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ORIGINAL ARTICLE

Placental infection with SARS-CoV-2, analysis of 16 cases and literature review

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Abstract

Purpose Since December 2019, the whole world has been affected by coronavirus [severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)]. However, the effects of COVID-19 infection on pregnancy and fetal transmission are still unclear. Therefore, this study was conducted to evaluate placenta samples regarding detection of SARS-CoV-2 RNA in women affected with COVID-19.

Method This study was a part of a cohort study carried out on pregnant women with a diagnosis of COVID-19 infection who had been admitted to the Imam Reza Hospital in Mashhad, Iran, from March 20 to August 5, 2020. Clinical and laboratory information of all the patients was collected and chest computed tomography (CT) scans were reviewed. Totally, 16 placental tissue were prepared for real time polymerase chain reaction (RT-PCR) testing. All samples were tested by PowerChek PCR real-time kit (South Korea) with 2 target genes (E gene and Rd Rp gene), and Pishtaz Teb kit, (Iran) with 2 target genes (N gene and RdRp gene).

Result In the first RT-PCR kit by PowerChek kit, 6 samples were positive for a single gene (E gene) and 2 samples were positive for both genes (E gene and Rd Rp gene). In the second RT-PCR kit by Pishtaz Teb kit, 3 samples were positive for two genes (N gene and RdRp gene).

Conclusion This present study showed that infection of placenta with SARS-CoV-2 may occur in pregnancy. However, whether this infection leads to neonatal infection and serious complication in pregnancy remains unclear.

Keywords Placenta · COVID-19 · Pregnancy · Vertical transmission

Introduction

Coronaviruses are important human and animal pathogens. At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, a city in Hubei Province in China. It was spread rapidly, resulting in an epidemic throughout China, followed by an increasing number of cases in other countries all over the world [1].

The body of evidence suggests that pregnancy does not increase the risk for acquiring COVID-19 infection but appears to worsen the clinical course of COVID-19 compared with nonpregnant females of the same age [2, 3].Risk factors for severe disease and death in pregnancy include older mean age (≥ 35 years), obesity, and preexisting medical comorbidities (particularly hypertension and diabetes) [4].

Possible vertical transmission has been reported in several cases of peripartum maternal infection in the third trimester, suggesting that congenital infection is possible but uncommon (0/310 cases in one review study) [5, 6]. Neonatal outcomes have been uniformly good for at-risk neonates in the absence of other issues, such as preterm birth or an abruption [5].

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In most women tested positive by PCR for SARS-CoV-2 in the nasopharynx, vaginal, and amniotic fluid samples have been negative by PCR testing to date [7–9] but, one patient with a positive vaginal swab has been reported [10].

Viremia rates appear to be low and transient in patients with COVID-19 (1 percent in one study) [11], suggesting that placental seeding and vertical transmission would not be common. Most placenta samples studied so far have had no evidence of infection, but the virus has been identified in a few cases [12].

In one study, Of 11 placental or membrane swabs sent for testing after delivery, 3 swabs returned with positive results for SARS-CoV-2, all in women with severe to critical COVID-19 at time of delivery. Placental swabs were obtained from the amniotic surface after clearing the surface of maternal blood (PCR of placental sample). Membrane swabs were obtained from between the amnion and chorion after manual separation of the membranes (PCR of membrane sample) [13].

Another report described two COVID-19 positive mothers in whom the samples obtained from fetal side (syncytiotrophoblast) of their placentas and neonates were also positive by PCR testing [14].

A few other possible cases of congenital infection based on newborn laboratory and/or clinical findings have been reported [15] but SARS-CoV-2 testing on fetal blood, amniotic fluid, and placenta in these cases was either negative or not performed. Although some had an elevated immunoglobulin M (IgM) level and/or pneumonia, positive IgM results are not definitive evidence of in utero infection (false positives and cross reactivity may occur), and in many of these cases, early infant infection may have been due to postnatal contact with the infected parents or caregivers [16]. Therefore, this study was done to investigate placenta samples of the patients with confirmed diagnosis of COVID19 regarding detection of SARS-CoV-2 RNA.

Methods

This study was a part of a cohort study carried out on pregnant women with a diagnosed with COVID-19 infection who had been admitted to the Imam Reza Hospital in Mashhad, Iran, from March 20 to August 5, 2020. Diagnosis of COVID-19 pneumonia was based on real-time reverse transcription-polymerase chain reaction (RT-PCR) test that was done on throat swab samples or through involvement of lung in high-resolution computed tomography (HRCT) finding.

All the subjects were informed about the objective and procedure of the study and an informed written consent was obtained from all of them regarding their enrollment in the study. The ethics committee of Mashhad University of Medical Sciences approved the study with the approval code of IR.MUMS.REC.1399.214.

