with PCOS were examined alongside 465 healthy women. They measured eighteen elements in follicular fluid using inductively coupled plasma mass spectrometry (ICP-MS). Through a multi-step statistical analysis and logistic regression modeling, identified the three most significant trace elements strongly associated with the risk of PCOS: Co, Sn, and Mn. Additionally, found that the three most important heavy metals linked to PCOS risk were Pb, Cd, and Ba. Notably, high concentrations of mixtures containing these six trace elements were found to be negatively associated with the risk of PCOS [24]. A female's fertility gradually decreases starting around age 32, with a more rapid decline occurring after age 37. The reduction in the quantity and quality of germ cells has the most substantial impact on changes in fertility [1]. This issue is particularly critical given the societal trend of postponing childbirth, which has led to an increase in permanent infertility. Various environmental factors can influence the aging process and the function of human eggs, including dietary habits, lifestyle choices, and exposure to environmental pollutants. Additionally, managing modifiable risk factors such as smoking, alcohol consumption, exposure to stress, and being overweight or underweight may help preserve egg quality. Also, hormonal dysfunction, such as luteinizing hormone (LH), secreted by the anterior pituitary gland, is an essential gonadotropin that plays a key role in female reproductive health. LH is vital for stimulating ovulation and the development of the corpus luteum in the ovaries [25]. Its levels fluctuate significantly throughout the menstrual cycle. LH is released during ovulation, prompting the ovaries to produce estradiol and release an egg. If fertilization occurs, LH levels typically remain low during pregnancy, usually measuring less than 1.5 IU/L [26]. In reproductive-age females with polycystic ovary syndrome (PCOS), there can be a surge in LH levels early in the menstrual cycle. Symptoms of PCOS often include irregular menstrual cycles, anovulation, weight gain, and excessive body and facial hair growth. Understanding the role of luteinizing hormone is crucial for managing reproductive health and fertility, as females with elevated LH levels may experience infertility [24–29]. This study aims to explore the relationship between cadmium and manganese concentrations and LH levels, contributing to potential solutions for females facing unexplained infertility.

2. Heavy metals (HMs)

2.1. Cadmium and manganese

The uterus is not the primary reservoir of cadmium in the human body; however, it is one of the most sensitive tissues to this metal. The danger of cadmium lies in its ability to mimic hormones, as it is classified as a mineral estrogen. This means it can bind to and activate estrogen receptors in the uterine lining, which can lead to abnormal cell growth, such as the thickening of the uterine lining. Cadmium concentrations tend to increase with age due to the adult biological half-life, which ranges from 10 to 30 years [30,31]. This duration can vary depending on the type of exposure and the specific form of cadmium that accumulates in the body. Recent endocrinological studies indicate that middle-aged females exposed to cadmium may experience a decrease in the number of eggs in their ovaries as they approach menopause. This condition, known as decreased ovarian reserve, results in fewer eggs than other females of the same age and is associated with various health risks [3,4]. According to the Centers for Disease Control and Prevention (CDC) Toxic Substances and Disease Registry, cadmium is the seventh-highest priority for reducing human exposure. The Dartmouth Toxic Metals Research Program notes that cadmium is a relatively rare metal that occurs naturally in the Earth's crust [4–7]. Manganese Mn is the twelfth most abundant element in the Earth's crust. Mn is an essential element that plays a crucial role in the body, also important for maintaining fertility, and deficiency occurs when the body does not obtain enough through the diet [8,9]. The European Food Safety Authority has established an adequate daily intake of manganese has not set an acceptable upper limit due to insufficient data. While manganese is essential in small amounts, excessive concentrations can lead to manganese toxicity [8–10]. Table 1 summarizes the most important properties of cadmium and manganese.

2.2. Luteinizing hormone (LH)

Luteinizing hormone (LH), also known as lutropin or lutrophin, is a naturally occurring chemical produced and secreted by the anterior pituitary gland. It is a heterogeneous glycoprotein composed of two main components: a glycan (sugar) portion made up of two polysaccharide chains and a polypeptide portion containing 92 to 96 amino acids, depending on the species. LH has a biological half-life of approximately 20 min. LH levels can be measured in either blood or urine [13,27]. Typically, LH levels are low during childhood and increase significantly in postmenopausal females. Blood LH levels are reported in international units (IU) Table 2.

3. Materials and methods

3.1. Study population

The sampling method was random, with stratification based on the type of infertility while considering factors such as age and weight. Samples were collected from Al-Zahraa Hospital in Najaf Center, Iraq, involving females aged 19 to 51 who were diagnosed with either primary or secondary infertility. The study included 40 females with primary infertility and 20 females with secondary infertility, who had no history of hysterectomy or bilateral oophorectomy. The first exclusion criterion was females with infertility who had a previous diagnosis of polycystic ovary syndrome

Table 1. Properties of cadmium and manganese [8−12].

