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Evaluation of maternal and neonatal outcomes of pregnant women with COVID-19 at Sayad Shirazi Hospital, Gorgan, in 2020-2022

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Abstract

Background: The coronavirus disease 2019 (COVID-19) can have a variety of effects on pregnant women, from mild symptoms to severe complications. Those with underlying medical conditions are at an increased risk for severe disease and adverse pregnancy outcomes. This study was designed to investigate the maternal and neonatal outcomes of pregnant mothers infected with COVID-19 who were referred to Sayad Shirazi Hospital, Gorgan (Iran), in 2019-2022.

Methods: This cross-sectional study was conducted on 160 pregnant women. The women who had just given birth were either suspected of having coronavirus or had a definite diagnosis of coronavirus either during hospitalization or during hospitalization who were included by the simple census. The data were collected from medical records and through telephone interviews. The chi-square test was used to compare categorical variables between the groups. The qualitative data were evaluated with quantitative variables by the analysis of variance. The significance level was less than 0.05.

Results: The difference in the access of minorities to medical and health service centers affected the pregnancy results (P=019). A high rate of Cesarean delivery (58.1%), a high prevalence of gestational diabetes (19.3%) and hypothyroidism (21.2%), and the need for high insulin injections during the COVID-19 infection (39.37%) were also found, which were independent of the patients. Diabetes and previous gestational diabetes showed high statistics.

Conclusion: This study demonstrated a high concentration in people with hypothyroidism and patients who need insulin due to hyperglycemia after contracting COVID-19 during pregnancy. According to this study, contracting COVID-19 increases the level of maternal and fetal problems, and women with hypothyroidism should be considered as a risk factor for contracting this disease and having a more serious condition, hyperglycemia, and the need for insulin, except for the remaining complications of this disease is expected.

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Highlights

What is current knowledge?

Current knowledge suggests that pregnant women with COVID-19 may be at an increased risk for adverse maternal and neonatal outcomes, including preterm birth, Cesarean delivery, and neonatal intensive care unit admission.

What is new here?

This study highlights the importance of early screening and management of COVID-19 conditions in pregnant women to prevent adverse outcomes. Overall, the findings of this study can inform clinical practice and improve the care provided to pregnant women with comorbidities during the COVID-19 pandemic.

Introduction

Pregnant women are a vulnerable population because their immune system is suppressed during pregnancy, which makes them susceptible to infections (1). The coronavirus disease 2019 (COVID-19) can have a variety of effects on pregnant women, from mild symptoms to severe complications. Pregnant women with COVID-19 may experience symptoms such as fever, cough, shortness of breath, fatigue, body aches, loss of taste or smell, and sore throat (2). In some cases, COVID-19 can lead to pneumonia and respiratory failure, which can be life-threatening. There is also evidence that COVID-19 can increase the risk of preterm birth and other pregnancy complications such as preeclampsia and stillbirth; the risk of hospitalization and intensive care unit (ICU) admission is also higher for pregnant women with COVID-19 compared to non-pregnant women (2, 3, and 4).

A recent study by the Centers for Disease Control and Prevention (CDC) found that pregnant women with COVID-19 are more likely to require invasive ventilation, ICU admission, and mechanical ventilation than non-pregnant women of reproductive age. In addition, pregnant women with underlying diseases such as diabetes, high blood pressure, and obesity are at risk of developing severe illness and adverse pregnancy outcomes such as premature delivery, fetal growth restriction, and stillbirth (5).

The impact of COVID-19 on Cesarean deliveries is also significant, especially among pregnant women with underlying conditions. According to a study published in the International Journal of Gynecology and Obstetrics, 30% of pregnant women with COVID-19 had a Cesarean delivery, of which 62.5% of cases were due to maternal or fetal distress. The association between elevated inflammatory markers, such as interleukin-6, and the need for Cesarean delivery among pregnant women with COVID-19 was also investigated (6, 7). Pregnant women with COVID-19 and those with underlying medical conditions are at an increased risk for severe disease and adverse pregnancy outcomes (8).

Another aspect to consider is the impact of COVID-19 on prenatal care. Due to the pandemic, many pregnant women have faced challenges accessing routine prenatal care, which can lead to missed opportunities for early diagnosis and management of complications (9, 10).

Considering the recent passing of the coronavirus pandemic and the disease being an emerging one globally, observing the various manifestations appropriate to each ethnicity and race can help to better understand this disease (11).

