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Study the changes in vitamin D3, magnesium, phosphorus, parathyroid hormone and calcium levels among women positive for SARS-2 n COVID-19 concerning rubella infection

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Abstract--Background: After February 2020, SarsnovelCovid-19 became pandemic; therefore, Iraqi people were implicated in this enigma. Rubella infection in women in Kirkuk Province as Bad Obstetric History (BOH) was more common. Suspecting co-infection for these two viruses' infections and the changes in the women's body like Vitamin D, Calcium, Magnesium, and other biochemical changes. Aims: this current study was carried on to assess and grant that. Setting: a cross-sectional observational study on 154 women of childbearing age with approved SARS n-covid-19 by real-time PCR was enrolled as a test group. Versus 45 women negative for covid with previous BOH were taken as a control group, and their ages ranged from 15 to 50 years. Methodology: Rubella IgM and IgG antibodies using ELISA kits were used for each woman. Vitamin D3, Parathyroid hormone (PTH) ELISA kit also used. For determining serum Calcium, Magnesium, and Phosphorus, routine spectrophotometry biochemical kits were tested. Results: The rate of Rubella infection was 77.92% IgG, contributed to 77.92 % of Rubella IgM versus 72.27 %. Controversy to the following rates 35.56 % and 8.88% of IgG and IgM

respectively, $P < 0.05$. The high rate of previous abortions 71.42 % and 22.72% stillbirth, were recorded as abnormal pregnancy outcomes; all were positive for Rubella IgM and IgG. In both the test and control groups, the mean levels of Vitamin D3 were insufficient, below 20 ng /mL. PTH mean level during Co-infection was low compared to control. Hyper-magnesium and inorganic phosphorus were recorded in the test group compared to the control. Calcium level was close to low normal level in Rubella+ covid patients compared to a normal state in sera of the patient in the control group. Conclusions: The rate of Rubella infection in women positive for covid -19 was high. Co-infection of Rubella+Covid-19 had a substantial impact on Vitamin D3, PTH, phosphorus and Calcium as a hypoparathyroidism in women aging from 41-45 years. Meanwhile, high Magnesium and Phosphorus should be checked in separate further researches for assurance.

Keywords---Rubella, Vitamin D, Magnesium, Calcium, ELISA.

Introduction

The family of positive-sense, single-stranded RNA-enclosed viruses known as coronaviruses (CoVs) is very varied. They threaten public health and veterinary and financial concerns since they infect people, other mammals, bird species, cattle, and companion animals. The family Coronaviridae belongs to the order Nidovirales and the suborder Coronavirineae. Alpha-coronavirus, beta-coronavirus, gamma-coronavirus, and delta-coronavirus are the four genera that make up the Ortho-coronavirinae subfamily, which includes the latter^[1]. Gamma-coronaviruses and delta-coronaviruses have a broader host range that includes avian species, while alpha-coronaviruses. However, beta-CoVs are the most relevant category since they contain the most dangerous human viruses, including COVID-19, MERS-CoV, and SARS-CoV. Rarely animal CoVs may be transferred to people; hence, the virus may spread during outbreaks such as MERS, SARS, and COVID-19. beta-coronaviruses only infect mammalian species. Human and animal coronavirus infections mostly bring on respiratory and intestinal illnesses^[2,3]. On December 31, 2019, an epidemic of pneumonia with an unknown etiology was reported to WHO for the first time in Wuhan City, located in Hubei Province in China. The authorities in China discovered a new coronavirus, which they have designated as 2019-nCoV. This virus is responsible for thousands of confirmed human illnesses throughout numerous provinces in China, then in Arab countries, including Iraq. There have been reports of infections with no symptoms, disorders with moderate symptoms, conditions with severe symptoms, and even some fatalities^[4]. The first record of the novel Corona-19 was recorded in the north of Iraq in February 2020, and the following figure-1 summarizes the subsequent infection in all of Iraq until June 2022. Rubella is caused by the rubella virus, an enveloped, single-stranded, positive-sense Paramyxovirus R.N.A. virus that is the only member of the genus Rubivirus^[5]. Rubella is usually a mild disease that subsides on its own. A rash denotes it. Rubella infection during pregnancy can cause congenital rubella syndrome (CRS), spontaneous abortion, and stillbirth^[6]. The classic triad comprises cataracts, heart problems, and sensorineural deafness. This is especially true when the