All vital signs of patients with COVID-19 symptoms including pulse rate (PR), respiratory rate (RR), blood pressure (BP), body temperature (T), and O2 saturation were monitored during hospitalization. Laboratory tests such as complete blood count differential(CBC diff), liver function test(LFT), blood urea nitrogen(BUN), creatinine(Cr), C-reactive protein(CRP), erythrocyte sedimentation rate(ESR), BG, and RH were investigated at the time of admission and then, they were repeated depending on clinical symptoms and the patient's condition. Every day the patients were visited by the gynecologist and infectious disease specialist at the hospital. Patients with severe dyspnea, respiratory rate > 30 per minute and blood oxygen saturation ≤ 93% on room air, were controlled in the intensive care unit (ICU).

Termination of pregnancy was performed according to obstetrical indication. All the people associated with childbirth, physicians, midwives and pediatricians were equipped with complete protective equipment, including clothing and an N95 mask. The patients also used the N95 mask during labor.

The babies were dried immediately after birth. Umbilical cord was quickly clamped and the babies were transferred to an isolation suite of neonatal intensive care unit (NICU) without having any contact with the mother. At the beginning of the coronavirus pandemic, our policy in our center was to separate the fetus from the mother because the rate of transmission of the virus to the mother was unknown. The placenta tissue was collected under sterile condition and tissue samples were taken from fetal side near the umbilical cord insertion. 1 cm^3 from the placenta fetal face was taken in a sterile dry tube immediately after delivery and was kept at − 70 freezer until performing laboratory studies. Placentas were obtained from Normal Vaginal Delivery (NVD) or Cesarean Section (CS) as Table 3.

Placenta samples were extracted from the ROJEH Company (Iran) using an RNA extraction kit with positive and negative control to confirm extraction procedures.

Totally, 16 samples were tested by PowerChek polymerase chain reaction (PCR) real-time kit (South Korea) with 2 target genes (E gene and RdRp gene), and Pishtaz Teb kit (Iran) with 2 target genes (N gene and RdRp gene) as negative and positive controls. Positive samples had risen as cycle 22–32. In addition, all the samples underwent standardized histopathological examination.

Data collection

We collected demographic data, clinical symptoms, laboratory tests, treatment, delivery method, and maternal outcome from their electronic medical record of the mother. All the

information about neonatal outcomes including, gestational age at the time of delivery, birth weight, Apgar score, stillbirth was also reviewed. In addition, if necessary, the patient was called in all cases and accuracy of the information was checked. Data were confirmed by a co-investigator.

Results

Totally, 16 patients were diagnosed with COVID-19 among whom 15 patients had positive PCR testing, and one patient had severe symptoms with positive findings on HRCT of the chest that was leading to her death. Mean age of women was between 18 and 42 years old, and age of pregnancy was between 22 and 41 weeks. Four women had a history of contact with the affected people in the family. Three patients had diabetes and two other patients had hypertension (HTN) during the pregnancy. Mean time of onset of delivery symptoms was 1–15 days and medium length of admission in hospital was between 2 and 17 days. Eight patients presented with fever, cough, and dyspnea, one person only had fever and the rest of them did not have fever. Four patients reported anosmia. Some patients reported other symptoms, such as sore throat, chills, nausea, and muscle aches.

Twelve patients did not have any problems after being diagnosed with COVID-19 during and after pregnancy (Table 1). One case had a premature rupture of the membrane (PROM) and one case had a postpartum hemorrhage. Among16 patients, 3 of them had a severe type of disease admitted in ICU and unfortunately, death was reported in 2 cases.

The results of laboratory tests showed that 8 out of 16 pregnant women with COVID-19 pneumonia had lymphopenia ($< 1{\cdot}0 \times 10^9$ cells per L). Results of all the liver tests were in normal range in the patients. Thirteen patients had the elevated concentrations of CRP (> 10 mg/L). CRP test was not performed on 3 patients. Result of PCR test was positive in 15 patients but it was negative in one patient as she had positive findings in HRCT. A chest CT scan was performed for 10 patients. Eight patients showed typical findings of chest CT images, but 2 patients had no pulmonary involvement in CT scan and HRCT was not performed for the rest of the patients due to unwillingness of the mother (Table 2).

Out of 16 patients, 5 infants were born preterm. Intrauterine fetal demise (IUFD) occurred in one case, which was a twin pregnancy at 23 weeks (Table 3). Two of the neonates had a birthweight lower than 2500 g. Twelve live births had a 1-min Apgar score of 8–9 and a 5 min Apgar score of 9–10, and 3 neonates had a lower Apgar score. None of these neonates showed any signs and symptoms of COVID-19 (Table 3).