Chemical Symbol	Impact on the Reproductive System	[a]IARC	Industrial Source	Natural Sources
Cd	Oxidative stress - inhibits normal growth and development of ovaries - disrupts morphology and function of ovaries and uterus - affects normal growth and development of follicles - inhibits oocyte growth - changes in ovarian vascular structure with shrinkage of the vascular zone - increased uterine weight - endometrial hyperplasia -Genetic toxicity - Hormonal dysfunction - Menstrual irregularities - Reduced fertility rates - Placental necrosis and damage - Increased risk of implantation failure - Low birth weight- decreased progesterone levels - decreased follicle-stimulating hormone levels - decreased luteinizing hormone levels - temporary infertility.	Group 1	Electroplating − welding -By-products from the refining of Pb, Zn, and Cu -fertilizer Industries - Pesticide manufacturer -Cadmium and Nickel batteries.	Weathering of rocks - airborne soil particles - desert soil - sea spray - forest fires - biogenic material − volcanoes and hydrothermal vents.
Mn	Hormonal dysfunction - decreased fertility - alters the levels of gonadotropin hormones (FSH and LH) - disrupts the metabolism and functions of the corpus luteum at high concentrations- increases the risk of miscarriage and puberty in children-reduces uterine weight change - low manganese intake increases the risk of anovulation - high levels can negatively impact egg quality and ovarian function -its deficiency leads to impaired reproductive function - birth defects. -regulates the synthesis of steroid hormones - affecting the hypothalamus, and Parkinson's disease gene mutations are associated with manganese deficiency.	No data	Mining operations - ocean floor nodule sediments - power grids - wolframite - hydrometallurgical processing - electrolysis - commercial carbon steel - alkaline and dry batteries.	Soil - oats - brown rice - leafy greens almonds - hazelnuts - legumes -pineapple- blueberries- fish and shellfish- sunflower seeds- flax seeds- black and green tea- turmeric- Black pepper- cloves- dark chocolate- drinking water- breast milk.

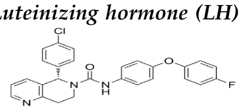
[a] International Agency for Research on Cancer (IARC).

(PCOS), which is a leading cause of female subfertility. Additionally, (20 Healthy females able to get pregnant). All participants were informed about the study and provided voluntary consent before participating. Sample analyses were conducted in the laboratories of the College of Science at the University of Kufa. Ethical approval for the study was obtained from Al-Qadisiyah University, College of Education, and Department of Physics. The purpose of the study was explained to each participant using informed consent forms for literate individuals and oral explanations for illiterate individuals. Participation was voluntary, and participants were not coerced into answering questions. The confidentiality of the information provided by the participants was ensured, and no financial benefits were offered or received.

3.2. Self-reported infertility

A self-reported questionnaire was used to assess the presence of infertility. To evaluate the

Table 2. Main consequences of hormonal abnormalities on female fertility [24,28].

Hormone	Low Level↓	High Level↑
Luteinizing hormone (LH) 	Problems with the pituitary gland or hypothalamus - difficulty with ovulation and menstrual cycles - irregular or absent periods -potentially infertility- hypothalamic dysfunction and anorexia -Pasqualini syndrome -Kallmann syndrome, hypopituitarism - eating disorders, the athlete's triad - hyperprolactinemia and hypogonadism.	Polycystic Ovary Syndrome (PCOS) - premature ovarian failure - menopause. Damage to the ovaries- autoimmune diseases - pituitary tumors.

prevalence of infertility among females aged 19 years and older, the following question was posed: "Have you ever attempted to become pregnant for at least one year without successfully conceiving?" Females who answered "yes" to this question were classified as primary infertile. The second question was asked: "Have you ever been successful in pregnancy once and then been unable to conceive?" Females who answered "yes" were classified as secondary infertile.

3.3. Other variables of interest

On the morning of the second or third day of their menstrual cycle, each participant visited the clinical laboratory at Al-Zahraa Teaching Hospital to provide a 5 ml blood sample for the measurement of sex hormones. Demographic and clinical information for all participants was collected from interviews with female participants. Their ages ranged from 19 to 51 years, and their weights varied between 54 and 94 kg. The participants experienced either primary or secondary infertility. It is important to note that all participants were non-smokers, did not have polycystic ovary syndrome (PCOS), were free from chronic diseases, were not taking any medications, and had no occupational exposure, as all the women involved in the study were housewives. Most participants come from the middle class, leading to similar dietary patterns based on family income.