infection happens during embryogenesis [7]. After 2003, many sera of individuals in Iraq reveal vitamin D deficiency, mainly among people in Kirkuk province. The rate was high, particularly among women of different ages [8]. Calcium homeostasis, conserving bone, and keeping it healthy are the sole functions of the steroidal hormone Vitamin D. Furthermore, recently, the relation between cancer and Vitamin D toxemia [9]. A lack of vitamin D or a low amount of vitamin D has progressed cancer between 30 and 50 percent of breast, prostate, and colon cancer cases, as well as rectal and colon cancer. Women with polycystic ovarian dysfunction (PCOS) may have anovulation throughout the fertile period [10]. The frequency of low vitamin D levels was documented at a high rate among women with (PCOS), and the rate was between 67 percent and 85 percent [11]. Obesity, increased risk of cardiovascular disease, insulin resistance, monthly irregularity, infertility, and hyper-androgenism are signs of vitamin D deficiency [12]. [Vitamin D has two key potential benefits: immunological modularity and prenatal immunologic response [13]. The connections between 25(OH) vitamin D and miscarriage in the first trimester are strong but mild in the second trimester. In contrast to the third trimester, this connection weakens, indicating vitamin D as a modifiable risk factor for miscarriage [14].

Calcium is an essential element that plays a significant role in the process of mineralization of skeletal tissue [15]. The human body has around 1 to 1.5 kilograms of Calcium, of which approximately 99 percent is kept in the bone as hydroxyapatite, and 1 percent is found in extracellular fluid. The former is what provides bones and teeth with their characteristic stiffness [16]. In children, a deficiency in Calcium may cause a disease known as rickets, and in adults, an imperfection in Calcium can lead to osteomalacia or osteoporosis [17]. Phosphorus is the second most plentiful and most abundant element. Building bones and teeth, using sugar and fat, protein synthesis, growth, maintenance, and healing of cells and tissues, Acting as an energy source (in the molecules adenosine triphosphate (ATP) and creatinine phosphate), muscle contractions, kidney function, control of heart rate, production of genetic material (DNA, RNA). Moreover, maintaining balance and helping the body use other vitamins and minerals (such as Calcium, Magnesium, potassium, and phosphorus) (within the phospholipid). All bodily metabolic functions, including energy production and cell division, depend on a proper acid-base balance (pH), which is also a need for numerous enzymes and hormones [18].

The parathyroid hormone is a peptide of 84 amino acids with a molecular weight of 9500.382. It is a hormone released by the parathyroid glands encoded by a gene on the short arm of chromosome 11. It is also known as parathormone or parathyrin. It modulates blood calcium at the bone, kidney, and gastrointestinal tract levels. PTH diseases include the following: Hypoparathyroidism is characterized by diminished parathyroid gland function and parathyroid hormone underproduction, which lead to low calcium levels in the blood, which can cause cramping and twitching of muscles, as well as tetany and other symptoms [19]. Hyperparathyroidism, on the other hand, is a disorder in which one or more parathyroid glands become hyperactive and release an excessive amount of parathyroid hormone (PTH). This raises the calcium levels in the blood, resulting in hypercalcemia [20].

Magnesium is an important nutrient that is necessary for a variety of physiologic actions in the body [21]. Magnesium homeostasis is primarily maintained by interactions between the colon, bone, and kidneys. Magnesium is a cofactor for approximately 600 enzymes in the body that perform a variety of tasks in a variety of systems [22,23]. The inflammatory system is one such system. Magnesium has been shown to have potent anti-inflammatory effects: low magnesium levels in rats have been associated with increased inflammation [24], and higher magnesium levels have been associated with a reduction in C-reactive protein (CRP) levels [25], which is one of the widely used biomarkers to measure inflammation, implying that Magnesium has the potential to reduce inflammation. This might imply that utilizing Magnesium to treat pulmonary inflammation in COVID-19 patients has therapeutic advantages. Magnesium's bioavailability varies depending on the form. According to studies, organic Magnesium, such as magnesium citrate, has better bioavailability than inorganic Magnesium, such as magnesium oxide [26,27].

The World Health Organization estimates that as of June 7 2020, a total of 12,366 persons in Iraq had contracted COVID-19, with women making up 44% of those cases. Although males now have greater COVID-19 death rates than women, the virus and its effects are more likely to have an overall negative impact on women [28]

Few studies on the effects of SARS novel covid-19 and women with a bad obstetric history (BOH) brought on by Rubella infection have been conducted in Kirkuk Province, thus the present research was conducted to evaluate and guarantee improvements.