Ten newborns (including twins) underwent throat swab test for COVID-19 after birth. Eight cases were negative but test was positive in 2 neonates (Table 4). The presence of SARS-CoV-2 was tested in the placenta of all deliveries. In the first RT-PCR kit by PowerChek kit, 6 samples were positive for a single gene, 2 samples were positive for both genes. In the second RT-PCR kit by Pishtaz Teb kit, 3 samples were positive for two genes (Table 5). Samples of the placenta were also positive in two patients who died.

In the pathological examination, there was no infiltration of acute inflammation in the membranes and in half samples, scattered focal ischemic necrosis was observed in 2 -5% villous placenta.

Discussion

This study showed that out of 16 patients diagnosed with COVID-19, placenta samples were positive in 8 patients, of which 6 patients were positive in a single gene and 2 patients were positive in 2 genes. Death was reported in 2 cases, both of which had placental involvement. In addition, in 2 cases, neonatal throat swab samples tested positive for SARS-CoV-2.

The risk of vertical transmission in pregnant women with COVID-19 has been very controversial in the literature. Placenta acts as a physical barrier between mother and fetus, and it is defined as the first line of defense, to prevent transmission of infectious agents from mother to fetus during pregnancy. Nevertheless, some infectious agents, such as Toxoplasma, rubella, cytomegalovirus, herpes, and Zika can pass through placental barrier leading to activation innate immune response and causing fetal and mother complications [17]. Therefore, there is an increasing concern about congenital infection and adverse pregnancy outcome in pregnant women with COVID-19. Diagnosis of intrauterine infection is based on detection of the virus in cord blood, amniotic fluid, or placenta [9].

So far, there has been little agreement on vertical transmission of SARS-CoV-2 in pregnancy [6, 18]. One mechanism for intrauterine infection is high expression of cell membrane angiotensin converting enzyme II (ACE2) as a receptor of SARS-CoV-2 in placental syncytiotrophoblast, and cytotrophoblasts. Also, spike protein of the virus is motivated by an enzyme called as *transmembrane serine protease* 2 (*TMPRSS2*) gene. It is assumed that the virus binds to this receptor to enter the cell [19–22].

Although, several studies have investigated the risk of vertical transmission with different methods, most of these studies have been limited to small number of cases. Preliminary studies have failed to detect the virus in the placenta and they have concluded vertical transmission of the virus during pregnancy is unusual [23–27].

Table 1 Clinical characteristics of patients

	Date of admission	Age (years)	Gestational age on admission	Other family members affected	First symptom(s)	Medical histories during pregnancy	Onset symptom to delivery (day)	Complications	Duration of hospitalization (day)
Patient 1	8 Aug	22	40.6	Negative	Fever, cough, dyspnea	HTN, Preeclampsia	1	Negative	4
Patient 2	25 Apr	21	39	Negative	Cough, Anosmia	Negative	1	Negative	8
Patient 3	12 May	25	41.1	Negative	Fever	Negative	1	Negative	4
Patient 4	3 June	21	35	Positive	Anosmia	Diabetic	4	Post-partum hemorrhage	7
Patient 5	7 June	23	36	Positive	Anosmia, dyspnea, myalgia	Diabetic	4	Negative	8
Patient 6	12 June	18	38	Negative	Fever, dyspnea, cough	Negative	2	Negative	7
Patient 7	21 June	35	32.3	Negative	Anosmia	Negative	15	PROM	10
Patient 8	19 July	29	37	Negative	Fever, chill, dyspnea	Negative	11	Negative	17
Patient 9	17 July	29	37	Negative	cough, dyspnea, myalgia	Negative	2	Negative	3
Patient 10	15 July	42	37	Negative	Fever, cough, dyspnea, myalgia	Negative	7	Negative	10
Patient 11	29 July	42	38	Positive	Fever, cough, dyspnea, myalgia, sore throat, headache, chill	Negative	12	Negative	4
Patient 12	28 July	34	41	Negative	cough, dyspnea, sore throat, headache, chill	Negative	2	Negative	6
Patient 13	24 July	26	41	Negative	myalgia, cough	Negative	9	Negative	2
Patient 14	21 July	25	34.6	Positive	cough, dyspnea, sore throat, headache, chill, vomiting	Negative	9	Negative	6
Patient 15	28 May	31	22.5	Negative	Fever, cough, dyspnea	HTN	7	Admission in ICU, mechanical ventilation, Severe preeclampsia, Acute Tubular Necrosis (ATN), death	8

Table 1 (continued)

	Date of admission	Age (years)	Gestational age on admission	Other family members affected	First symptom(s)	Medical histories during pregnancy	Onset symptom to delivery (day)	Complications	Duration of hospitalization (day)
Patient 16	30 July	33	33	Negative	Fever, cough, dyspnea, myalgia, sore throat, headache, chill	GDM	1	Admission in ICU, preeclampsia, mechanical ventilation, death	4