3.4. Measurements of cadmium, manganese, and luteinizing hormone levels

Cadmium and manganese concentrations were measured using an atomic absorption spectrometer (AAS) from Buck Scientific Inc., USA. Cadmium was assessed at a wavelength of 228.8 nm, while manganese was estimated at 270.5 nm. Luteinizing hormone level was measured by the Snibe Maglumi 800 Chemiluminescence Immunoassay (CLIA) system; all reported results adhered to the quality control standards established by the Environmental Health Laboratory Sciences Division. Preparing blood serum samples and digestion involves several basic steps, as shown in Table 3.

4. Result and discussion

Table 4 examines the concentrations of cadmium and manganese in healthy females of childbearing potential aged 19 to 39 years in the control group. The cadmium concentration at a rate of 0.1307 ppm was slightly higher than the recommended value of 0.12 ppm, according to the Agency for Toxic Substances and Disease Registry ATSDR [32]. Elevated cadmium concentrations are even in healthy females. Numerous studies have documented high levels of cadmium in Iraq, primarily due to the direct discharge of untreated industrial and oil waste into the Tigris and Euphrates rivers. This pollution mostly originates from refineries and power plants that use heavy fuel oil. Additionally, dust storms, which are common in Iraq, contribute to the redistribution and inhalation of pollutants present on the soil's surface. The concentration of manganese was 0.00049 ppm, with an acceptable level compared with (0.0004-0.0012) ppm for females, as recommended by ATSDR. The luteinizing hormone concentration is approximately 6.769 IU/L compared to the expected levels of luteinizing hormone during the follicular phase of the menstrual cycle, which is 3.5 − 12.5 IU/L according to WHO recommendations [33]. The data were analyzed using logistic modeling with SPSS 27. Fig. 1 displays the standard residuals of a regression model where the dependent variable is "LH." In statistics, this graph is primarily used to assess the

Table 3. Preparing blood serum samples and digestion.

Basic steps	Description [5–8]
Sample volume	5 mL of the blood sample was placed into SST or red-capped tubes.
Mechanism of preservation	Do not stir the blood excessively, as this can lead to hemolysis (the destruction of red blood cells), affecting hormone concentrations. After blood collection, allow it to clot at room temperature for 20-30 min. This allows the clotting factors in the blood to work and separate the serum from the cells.
Centrifugation	Samples centrifuged at 6000 rpm at room temperature for 10 − 15 min.
Serum extraction	1 ml of serum was placed in a separate cup tube.
Digestion	1 ml hydrogen peroxide, 3 mL of nitric acid, and 10 mL of distilled water.
Dilution	Adding distilled water, 25 mL, to a final volume.
Thermal digestion	Samples were digested at 200 °C for 1 h.
Sample storage	Then carefully transfer the serum to a clean, labeled tube using a pipette to avoid contamination. Freeze serum samples at −20 °C or −80 °C to maintain sample integrity, then gently thaw frozen samples at room temperature; the serum must be thoroughly mixed before use. Gently mix the sample by inverting the tube 5–10 times.

Table 4. All parameters for healthy females (Control Group).

ID	Cd (ppm)	Mn (ppm)	LH (IU\L)	Age (y)	Weight (kg)	Economic situation
H_1	0.2941	0.0009	6.03	24	65	Mid.
H_2	0.0441	0.0007	0.967	19	62	Mid.
H_3	0.2941	0.0006	7.64	25	85	Mid.
H_4	0.0519	0.0003	3.18	28	75	good
H_5	0.0254	0.0005	6.98	30	59	Mid
H_6	0.0500	0.0008	7.94	30	60	Mid
H_7	0.0367	0.0002	13.8	29	70	Mid.
H_8	0.1618	0.0001	3.38	31	82	good
H_9	0.0312	0.0009	11.38	26	94	Mid.
H_{10}	0.2802	0.0004	6.450	28	88	Mid.
H_{11}	0.2840	0.0005	6.050	20	62	Mid.
H_{12}	0.0421	0.0004	1.962	22	60	Mid.
H_{13}	0.2509	0.0003	6.641	25	80	Mid.
H_{14}	0.1801	0.0007	3.282	28	71	good
H_{15}	0.0373	0.0002	6.991	32	56	good
H_{16}	0.0434	0.0003	7.09	19	55	good
H_{17}	0.0202	0.0003	13.89	39	71	Mid.
H_{18}	0.1724	0.0004	3.881	35	72	good
H_{19}	0.0519	0.0001	11.281	26	90	Mid.
H_{20}	0.2612	0.0006	6.570	24	85	Mid.
Ave.	***0.1307***	***0.00049***	***6.769***	**27**	**72**	-