Active Cases in Iraq

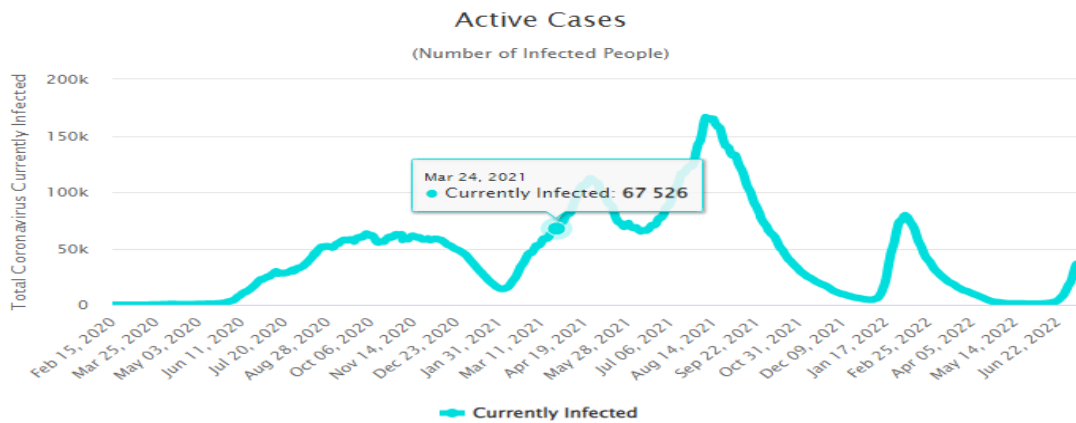


Figure 1 summarizes the incidence of SARS2-Novel Covid-19 in Iraq

Materials and Methods

Data collection

comprehensive data, including (name, age, sex, address, residence, signs and symptoms, and other information), was collected from each patient and organized in a unique questionnaire explicitly designed for the goal of gathering this information.

Samples collection

Nasopharyngeal, oropharyngeal for n-Covid-19 was collected using a protocol of WHO,2019 in medical care centers and Public health lab in Kirkuk city and processed for nCovid-19 using RT-PCR.

Blood sample: From each participant, 5 ml of whole blood in both groups was drawn using a sterile technique left after 10 minutes, then centrifuged for 5 minutes using the speed of 3000 RPM. Good clear quality serum was obtained, avoiding icteric, lipemic, and hemolyzed sera; transferred into another sterile plan tube and kept at -20°C until use [30].

Timeframe and location:

The present investigation was conducted at the Ibn-Nafees private medical lab from March 2019 until June 30 2021.

Study participants and design: Two groups of women were the subjects of a cross-sectional observation; the first group included those with a negative nCovid-19 test and those with a negative bad congenital anomaly. Their age ranged from 15 and over 50 years.

Exclusion standards:

Women who tested positive for Cytomegalovirus, Toxoplasma, or human Herpes simplex viruses—other etiological agents of BOH—were excluded from the trial and did not participate.

Ethical approval: According to the roles of the scientific committee of medical ethics at the Kirkuk Medical College, permission was given for the study. Each woman's signature on the permission form indicates her willingness to participate in the research.

Laboratory tests: Rubella IgM and IgG Abs using ELISA kits and sandwich technique, Vitamin D3 using ELISA-fluorescent process in Mini-Vidas machine, Calcium, inorganic phosphate, and Magnesium using a spectrophotometric method, and parathyroid hormone using sandwich ELISA kit. All tests followed each kit's instructions.

Vitamin D determination: The amount of vitamin D in each serum was measured using an automated system called Mini-Vidas; the detection technique was a hybrid of ELISA and fluorescence principles. This technique was carried out in accordance with the kit's instructions as recommended by the French business Biomuriex. After 45 minutes, the results of each serum were shown on the LCD

screen of the machine. According to the results, vitamin D deficiency is indicated by levels below 20, insufficiency is characterized by levels between 20 and 30, adequacy is indicated by levels between 30 and 70, and vitamin D toxicity is indicated by levels over 70 ng/100 ml. The test procedure was conducted as per the manufacturer's instructions^[18].

Parathyroid hormone assessment:

Method: ELISA-Biotech sandwich-Spain.