Sayad Shirazi Hospital, the research unit of this study, was both the coronavirus center of Golestan Province (Iran) during the pandemic and the obstetrics and gynecology center of the province. As such, examining the data from the pandemic in this center could yield important results to provide a better view of this disease. This study was designed to investigate the maternal and neonatal outcomes of pregnant women with COVID-19 referred to Sayad-Shirazi Hospital in Gorgan from 2019 to 2021.

Methods

This study was conducted in Gorgan, Iran, to investigate the effects of COVID-19 on pregnant women. This was a cross-sectional study that included 160 pregnant women with or suspected of having COVID-19 in a 3-year interval from 2019 to 2022 (the pandemic years) who needed hospitalization. The data were collected during 3 months from December to March 2023 after obtaining the approval of the Ethics Committee of Golestan University of Medical Sciences (ethical code: IR.GOUMS.REC.1401.284). All the patients were hospitalized in Sayad Shirazi Hospital of Gorgan, which is the maternity and gynecology center and the main center of COVID-19 in the province. This study was a single-center research. All the eligible patients were included by

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complete enumeration (a census).

Inclusion criteria:

Pregnant women or women who had just given birth (who were infected during pregnancy, but the conditions of delivery took priority), were suspected of having COVID-19 (having at least 3 of the symptoms related to COVID-19 that have not been proven, or lung involvement in favor of COVID-19 confirmed by a computed tomography (CT) scan or chest X-ray), or had received a definitive diagnosis of COVID-19 by polymerase chain reaction (PCR) either at hospitalization or during hospitalization were included. Due to the sanctions and the lack of PCR diagnostic kits, most patients during the epidemic in the Islamic Republic of Iran were diagnosed with chest X-ray. The pregnancy of all the women had been confirmed (with β-hCG or seeing the gestational sac in the ultrasound before hospitalization).

Exclusion criteria:

In case of defects in the hospital file, noncooperation, or lack of access to correct and sufficient information during a phone call, and in case of diagnosis during hospitalization with another disease that justified the symptoms (such as cold and influenza), the women would be excluded.

Data analysis:

Data analysis was performed in SPSS v. 16 (SPSS Inc., Chicago, IL, USA) with descriptive statistics, including frequency, percentage, mean, and standard deviation (SD). Chi-square tests were also used to examine the relationship between the outcomes of COVID-19 and various factors such as age, gestational age, and comorbidities. The chi-square test and Fisher's exact test were used to compare categorical variables between the groups, and the independent *t*-test was run to compare continuous variables between the groups. The relationship between qualitative data and quantitative variables analyzed by the (ANOVA) test. The significance level in all the analyses was less than 0.05.

Materials:

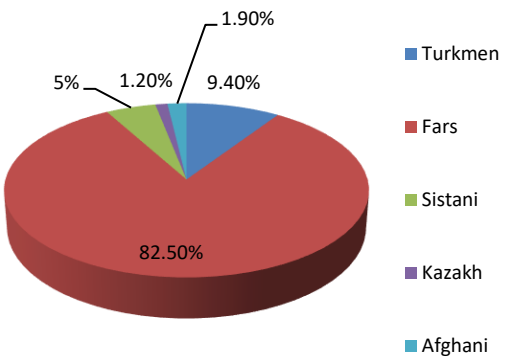
The materials used in this study included medical records and a structured telephone interview questionnaire. The medical records contained information about the participants' demographics, medical history, symptoms, and outcomes of COVID-19. The following variables were obtained from the medical records: maternal age, gestational age at the time of COVID-19 diagnosis, symptoms, comorbidities, mode of delivery, neonatal outcomes, length of hospitalization, need for ICU admission, oxygen saturation, mode of delivery, medicines used, and the neonatal Apgar scores. The telephone interview questionnaire was designed to collect additional information about the participants' experiences with COVID-19, including long-term complications, subsequent complications of the children born, complications of childbirth after hospitalization, gestational age at delivery that occurred during subsequent hospitalizations, or malformations.

The telephone interview questionnaire included closed questions that were easy to understand and answer and covered a range of topics related to COVID-19, including symptoms and complications, as well as place of residence and ethnicity. It was tested on a small sample of pregnant women to ensure its clarity and comprehensiveness.

Results

A total of 160 women were deemed eligible to enter the study. Their average was 30.66 years with a variation range of ± 6.501. The minimum age was 14 years, and the maximum age was 49 years. The racial distribution is shown in (Figure 1).

Figure 1. Races



45% of these mothers lived in the urban area of the center of the province (Gorgan), 27.5% lived in the surrounding cities and 27.5% lived in the villages of Golestan province.