normality assumption in a linear regression model. The mean value indicates that the residuals are appropriately centered, while the standard deviation suggests that the spread is slightly narrower than what is observed in a perfect standard normal distribution, though it remains relatively close. The black curve represents the normal distribution (bell curve). To meet the assumptions of linear regression, the blue bars should generally align with this curve. A noticeable peak or cluster at zero suggests that a significant portion of the predictions is highly accurate. The distribution appears fairly symmetrical. While it may not be a perfect bell curve, it is generally considered acceptable for a small sample size of (N = 20). Additionally, there are no extreme outliers or sharp deviations that would immediately invalidate the regression model. Table 5 presented the descriptive statistics for Cd, Mn, and LH in healthy females (control group).

Table 6 presented data on female's aged 19-51 experiencing primary infertility. The cadmium concentration is 0.2453 ppm, significantly higher than the recommended value of ATSDR. In contrast, the manganese concentration indicated as

Table 5. Descriptive statistics for healthy females (control group).

Biochemical parameters	No.	Min.	Mean	Max.	± SD
Cd(ppm)	Twenty	0.02	0.1307	0.29	0.0108
Mn(ppm)		0.0001	0.00049	0.0009	0.0027
LH (IU\L)		0.97	6.7693	13.89	3.6036

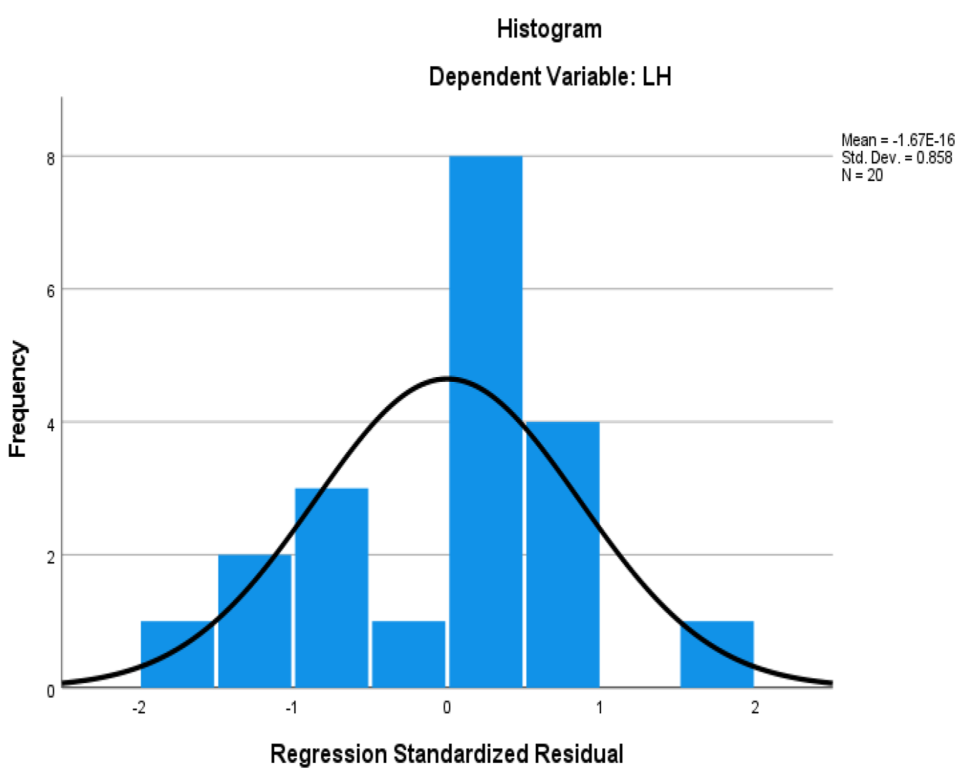


Fig. 1. The normal distribution of dependent variable luteinizing hormone levels in the logistic regression model for healthy females.

Table 6. All parameters for the two types of female infertility.