Principle: Intact PTH is a sandwich ELISA. Standards and patient samples were treated with enzyme-labeled detection antibody and biotin-coupled capture antibody in a streptavidin-coated microplate well. The micro-well is rinsed to eliminate unattached components and the enzyme coupled to the solid phase is treated with tetra-methyl-benzidine (TMB). An acidic stopping solution then turns the hue yellow. The yellow tint indicates the sample's intact PTH content. Standards provide an absorbance unit vs. concentration dose-response curve. Standard calibration curves determine intact PTH concentrations in control and patient samples. 10-90 pg/ml is common. This manual follows the kit's pamphlet and ^[30]

Serum calcium: RandoxKit-UK, the business that created the kit, provided the instructions for the serum calcium evaluation. The normal result varied from 8.2 to 10.5 mg/dL .

Detection of serum inorganic phosphorus: A Bi-Labo-France kit for UV-spectrophotometry was used to find out how much inorganic phosphate was in the patient's serum. 2.5 to 4.3 mg/dl is considered the normal range for this parameter

Serum Magnesium determination: Magnesium ions create a colorful complex with xylydyl blue in an alkaline media, which is used to monitor serum magnesium levels. Using GEDTA as a calcium ion masking agent increases the absorbance in direct proportion to the magnesium content in the sample. The normal range was between 1.9 and 2.33 mg/dl.

Statistical analysis

All received data were consolidated and presented in specified tables using a Microsoft Excel file for statistical analysis. Using the manual statistical equations chi-square and t-student test for imposing variances and significances at the levels P 0.05 and P 0.01, differences in the parameters between the study group and the control group were analyzed.

Results

The total rate of rubella infection was 78.57 percent, with Rubella IgM and IgG antibodies present in the test group at 79.22 percent and 77.92 percent, respectively. Rubella IgM and IgG contradiction to control group SARS2-Covid 19 negative exhibited 8.88 and 35.56 percent, respectively; P< 0.05. Table.1.

Table.1 Total and percentage of Rubella infection among women positive for SAR2-Covid 19

Type of antibodies	Test group			Control group		
	No. +ve	%	Mean \pm s.e	No +ve	%	Mean \pm s.e
Rubella IgM	109 70.77		1.45 \pm 0.41	3	6.66	0.63 \pm 0.03
Rubella IgG	95 61.68		1.44 \pm 0.3	15	33.34	0.70 \pm 0.15
Equivocal IgM +ve	13 8.44		0.91 \pm 0.03	1.0	2.22	0.98 \pm 0.02
Equivocal IgG +ve	25 16.23		0.94 \pm 0.03	1.0	2.22	0.93 \pm 0.02
Total IgM +ve	112 72.27		1.18 \pm 0.22	8.88	0.4	0.80 \pm 0.03
Total IgG +ve	120 77.92		1.19 \pm 0.016	35.56	16	0.82 \pm 0.08
All total positive	121 75.09		1.175 \pm 0.	20 44.45		0.081 \pm 0.06

Total No. exam=199, test group=154, control group=45 Equivocal (0.9—0.99 IU/ml)

Table 2 depicts women in crisis with rubella infection as they had past pregnancy outcomes, notably stillbirth, which occurred in 35 women at a rate of 22.72 percent, in contrast to the number of abortions, which totaled 110 instances. The overall mean of Rubella IgM and IgG was 1.24 percent for both antibodies; the total standard of Rubella IgM and IgG was 1.24 percent for both types of antibodies. In contrast, nine women had no congenital malformations or abortions, and their sera exhibited the following means: 1.59 IU/ml and 1.10 IU/ml, respectively; $P < 0.05$.

Rubella antibody distributions were shown in table-3 according to the women's ages, with young women and the elderly displaying somewhat lower mean IgM antibodies. The rates were 0.98 and 0.87 IU/ml, respectively, whereas other age groups had high mean Rubella IgM levels. Meanwhile, the opposite findings of IgG antibody levels were more remarkable in young and older women, with rates of 1.31 and 1.83 IU/ml reported, respectively, with $P < 0.05$.