There was no statistically significant difference between fetal complications and different races on chi-square test, but by re-categorizing and comparing Fars and being a minority, the result changed and became significant (P=.049). The sum of minorities was lesser than Fars's race and the difference in the distance of the people's residence from the center of the province that has equipped medical service centers couldn't be seen with different races (which was not an acceptable result due to the lack of samples with other races) But after re-categorizing and comparing the Fars race with the minorities, the impact was meaningful (P=0.019) but the place of the residence itself was not related to fetal problems, and also this significant difference was not reliable due to the small number of people in the sample and the needs Studies being larger. (Tables 1) [Chi-square tests performed: Fetal problems and race (P=0.514), place of residence and race (comparing all races: P=0.004 comparing the Fars race with minorities: P=0.019), fetal problems and place of residence (P=0.485)]

Table 1. Admission gestational ages distributions

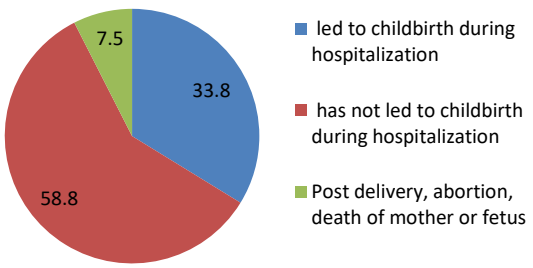
Admission gestational age (Weeks)	frequency	percent	The cumulative percentage
≥12	9	5.6	10.6
12-24	29	18.1	28.8
24-27	20	12.5	41.2
27-30	19	11.9	53.1
30-32	15	9.4	62.5
32-34	20	12.5	75.0
34-37	26	16.2	91.2
37-40	12	7.5	98.8
>40	2	1.2	100.0
After birth admission	8	5.0	5.0
total	160	100.0	

The average gestational age of the mothers at the time of admission was 26 weeks and 4 days with a standard deviation of 70.4 days. Among these mothers, 8 mothers were hospitalized after giving birth, one mother had a normal delivery and was sent with corona symptoms and 40-50% lung involvement, the rest had a cesarean section, one case was due to uterine atony and one case was due to uterine atony and fever. And they had corona symptoms and one case of cesarean section infection, and the other 4 patients were hospitalized due to corona symptoms. Of total 160 people, 93 people had a cesarean delivery (58.1 percent) (this statistic includes cesarean section after hospitalization and 43 people had a cesarean section during the initial hospitalization), of which 31 people (33.3 percent of cesarean deliveries and 19.3 percent of the total) Of these, they did not have previous indications for cesarean delivery (previous cesarean section, twins, abnormal placenta (precipitous placenta, placenta accreta), abnormal childbirth due to reasons such as head and pelvis mismatch, 12 of these people had contraindications to childbirth. They were not removed from these numbers due to the possibility of covid-19 involvement in their development (such as severe eclampsia). Most of these patients were before 37 weeks (91.2 percent) and the largest category was 18.1 percent of them between weeks. They were 12 to 24 weeks.

7 mothers (4.4 percent) died, which led to the abortion of one and two Intra-uterine-fetal-death (IUFD) (1.8 percent), but 4 other children survived; 109 mothers (68.1 percent) recovered from discharge, including seven abortions in the hospital (4.4 percent), and one abortion that occurred at 4 months of pregnancy outside the hospital (0.6 percent) and did not require dilatation and There was no curettage, 43 mothers (26.9%) were discharged with personal consent, they did not have an abortion, and one mother (0.6%) ran away from the hospital for unknown reasons, and the fetus was healthy at the last visit.

Among hospitalized mothers, 54 mothers (33.8%) gave birth during their first hospitalization (Figure 2).

Figure 2. Last results



34 fetuses with problems such as distress (respiratory, meconial, and chorio-amniotic), icterus, abortion, low FHR, unsatisfactory NST; Lack of growth and IUGR, as well as three cases of macrosomic fetuses and one case of

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stroke in a patient during childbirth following cardiac arrest, who was unable to walk until one year and 8 months old (at the last contact) and incomplete cognitive development. 38 fetuses needed hospitalization after birth. 39 fetuses were in preterm labor (born before 37 weeks), of which 4 fetuses were before 28 weeks (extreme preterm labor). Six fetuses had Apgar scores less than 6.7 if they were born during the first hospitalization (without Apgar scores of 0.0, the Apgar scores of subsequent deliveries were not included due to the unreliability of the mothers' statements in the phone call.)