ID	Cd(ppm)	Mn(ppm)	LH (IU\L)	Age (y)	Weight (kg)	Economic situation	Type of Infertility
F_1	0.2794	0.00031	3.77	41	72	Mid.	Primary infertility
F_2	0.3235	0.00004	9.3	51	70	Mid.	
F_3	0.2059	0.00011	9.121	24	60	Mid.	
F_4	0.2059	0.00013	12.7	30	58	good	
F_5	0.1176	0.00003	6.22	19	63	Mid	
F_6	0.3971	0.00014	5.78	30	64	Mid	
F_7	0.2647	0.00001	10.58	20	71	Mid	
F_8	0.3088	0.00003	83.67	26	82	Mid	
F_9	0.1471	0.00003	21.29	30	79	Mid	
F_{10}	0.2794	0.00014	13.92	28	75	Mid.	
F_{11}	0.1324	0.00001	37.89	34	74	Mid.	
F_{12}	0.1618	0.00033	7.635	21	65	Mid.	
F_{13}	0.3676	0.00003	8.059	32	65	good	
F_{14}	0.2647	0.00014	7.239	30	80	good	
F_{15}	0.1471	0.00001	4.38	26	62	Mid.	
F_{16}	0.3824	0.00093	10.42	23	84	Mid.	
F_{17}	0.0147	0.00024	6.680	21	70	good	
F_{18}	0.2647	0.00041	4.334	24	78	Mid.	
F_{19}	0.3961	0.00012	6.847	26	75	Mid.	
F_{20}	0.3875	0.00010	6.847	25	70	Mid.	
F_{21}	0.1994	0.00031	4.799	26	72	Mid.	
F_{22}	0.2235	0.00004	10.35	37	67	Mid.	
F_{23}	0.2959	0.00007	15.129	25	62	Mid.	
F_{24}	0.2234	0.00013	13.73	30	58	good	
F_{25}	0.1272	0.00003	7.221	29	61	Mid	
F_{26}	0.1971	0.00014	14.780	31	64	Mid	
F_{27}	0.2557	0.00001	15.54	28	70	Mid.	
F_{28}	0.3478	0.00003	60.67	26	80	Mid	
F_{29}	0.1462	0.00003	19.30	32	75	good	
F_{30}	0.2737	0.00014	19.90	29	75	Mid.	
F_{31}	0.1384	0.00001	30.89	35	72	Mid.	
F_{32}	0.1692	0.00031	10.537	27	65	Mid.	
F_{33}	0.3666	0.00003	9.155	36	63	good	
F_{34}	0.2287	0.00014	7.238	30	80	good	
F_{35}	0.3598	0.00002	6.190	27	60	Mid.	
F_{36}	0.3668	0.00083	13.03	22	75	Mid.	
F_{37}	0.2187	0.00022	6.661	23	70	Mid	
F_{38}	0.2547	0.00009	12.332	25	08	Mid.	
F_{37}	0.1340	0.00021	6.589	22	73	good	
F_{38}	0.2542	0.00041	8.234	24	55	Mid.	
F_{39}	0.1346	0.00025	13.781	24	74	Mid.	
F_{40}	0.2547	0.00041	8.034	27	28	Mid.	
Ave.	0.2453	0.000165	14.2018	28	68	-	
F_1	0.0869	0.00030	19.390	32	72	Mid.	Secondary infertility
F_2	0.3038	0.00040	15.101	39	70	good	
F_3	0.2842	0.00011	15.11	26	80	Mid.	
F_4	0.0118	0.00033	16.096	30	50	Mid.	
F_5	0.2740	0.00032	16.051	23	52	Mid.	
F_6	0.1322	0.00048	22.92	19	57	good	
F_7	0.2053	0.00030	13.702	25	65	Mid.	
F_8	0.3509	0.00031	2.870	22	58	good	
F_9	0.6021	0.00042	15.404	26	61	Mid.	
F_{10}	0.2053	0.00030	4.705	27	64	Mid.	
F_{11}	0.3412	0.00035	16.879	23	60	Mid	
F_{12}	0.6011	0.00044	15.404	29	62	Mid.	
F_{13}	0.0013	0.00010	14.640	26	67	good	
F_{14}	0.2402	0.00031	11.02	31	76	Mid.	
F_{15}	0.0300	0.00032	19.19	22	60	Mid.	
F_{16}	0.3004	0.00041	14.60	38	64	good	
F_{17}	0.2292	0.00017	13.13	24	73	Mid.	
F_{18}	0.0107	0.00033	20.903	30	54	Mid.	
F_{19}	0.2510	0.00037	16.200	26	56	Mid.	
F_{20}	0.1302	0.00048	19.131	22	59	good	
Ave.	0.2296	0.000307	15.122	27	63	-	