Table. 2- Congenital abnormality and previous abortion among test group

Abnormal previous history	No. %		Rubella IgM	Rubella IgG
			Mean \pm s.e	Mean \pm s.e
Stillbirth	35	22.72	1.16 \pm 0.21	1.32 \pm 0.19
Abortion				
One abortion	51	44.11	1.2 \pm 0.49	1.22 \pm 0.49
Double abortion	33	21.42	1.17 \pm 0.5	1.20 \pm 0.45
Triple abortion	12	7.69	1.33 \pm 0.28	1.29 \pm 0.53
Fourth abortion	8	5.19	1.29 \pm 0.43	1.18 \pm 0.46

Fifth abortion	5	3.24	1.29 ±0.29	1.16±0.21
Six abortion	1	0.65	1.16±0.00	1.39±0.0
Total for abortion	110	71.42	1.24±0.33	1.24± 0.35
No abortion	9	5.88	1.59 ± 0.75	1.10±0.20

Total No. Exam =154.

The mean level of vitamin D3 was 17.19 ng/ml in women who tested positive for Covid and Rubella. Serum vitamin D3 concentrations ranged from 8.54 ng/ml in women aged 15 to 20 years to 10.41 ng/ml in women aged 21 to 25, followed by 11.73 ng/ml aged 50 and beyond. Covid positive and women's ages were statistically linked to rubella infection. $P < 0.05$. Serum magnesium and phosphorus levels related to rubella infection were not statistically significant ($p > 0.05$). 2.45 mg/100ml of Magnesium was over the standard upper limit. Meanwhile, the mean S. phosphorous concentration of 4.20 mg/100 ml was near the upper range of normal. Table-3.

Table.3-Impact of rubella infection on Vitamin D3, Magnesium, and phosphorus levels according to women's ages.

Age group	Age (year) Mean ± STDE	Rubella IgM (IU/ml) Mean ± STDE	Rubella IgG (IU/ml) Mean ± STDE	Vitamin D (ng/ml) Mean ± STDE	Magnesium (mg/dl) Mean ± STDE	Phosphorus (mg/dL) Mean ± STDE
15-20 Y	17.74 ± 0.59	0.98 ± 0.07	1.31 ± 0.148	8.54 ± 0.52	2.42 ± 0.063	4.19 ± 0.151
21-25 Y	23.44 ± 0.38	1.03 ± 0.14	1.09 ± 0.11	10.41 ± 1.19	2.54 ± 0.12	4.37 ± 0.19
26-30 Y	27.9 ± 0.21	1.29 ± 0.1	1.23 ± 0.08	14.89 ± 1.39	2.46 ± 0.044	3.89 ± 0.145
31-35 Y	33.37 ± 0.29	1.2 ± 0.091	1.2 ± 0.073	23.03 ± 4.01	2.46 ± 0.04	4.35 ± 0.11
36-40 Y	38.49 ± 0.47	1.33 ± 0.16	1.32 ± 0.13	20.51 ± 6.69	2.42 ± 0.09	4.62 ± 0.146
41-45 Y	43.29 ± 0.54	1.19 ± 0.12	1.13 ± 0.14	14.57 ± 2.48	2.48 ± 0.075	3.9 ± 0.34
46-50 Y	48.4 ± 0.5	1.29 ± 0.11	1.21 ± 0.14	21.07 ± 6.25	2.41 ± 0.06	4.26 ± 0.29
Above 50 Y	53.67 ± 0.47	0.87 ± 0.08	1.81 ± 0.25	11.73 ± 1.96	2.54 ± 0.05	3.99 ± 0.22
TOTAL	34.12 ± 0.8	1.24 ± 0.039	1.2 ± 0.032	17.19 ± 1.28	2.45 ± 0.021	4.2 ± 0.06
P @ 0.05	P= <0.001	P= 0.028	P=0.025	P=0.004	P=0.63	P=0.39
	Significant	Significant	Significant	Significant	Not Significant	Not Significant

In control group mean± s.e of Vit D3=13.91±2.77 ng/mL, Mg⁺²=1.96±0.29 mg/dL and S.Phosphorus:3.88±1.8mg/dL.

The borderline lower normal Calcium limit in the test group, 8.21± 0.08mg/dl versus 8.5± 1.04 mg/dl in the control group, was reported and obvious in table-4,

$p < 0.05$. Also, Parathyroid hormone levels exerted 15.81 pg/mL in a test group were close to the lower standard limit from 9 to-55 pg/mL compared to 24.04 ± 2.26 pg/mL. Statistical analysis of the differences between the test and control group regarding PTH and Calcium was significant, $p < 0.05$. Women aged from 26-30 years and women in age groups from 41 over 50 years showed the lowest mean of Calcium 7.77 ± 0.26 mg/dl. For women aged 41 to 45 years, their sera reveal a hypo-PTH mean of 7.29 ± 0.91 pg/mL, $P < 0.05$.