There was no significant relationship between the presence or absence of fetal problems such as the need for hospitalization, being preterm, low Apgar, stillbirth, and abortion with the type of mother's underlying disease according to the Chi-square test, although paying attention to the fact that mothers who need were included in this study and 38 fetuses had problems out of 61 mothers without underlying diseases, this can be explained on the one hand, there is a statistically significant relationship between the number of underlying diseases and the presence or absence of fetal problems and the more the number of underlying diseases of the mother, the more likely problems will arise for the fetus (Table 2 and 3) (Chi-square test: P=0.019).

Table 2. Mother's past medical histories & Fetus complications

Fetus complications Mothers Past Medical histories	Without complications	Fetus complications	Fetus hospitalization	Preterm labor	Extreme preterm labor (before 28 weeks)**	Fetal death, IUFD, abortion	Low Apgar score***	Total*
Without PMH	38	6	11	8	2	3	1	61
HTN	4	3	3	4	1	1	1	11
DM	2	1	2	1	0	0	0	5
GDM	15	9	12	9	1	1	2	31
Cardiovascular diseases	4	0	0	0	0	0	0	4
Respiratory diseases	0	2	1	2	0	0	0	2
Renal diseases	0	0	0	1	0	0	0	1
History of preeclampsia in previous pregnancies	2	2	2	3	1	1	0	7
Preeclampsia	4	6	11	12	0	0	3	21
hypothyroidism	18	6	4	7	0	3	1	34
IVF	1	۴	2	2	0	0	1	5
IUFD	0	0	1	1	0	0	1	2
infertility	0	1	1	2	0	0	0	2
Hepatitis B	0	0	1	1	0	0	0	1
Thalassemia	2	1	1	3	0	2	0	8
Lupus	0	1	1	1	0	0	0	1
seizure	0	2	0	1	0	1	0	4
Anemia	3	0	1	1	0	0	0	4
UTI	1	0	0	1	0	0	0	2
Willson	0	1	1	0	0	0	1	1
Depression	0	0	0	0	0	1	0	1
PRESS syndrome	0	1	0	0	0	0	0	1
Lichen planus	0	1	1	1	0	0	0	1
strabismus	1	0	0	0	0	0	0	1
Placenta previa	1	1	0	1	0	0	0	2
thrombophilia	0	1	0	1	0	0	0	1
Bicornuate uterus	0	1	0	1	0	0	0	1
Previous history of abortion	1	1	0	1	0	0	0	2
History of cholestasis	1	0	0	0	0	0	0	1
Uterine fibroids	1	0	0	0	0	0	0	1

*It should be noted that some patients have several underlying diseases, the total number of people is written, not the number of diseases.
**Apgar 0.0 is not included
***Less than Apgar 6/7

Table 3. Fetus complications & maternal underlying disease's count

Number of maternal underlying diseases	Fetus complications		Total
	No	Yes	
0	38	23	61
1	32	31	63
2	12	7	19
3	2	10	12
4	0	4	4
7	0	1	1
Total	84	76	160

The reasons for hospitalization and visits of mothers were mostly related to the coronavirus (86.2 percent) were either suspected of coronavirus or had a definite diagnosis of coronavirus, of which 5 people (3.1 percent) also had labor symptoms. They showed it simultaneously and one patient was after delivery and with corona symptoms, the rest of the patients, 30 patients (18.7%) had labor symptoms, except for the 5 mentioned, one patient also had NST drop at the same time. and 3 people (1.9 percent) only had symptoms related to the fetus, which included NST drop, lack of fetal growth, meconial and fetal distress, and expulsion of the amniotic sac without dilatation and curettage, 4 people (2.5 percent) Others were hospitalized after childbirth with childbirth complications such as cesarean section infection and uterine atony.

13 people (8.1%) had AFI outside the normal range, which did not have a significant relationship with the final problems of the fetus according to the chi square test. (P=0.290)

5 mothers (3.1%) were pregnant with twins, although the second-born babies showed a lower Apgar score, however, no significant relationship between twins and fetal complications was observed.