0.000165 ppm is below the expected level. The main causes of manganese deficiency are diets low in manganese-rich foods, impaired absorption due to medical conditions or gastrointestinal issues, and a high intake of processed foods. The luteinizing hormone level is recorded at 14.2018 IU/L, which is relatively high for 3.5 − 12.5 IU/L according to WHO. These findings are concerning, as elevated cadmium levels and low manganese concentrations are known to impact female fertility. A high LH level in females of childbearing age may indicate a lack of ovulation, potential issues with the ovaries, polycystic ovary syndrome, or Turner syndrome. Fig. 2 illustrates the distribution of residuals in a regression model where "LH" is the dependent variable. A normal distribution is a crucial assumption in linear regression. In this case, the data is right-skewed, meaning it leans towards the right. There is a long tail extending to the right, with values ranging between 2 and 4, while most of the data is concentrated on the left side. The black bell curve represents the ideal normal distribution for this dataset. However, the blue bars do not align well with this ideal; they show excessively high peaks on the left and gaps or outliers on the right. Because the residuals do not perfectly follow a normal distribution and include an outlier, the reliability of the p-values and confidence intervals may be compromised. Table 7 presented the descriptive statistics for Cd, Mn, and LH in Primary Infertile Females.

From Table 8: Cadmium concentration was 0.2296 ppm for females with secondary infertility exceeds the recommended level. In contrast, the manganese concentration was 0.000307 ppm, lower than the expected value of ATSDR. The luteinizing

Table 7. Descriptive statistics for primary females' infertility.

Biochemical parameters	No.	Min.	Max.	Mean	± SD
Cd(ppm)	Forty	0.0147	0.3971	0.2453	0.10311
Mn(ppm)		0.00001	0.00093	0.000165	0.00023
LH (IU\L)		3.77	83.67	14.2018	3.00001

hormone level was 15.122 IU/L, which is relatively high from the recommended level of 3.5 − 12.5 IU/L of the WHO. These findings are concerning, as both high cadmium and low manganese levels significantly impact female fertility and cause hormonal dysfunction. High levels of luteinizing hormone are associated with not getting pregnant again. Cadmium inhibits progesterone synthesis by suppressing the expression of the steroidogenic acute regulatory protein (StAR) and the cytochrome P450 enzyme known as P450scc, which is responsible for the conversion of cholesterol. StAR transports cholesterol into mitochondria, while P450scc is a mitochondrial membrane enzyme that converts cholesterol into pregnenolone, the first step in the steroidogenesis pathway in mammalian tissues. Cadmium also accumulates in ovarian granulosa cells, leading to a significant decrease in gonadotropins, which disrupts the activity of steroidogenesis enzymes. Additionally, cadmium indirectly induces oxidative stress by reducing intracellular glutathione levels. It does this by binding to thiol groups in antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, thereby impairing their normal function [34]. High levels of cadmium and low levels of manganese have been linked to increased luteinizing hormone levels, which can lead to decreased ovarian reserve, premature ovarian failure, polycystic ovary

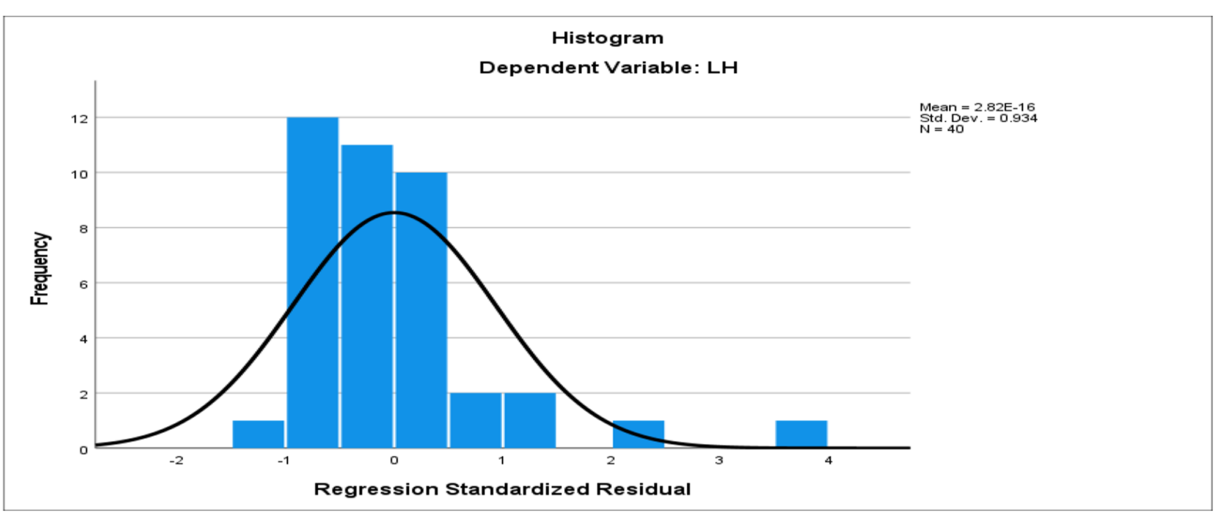


Fig. 2. The normal distribution of dependent variable luteinizing hormone levels in the logistic regression model for primary infertile females.