Table.4- Parathyroid (PTH) hormone and Calcium levels among patients positive for Rubella and SARS-2 Covid -19

Age group	Age (year)	Rubella IgM (IU/ml) Mean \pm STDE	Rubella IgG (IU/ml) Mean \pm STDE	Parathyroid (pg/mL) Mean \pm STDE	Calcium (mg/dL) Mean \pm STDE
15-20 Y	17.74 \pm 0.59	0.98 \pm 0.07	1.31 \pm 0.148	22.28 \pm 6.06	8.56 \pm 0.23
21-25 Y	23.44 \pm 0.38	1.03 \pm 0.14	1.09 \pm 0.11	13.44 \pm 4.54	8.34 \pm 0.26
26-30 Y	27.9 \pm 0.21	1.29 \pm 0.1	1.23 \pm 0.08	20.21 \pm 3.99	8.12 \pm 0.23
31-35 Y	33.37 \pm 0.29	1.2 \pm 0.091	1.2 \pm 0.073	15.96 \pm 4.25	8.07 \pm 0.21
36-40 Y	38.49 \pm 0.47	1.33 \pm 0.16	1.32 \pm 0.13	14.39 \pm 4.51	8.62 \pm 0.24
41-45 Y	43.29 \pm 0.54	1.19 \pm 0.12	1.13 \pm 0.14	7.29 \pm 0.91	8.45 \pm 0.41
46-50 Y	48.4 \pm 0.5	1.29 \pm 0.11	1.21 \pm 0.14	15.25 \pm 8.48	7.77 \pm 0.29
Above 50 Y	53.67 \pm 0.47	0.87 \pm 0.08	1.81 \pm 0.25	19.17 \pm 4.94	7.77 \pm 0.23
TOTAL	34.12 \pm 0.8	1.24 \pm 0.039	1.2 \pm 0.032	15.81 \pm 1.51	8.21 \pm 0.08
	P= <0.001	P= 0.028	P=0.025	P=0.008	P=0.18
	Significant	Significant	Significant	Significant	Not Significant

In control group mean \pm s.e of PTH = 24.04 ± 2.26 pg/mL, $Ca^{+2} = 8.5 \pm 1.04$ mg/dl. From the information in Table 5, it's clear that women with Rubella plus covid have a high level of Magnesium in their blood, 2.45 mg/100 ml, compared to 1.96 mg/100 ml in the control group. Even though the later mean was in the normal range, the relationship was significant compared to the test group. $P < 0.05$.

Table.5 -Serum magnesium levels changes in test and control groups during Rubella infection.

Age group	No.Exam	Age (year) Mean ± STDE	Rubella IgM	Rubella IgG	Magnesium Test group	Magnesium Control group
			(IU/ml) Mean ± STDE	(IU/ml) Mean ± STDE	(mg/dL) Mean ± STDE	(mg/dL) Mean ± STDE
15-20 Y	10	17.74 ± 0.59	0.98 ± 0.07	1.31 ± 0.148	2.42 ± 0.2	0.0 ± 0.0.0*
21-25 Y	26	23.44 ± 0.38	1.03 ± 0.14	1.09 ± 0.11	2.54 ± 0.34	2.18 ± 0.32
26-30 Y	37	27.9 ± 0.21	1.29 ± 0.1	1.23 ± 0.08	2.43 ± 0.28	2.20±0.44
31-35 Y	34	33.37 ± 0.29	1.2 ± 0.091	1.2 ± 0.073	2.46 ± 0.19	2.41 ± 0.16
36-40 Y	34	38.49 ± 0.47	1.33 ± 0.16	1.32 ± 0.13	2.42± 0.34	2.38± 0.24
41-45 Y	16	43.29 ± 0.54	1.19 ± 0.12	1.13 ± 0.14	2.48 ± 0.24	2.28 ± 0.0.8
46-50 Y	22	48.4 ± 0.5	1.29 ± 0.11	1.21 ± 0.14	2.41 ± 0.18	2.33 ± 0.32
Above 50 Y	20	53.67 ± 0.47	0.87 ± 0.08	1.81 ± 0.25	2.54 ± 0.17	0.0 ± 0.0.0*
TOTAL	199	34.12 ± 0.8	1.24 ± 0.039	1.2 ± 0.032	2.45 ± 0.26	1.96 ± 0.29
		P= <0.001	P= 0.028	P=0.025	P=0.229	P=0.18
		Significant	Significant	Significant	Not significant	Not Significant