Mothers who had lower O2 saturations were more often hospitalized in ICU and had a significant relationship with fetal problems, and among 52 mothers who were hospitalized in ICU, 32 fetuses had problems (One-way ANOVA test: P=0.014)

The drop in saturation occurred during hospitalization for 11 mothers, the highest range of drop was from 98% to 70% and the lowest range was from 100% to 97%, the rest of the mothers did not have a clear drop in saturation, although a wide range was recorded at the time of arrival. One mother with 50% saturation which led to the death of the mother and fetus and one mother with 70% saturation from the time of arrival, the delivery did not happen and the fetus had no problems in the end, the rest of the mothers are 7 (4.3%) in the range of 77 % to 88%, 11 people (6.9%) in the range of 88% to 92%, 24 people (15%) in the range of 92% to 95% and the remaining 105 people (65.6%) which was the largest number. The percentages were in the normal range above 95%.

Most of the mothers were hospitalized for less than 5 days (75%). The relationship between the problems caused to the fetus and the duration of the mother's stay in the hospital was not significant. (ANOVA Test: P=0.599) (Table 4)

Table 4. During hospitalization

Fetus complications drugs used during mother's hospitalization	Without complications	Fetus complications	Fetus hospitalization	Preterm labor	Extreme preterm labor (before 28 weeks)**	Fetal death, IUFD, abortion	Low Apgar score***	Total*
Without any drugs	5	1	0	0	0	3	0	9
Insulin	33	13	14	12	1	2	0	59
Methyldopa	4	3	3	4	0	1	1	11
Prednisolone	1	1	1	1	0	0	0	2
hydroxychloroquine	1	1	3	4	0	1	0	7
Aspirin	0	2	1	1	0	0	0	2
Tenofovir	0	0	1	1	0	0	0	1
Anti-biotics	11	3	5	2	0	0	1	19
Penicillamine	0	1	1	0	0	0	1	1
IVIG	1	0	0	0	0	0	1	2
Labetalol	0	0	0	0	0	0	1	1
Metoral	1	0	0	0	0	0	0	1
Amlodipine	0	0	1	1	0	0	0	1
Valsartan	0	0	1	1	0	0	0	1
Interferon	14	3	6	4	1	3	1	25
Remdesivier	74	25	24	25	4	3	6	126
Anti-viral	1	2	3	3	0	1	0	7
Tocilizumab	4	1	2	2	0	1	0	8
Corticosteroids IV	60	23	28	22	4	5	0	112
Magnesium sulfate	2	5	9	10	0	1	1	17
Heparin	45	19	21	15	4	1	1	82

It should be noted that some patients have been injected with several drugs, the total number of people is written in the total number of people, not the number of diseases.
**Apgar 0.0 is not included
***Less than Apgar 6/7

The results of the comparison of the variables with chi-square test are shown in the Table 5.

Table 5. Chi-Square test results

	Value	df	Asymp. Sig. (2-sided)
Race & fetal complications	3.266[a]	4	0.049
Race & place of residence	22.720[a]	8	0.004
Place of residence & fetal complications	1.447[a]	2	0.485
Fetal complications and number of mothers underlying diseases	15.113[a]	6	0.019
AFI & fetal complications	1.118[a]	1	0.290
Mothers ICU admissions and fetal complications	6.088[a]	1	0.014
ANOVA tests results	mean		p-value
Durations of hospitalization & fetal complications	4.99		0.599
O2 saturation and fetal complications	96%		0.14

Discussion

The outbreak of COVID-19 has brought about significant changes in the way we live. From social distancing to wearing masks, the pandemic has impacted every aspect of society. One group that has been particularly affected by this

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virus is pregnant women and their newborns. The effects of COVID-19 on pregnancy and childbirth are still being studied, and it is important to understand the potential risks and precautions that need to be taken to ensure the health and safety of both mother and child (12, 13).

There was a significant relationship between the race of people and their place of residence. There was no relationship between race and place of residence with fetal complications, but with re-categorization and comparison of two levels of Fars as the majority race and the remaining races, this difference had a significant relationship. Although less access of minorities to health care services has been expected, the result of this change in classification can be observed in the significance of the comparison of being a minority and being Fars with fetal problems. According to various studies, it was expected that during the pandemic, people's access to appropriate medical services would be of great importance; racial differences have been pointed out in some studies in this case, which was caused by the less access of minorities (19, 20, and 21).

In total, 93 births (58.1%) were performed by Cesarean section, and 31 (19.3%) of these Cesarean sections can be considered a result of COVID-19 infection. The conditions of the mothers were diverse, and it is not possible to consider all of these 31 cases to be the outcome of COVID-19 infection. For example, severe eclampsia may not be caused by COVID-19 infection. However, the high number of Cesarean sections in this study compared to other similar studies was evident, which shows that infecting with covid-19 could be the cause of the increase in Cesarean section in the province (17 and 18).