Table 8. Descriptive statistics for secondary females' infertility.

Biochemical parameters	No.	Min.	Max.	Mean	± SD
Cd(ppm)	Twenty	0.0013	0.6021	0.2296	0.07170
Mn(ppm)		0.00001	0.00048	0.000307	0.000012
LH (IU\L)		2.870	22.92	15.122	2.74890

syndrome PCOS, pituitary gland problems, signs of chromosomal abnormalities, and thyroid disorders. All of these factors are known to cause primary and secondary infertility. From Fig. 3, the black curve illustrates a normal distribution, also known as the bell curve. To satisfy the normality requirement, the blue bars should generally align with this curve. The residuals are centered around zero, and most of the data falls between −2 and +2, which is expected for standardized scores. There is a slight rightward slant in the distribution, indicating that the tail extends a bit to the right, along with a noticeable sharp drop on the left side, around −1.5. Although the distribution curve is not perfect, it is generally acceptable for a sample size of only 20, as smaller samples often do not appear perfectly normal. Since the residuals are nearly normal, p-values and confidence intervals are likely to be reliable. This graph demonstrates that the regression model, which includes cadmium and manganese as predictor variables, is statistically valid in terms of its error distribution. The relationship between these predictive variables and LH levels is clearly established, and there is no indication of systematic error bias. Table 8 showed the descriptive statistics for Cd, Mn, and LH in secondary infertile females.

Table 9 shows the logistic regression analysis of all biochemical parameters studied, where, for healthy females, Luteinizing hormone and Cd ($r = -0.239$): weak negative correlation. Luteinizing hormone and age (person correlation coefficient $= 0.357$, $p = 0.061$): moderate positive correlation, but not statistically significant because the p = slightly above 0.05 ($r = 0.357$, $p = 0.061$). This may be a trend worth investigating in a larger sample. Logistic regression analysis or logistic modeling based on the p-value of reveals that the regression is not significant and the independent variable is not correlated with the dependent variables Cd, Mn, weight, and economic situation, except for age, where the regression is significant, and the level of luteinizing hormone is correlated with age, and there is a statistically significant relationship between them.

For primary infertile females, weight and LH ($r = 0.258$, $p = 0.054$): This relationship is very close to statistical significance, but it does not reach the standard lower bound of 0.05. This relationship indicates a weak positive trend. Most of the other values (e.g., Cd vs. LH) have p-values much higher than 0.05 (e.g., 0.354), which means that these correlations are likely random and not statistically