*Means no patients enrolled in the study for these age groups •

Discussion

Since the imposition of economic sanctions on Iraq in 1991 and the subsequent instability that followed in 2003, the incidence of certain infectious agents, particularly viruses like *HumanHerpes simplex-2*, cytomegalovirus, Rubella, and hepatitis B, has risen significantly [31]. This rise can be attributed partly to poverty, a lack of effective medications, a lack of celery, and other factors. Our final destination is regarding the growing frequency of nCovid-19 infections in Kirkuk province. Concerning the nCovid-19 increase in the number of conditions in our destination, the area of Kirkuk reflects the poor hygiene and low-level sanitation in considering the disregard for wearing gloves and face masks and poorly manufactured antiseptic solutions as rejecting vaccination or visiting either government or private clinics. This reflects the poor hygiene and low-level sanitation in the province. Each of these factors might be responsible, in whole or in part, for this increase. There was no genuine evidence of the role of Rubella in producing congenital abnormalities or pregnancy loss before the rubella vaccination application program for pregnant women in Iraq in 2002. Most cases were attributable to the BOH (TORCH) causative agent, which included Rubella. The recommended Rubella vaccination decreased the prevalence of rubella infection, one of the most common and deadly congenital disorders associated with fetal loss and teratogenicity[32]. In the current study, 75.09 percent of continuing rubella cases were caused by activities in various countries. This incidence was lower than a study in western Saudi Arabia, which found that 88.9 percent of women are immune to Rubella and 24 percent are vulnerable[33]. WHO reported 62 rubella cases in Saudi Arabia in 2019 and four instances of congenital rubella syndrome in 2017-2018. 22 This study investigated the rubella seroprevalence in Saudi Arabia's eastern region and documented cases of acute rubella infections to help guide and evaluate national vaccination efforts[34]. [35] found 21.03 percent of Rubella seropositive infections in a different investigation in Kirkuk, Iraq, in 2014. The discrepancy is mostly related to the 2014

occurrence, during which most of the family was moved and relocated to a region with bad hygiene and living under the poverty level. As a result, they were unable to vaccinate against Rubella. Furthermore, co-infection with nCovid-19 in this city may have had a role in the increased rubella incidence.

Even though the rate of Rubella IgM antibodies is 72.27 percent and the rate of Rubella IgG antibodies is 77.92 percent. The latter may be considered protection against past infection since Rubella produces lifetime immunity, much like CMV^[36]. At the same time, 72.27 percent of Rubella IgM was helpful during pregnancy, which could cause an abortion or other problems after birth. The IgM rate is 72.27%, although it is pretty low compared to the rate obtained in Australia by^[37], who recorded a range of rates (84.2 percent to 96.5 percent) from testing 220 blood samples using a mini-vides kit. Congenital squeals as stillbirth occurred in 35 (22.27 percent) of the women in the present research, which was greater than the 6.67 percent observed in 17 women in 2014 by^[35]. Congenital squeals as stillbirth in 35 (22.27%) in the current study was higher than that recorded in 2014 by^[35], who recorded 6.67 % in 17 women. Furthermore, 110 women have had previous multiple abortions, 71.42%; this rate was higher than 34.12% recorded between 207 and 2014 by^[36]. Despite this variance, other infectious agents should be considered, such as Toxoplasma, Cytomegalovirus, and HHSV-1and2, which also impacted rising congenital anomalies.

Regarding the women's age and rubella infection, negative rubella IgM and positive IgG Abs in young and women over 50 refer to less sexual activity, menopause, and sterile immunity. While mid-aged women are having both IgM and IgG positive indicate that those women were more susceptible to getting infectious agents, including Rubella virus.

The link between Vitamin D problems and Rubella antibodies suggests a strong connection between the two. Table 3 shows that most vitamin D problems happen between the ages of 15 and 20. Most vitamin deficiencies and insufficiencies occur in women between the ages of 20 and 30 and over 50. A lack of sun exposure could cause this finding because of changes to building designs in Iraq after 2003, the wrong way to use tonics with Vitamin D and other minerals, or problems with how the parathyroid gland secretes its hormones^[18]. Another possibility is that all the participants tested positive for nCovid-19 because co-infection worsened the harmful effects on Vitamin D3 metabolism and absorbers^[30]. On the other hand, it could be because cortisol and other drugs were used to treat COVID patients. Overall, the effects of Rubella+Covid showed these deficiencies and inadequacies.