Seven mothers died, 2 of their children also died with them, and 5 other infants were saved. Hospitalization during an epidemic requires higher levels of importance, and unnecessary hospitalization has been avoided as much as possible. Therefore, the obtained statistics can be compared with the rest of the centers, although the improvement of the performance of all centers should be considered.

The distribution of maternal diseases has shown an increase in hypothyroidism more than expected, as 34 people out of 160 hospitalized patients (21.2%) had this disease. Nevertheless, Teng et al. found the prevalence of hypothyroidism in pregnant women to be around 5-3% (22), which can be looked at in several ways. One possibility could be that the condition of pregnant mothers with COVID-19 who also had the underlying hypothyroid disease is worse, which has led to more hospitalization of these women. Another potential scenario is that individuals who have hypothyroidism (both pregnant and non-pregnant) are more susceptible to contracting Covid-19. This is primarily because this condition is already present in all affected mothers prior to pregnancy, except for four cases where accurate information was lacking and pre-pregnancy examinations were not conducted. Hence, the likelihood of infection is higher in such individuals. Yet another possibility can also indicate a cause-and-effect relationship between contracting COVID-19 and hypothyroidism, which seems more unlikely than the other two possibilities. Many studies have shown the first and second hypotheses, and the poorer outcomes of contracting COVID-19 have been greater in people with hypothyroidism (23, 24, and 25).

The distribution of people without underlying diseases and with a normal pregnancy was 38 out of 160 mothers, which is due to the hospitalization of people who are sicker and more outpatient visits during the pandemic, people's fear of going to medical centers, self-treatment, and not deeming it necessary to refer people. Underlying problems were more related to fetal problems, which were expected (26).

The AFI is an indicator of fetal health, which is proportional to the expectation of more fetal disorders in people with abnormal size. Nevertheless, it was not significant in this study. The metric and the need to be admitted to the ICU both indicated the worsening of the mother's condition, who had fetuses with more problems. Although it was thought that the maternal length of hospitalization was also related to the fetal problems, this relationship was not observed. Note that the medical community's attempt during the epidemic was to discharge patients who were able to be discharged faster, and this could be a confounding factor (28).

In this research, the utilization of remdesivir, corticosteroids, heparin, enoxaparin, and blood anticoagulants were found to be more prevalent compared to other cases. This finding reflects the treatment approach adopted for managing COVID-19 during the epidemic period, which aligns with the expectations within the studied population. (16).

There are extensive studies that show hyperglycemia after contracting COVID-19. The stress of the pandemic, on the one hand, and pregnancy, on the other hand, make people susceptible to hyperglycemia and even diabetes and gestational diabetes. (29, 30, 31, 32) In the current study, 59 mothers needed to take insulin during hospitalization, 5 had previous diabetes, 4 of them had a diet, only 1 of them had pre-injected insulin, and 31 had gestational diabetes (19.3 %), which is considered a high percentage (13.4% in the world (2021) (33), 2-10% in the US according to CDC statistics, and 3.4-7.9% in Iran (34 and 35)). This indicates that, similar to hypothyroidism, gestational diabetes is also a risk factor for contracting the coronavirus. It was observed that the majority of these mothers required insulin only during their hospital stay and discontinued its use after being discharged. However, the percentage of mothers requiring insulin during hospitalization is considered high and exceeds expectations based on other studies. For instance, the study conducted by Helmet and colleagues reported a prevalence rate of 9.4% in 2020. (36).

Conclusion

This study demonstrated a high concentration of people with hypothyroidism and patients who needed insulin due to hyperglycemia after contracting COVID-19 during pregnancy. According to this study, contracting COVID-19 increases the level of maternal and fetal problems, and mothers with hypothyroidism should be considered a risk factor for contracting this disease and more serious conditions, hyperglycemia, and the need for insulin, be expected in this disease. However, more extensive studies can help identify these connections more definitively and help with understanding this new disease.

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Ethical statement

This study was carried out after obtaining the necessary permits from the Disciplinary and Ethical Committee of Gorgan University of Medical Sciences (Ethical code: IR.GOUMS.REC.1401.284).

Conflicts of interest

There were no conflicts of interest/competing interests for this study.

Author contributions

The first author, Shohreh Vosough, provided supervision and guidance throughout the study, contributed to the study design, data analysis, and manuscript writing, and provided critical feedback on the manuscript. The corresponding author, Azadeh Kaghazi, contributed to the study design, data collection, analysis, and manuscript writing and served as the primary author of the manuscript. Both authors have read and approved the final version of the manuscript.

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