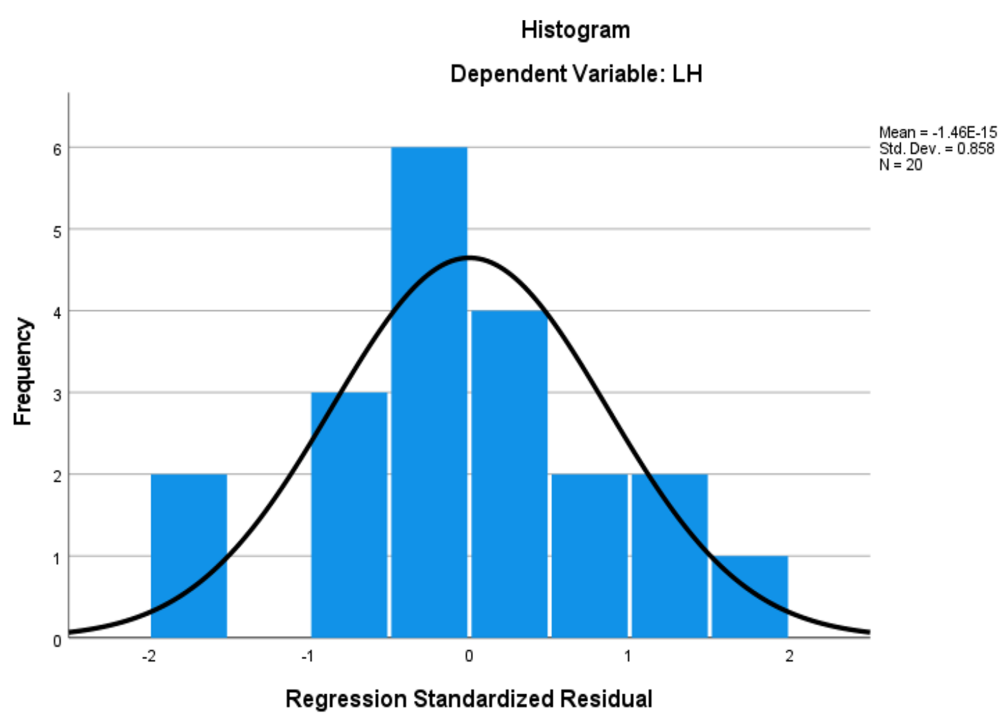


Fig. 3. The normal distribution of dependent variable luteinizing hormone levels in the logistic regression modelfor secondary infertile females.

Table 9. Logistic regression analysis of all biochemical parameters.

Dependent Variable: LH	Mn	Cd	Age	Weight	Economic situation
Healthy Females					
Pearson Correlation	0.058	−0.239	0.357	0.269	0.019
Sig. (1-tailed)	0.404	0.156	0.061	0.125	0.469
B	672.488	−13.852	0.195	0.117	0.200
Primary Infertility					
Pearson Correlation	−0.228	0.061	0.040	0.258	−0.152
Sig. (1-tailed)	0.079	0.354	0.403	0.054	0.175
B	−23177.377	20.268	−0.275	0.419	−0.439
Secondary Infertility					
Pearson Correlation	0.397	−0.261	0.047	0.106	0.180
Sig. (1-tailed)	0.041	0.133	0.421	0.329	0.224
B	52131.626	−20.087	−0.215	0.452	0.214

a. Dependent Variable: LH.
b. Predictors: (Constant), Mn, Cd, Age, weight, Economic situation.

significant. Logistic regression analysis based on the p-value reveals that the regression is not significant and the independent variable is not correlated with the dependent variables Cd, age, and economic situation, except for Mn and weight, they added a fundamental explanation for the variance in the dependent variable, where the regression is significant, and the level of luteinizing hormone is correlated with Mn and weight, and there is a statistically significant relationship between them.

While for secondary infertile females, Luteinizing hormone (LH) and Mn (weak to moderate positive correlation): There is a statistically significant positive correlation ($r = 0.397$, $p = 0.041$). Age and LH ($r = 0.047$) show no relationship or statistical significance. The same applies to the other parameters. The regression was not statistically significant and was not related to the dependent variables Cd, age, weight, and economic status, except for Mn. It provided a basic explanation for the variance in the dependent variable, and the regression was statistically significant, and the level of luteinizing hormone was related to Mn, and there was a statistically significant relationship between them.

The study was conducted in the city of Najaf, and as such, the results can primarily be applied to the central and southern governorates of Iraq, due to their similar climate and dietary culture. Additionally, some data, including economic status, medical history, and marriage history, were collected orally, which may introduce a margin of error. Therefore, it is recommended that future studies rely on official documents to ensure the accuracy and transparency of the results.

5. Conclusion

Heavy metals are risk factor for female fertility by disrupting reproductive hormones. Therefore, this study aimed to analyze the relationship between heavy metal (Cd and Mn) and luteinizing hormone LH levels in infertile females (primary and secondary infertility) to identify indicators that could aid in early diagnosis of infertility or help alleviate its symptoms. Cadmium concentrations were found to be higher than the safe limits, which negatively affect female fertility, especially ovulation, reproductive hormone levels. In contrast, manganese concentrations were significantly lower than the body's needs a healthy threshold that affects female fertility. The study demonstrated a relationship between Cd and Mn with increase LH levels in both primary and secondary infertility cases. To support reproductive health, it is important to increase your intake of antioxidant-rich foods and ensure you maintain adequate levels of essential minerals.

Source of Funding

The study was conducted without any external funding.

Conflicts of Interest

The authors have no competing interests to declare.

Ethical Approval

The principles outlined in the Declaration of Helsinki were adhered to during this investigation. The Ethics Committee of Al-Zahraa Hospital in Najaf Center, Iraq. The Health Directorate approved the project. The local ethics committee reviewed the research methodology, as well as details of the subjects and the permission form, and granted a seal of approval (Najaf Health Directorate). Form No. 2025-08: Decision No. 2025-260.

Data Availability

No datasets were created or analyzed in the current study.

Author Contributions

Shatha F. Alhous authored the main manuscript text, conducted statistical analyses, cited references, and collected samples; author Anees A. Al-Hamzawi reviewed the manuscript. Murtadha Sh Aswood reviewed and collected the samples.

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