Magnesium ions in the body of Covid patient were potentially deficient in Magnesium. Patients hospitalized in Tehran, Iran's Shahid Modarres Hospital, between January 30, 2020, and April 5, 2020, were the focus of independent research. The amount of Magnesium in a patient's blood at the time of their admittance to the hospital was tested. Based on these findings, Magnesium seems to provide some protection against COVID-19^[38]. For 306 patients hospitalized at Tongji Hospital in Wuhan, China, a retrospective cohort research evaluated the amounts of necessary and harmful metals in blood samples. The study found that patients with more severe COVID-19 symptoms had lower magnesium levels^[39].

The result of the current study has not agreed with the conclusions of [38,39]. It is well known that Magnesium activates vitamin D, improving immunological functioning [22, 23]. Low vitamin D levels are linked to autoimmune disorders such as MS, RA, diabetes, and IBD [37-39]. Several studies have demonstrated that vitamin D reduces inflammatory cytokines and increases anti-inflammatory cytokines [40], suggesting its therapeutic utility in treating disorders that produce excessive inflammation, such as COVID-19. Vitamin D lowers inflammatory cytokines by increasing T-regulatory cells[41]. Magnesium activates vitamin D, which improves immunological function and reduces COVID-19 cytokine storm intensity [42]. A cytokine storm drains energy and ATP. Magnesium and phosphate are essential for ATP regeneration; therefore, Magnesium may replenish energy reserves to increase immune system functioning [43, 44]. The justification for the higher incidence of Rubella IgM may be seen in the following, which argues that viruses can undermine the integrity of tight junctions upon invading cells and that vitamin D has been proven in some studies to minimize viral infections by maintaining tight junctions [45]. Studies have demonstrated that vitamin D might prevent certain viral infections, which supports the idea that vitamin D might inhibit viral infection by stabilizing tight junctions [46].

In the current study, obtaining low levels of Calcium and increased serum phosphorus levels among patients aged from 41 to 45 years refers to hypoparathyroidism among women positive for Sars2-nCovid-19. As acknowledged, low levels of blood calcium, elevated levels of serum phosphorus, and the absence or improperly low levels of serum parathyroid hormone (PTH) are the hallmarks of the uncommon endocrine deficiency illness known as hypoparathyroidism. The parathyroid gland is often damaged by autoimmune or post-surgical processes, leading to hypoparathyroidism [47]. The most common cause of non-surgical hypoparathyroidism is autoimmune hypoparathyroidism. Hypoparathyroidism may also be induced by external or internal radiation, but this is seldom noted since parathyroid glands are radiation-resistant [48]. Hypoparathyroidism may also be caused by copper (Wilson's disease) and iron (hemochromatosis) deposits in the parathyroid gland. Hypoparathyroidism is also linked to mitochondrial disorders. Such people presumably have an autoimmune illness [49]. To the best of our knowledge, the case that is presented in this article is the fourth to disclose primary hypoparathyroidism caused by SARS-CoV-2 infection. The 16 patients in this study who had COVID-19 pneumonia and were between the ages of 41 and 45 all had low levels of PTH and low levels of Calcium and phosphorus in their blood. This is similar to what was shown in the previous instances [50,51,52]. It was determined that the patient did not have cancer, paraneoplastic syndrome, or autoimmune conditions.

Conclusions

The incidence of rubella infection among COVID-19-positive women was high. Rubella+Covid-19 co-infection had a significant effect on Vitamin D3, PTH, phosphorus, and calcium in women aged 41-45, manifesting as hypoparathyroidism. In the meantime, high Magnesium and Phosphorus should be verified through additional study.

Author Contributions

Tahseen, Salman, and Amin designed the study. Amin picks women and directs them to Jirjees and Ali for research laboratory sampling and a few lab test procedures. Tahseen Amin and Salman are responsible for data analysis and paper production. Tahseen and Salman proposed methods and interpretations for locating laboratories. Salman examined the document. Each author contributed to the article and approved the version that was submitted.

Conflict of Interest

There were no commercial or financial ties that may be considered as a possible conflict of interest throughout the study process.

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