Table 2 Laboratory and imaging characteristics

Laboratory and imaging characteristics	White blood cell count ($\times 10^9$ cells per L)	HB	PLT	Lymphocyte count ($\times 10^9$ cells per L)	ALT (U/L)	AST (U/L)	C-reactive protein concentration (mg/L)	SARS-CoV-2 RT-PCR	CT
Patient 1	10,600	13.9	146,000	23.5	11	21	90.3	Positive	Positive
Patient 2	10.2	12.6	238,000	12.7	16	20	55	Positive	Positive
Patient 3	22.8	11.6	160,000	5	5	11	25	Positive	Positive
Patient 4	2.3	11.2	215,000	23.7	53	33	–	Positive	–
Patient 5	5.5	10.7	99,000	27.3	11	15	–	Positive	–
Patient 6	4.6	10.9	69,000	13.7	7	14	–	Positive	–
Patient 7	9.2	12.5	182,000	17	22	22	13.2	Positive	Negative
Patient 8	5.6	11	172,000	16.4	6	10	144.5	Positive	Negative
Patient 9	8.9	11.3	175,000	15/2	12	15	61/3	Positive	–
Patient 10	11	14,1	180,000	12.1	13	25	92.8	Positive	Positive
Patient 11	10.9	10.7	217,000	7.1	8	16	34.5	Positive	Positive
Patient 12	9.2	11.7	209,000	17.9	13	28	77.2	Positive	Negative
Patient 13	10	12.1	170,000	23.9	7	14	74.3	Positive	Negative
Patient 14	16.6	10.9	225,000	9.8	18	22	87.9	Positive	–
Patient 15	11.1	9.2	242,000	10.4	12	18	107	Negative	Positive
Patient 16	7.2	11.5	235,000	10.3	17	30	145.2	Positive	–

However, there are some reports of about detection of SARS-CoV-2 RNA in various fetal samples including the placenta [10, 13, 28–33].

In a case series study conducted by Penfield, 32 patients with COVID-19 were admitted, among whom membrane or placenta swabs were tested in 11 cases. Three out of 11 swabs were positive for SARS-CoV-2, nevertheless, the neonatal tests were negative for COVID-19 infection by RT-PCR analysis [13]. In our study, in 2 cases with mild symptom in the mother. The neonates' tests were positive but had no symptoms and sign of COVID-19. In another case series study conducted by Patanè et al., 2 infants with COVID-19 PCR positive test were reported from others diagnosed with COVID-19. SARS-CoV-2 RNA was detected on fetal side of the placenta as well as neonates tests. Also, in this study, contrary to our research, immunohistochemistry was performed that showed chronic intervillositis with CD68þ macrophage infiltration. Similar to other studies, despite the positive test, the infants had no symptoms. The first infant and the second one were discharged from hospital after 10 and 20 days, respectively [14].

In contrast to the above-mentioned studies, Richtmann described 5 cases of IUFD in women with confirmed diagnosis of COVID-19 without a history of obstetric disorders. They found SARS-CoV-2 in one amniotic fluid and 2 placental swab samples. Acute chorioamnionitis was reported in histological assessment of placenta of all the cases. They proposed that SARS-CoV-2 infection in pregnancy can be associated with adverse neonatal outcomes, such as fetal death [34]. In our study, only one case of IUFD was observed in a woman with severe type of disease who was

Table 3 Outcome of pregnancy

	Treatment					Admission in ICU	Delivery		Neonates outcome
	Oxygen support (nasal cannula)	Antibiotic therapy	Antiviral therapy	Colchicine	Corticoid therapy		Method	Indication for C-section	
Patient 1	Positive	Positive	Negative	Negative	Negative	NO	CS	Preeclampsia	Term
Patient 2	Negative	Positive	Positive	Positive	Negative	NO	NVD	–	Term
Patient 3	Negative	Positive	Negative	Negative	Negative	NO	CS	Fetal distress	Term
Patient 4	Negative	Positive	Negative	Positive	Negative	NO	NVD	–	Preterm
Patient 5	Negative	Positive	Negative	Positive	Negative	NO	CS	Fetal distress	Preterm
Patient 6	Negative	Positive	Negative	Negative	Negative	NO	CS	Fetal distress	Term
Patient 7	Negative	Positive	Negative	Negative	Negative	NO	CS	History of C-section	Preterm
Patient 8	Positive	Positive	Positive	Positive	Negative	YES	CS	Fetal distress	Term
Patient 9	Negative	Positive	Negative	Negative	Negative	NO	CS	History of C-section	Term
Patient 10	Negative	Positive	Negative	Negative	Negative	NO	CS	History of C-section	Term
Patient 11	Negative	Positive	Negative	Negative	Negative	NO	NVD	–	Term
Patient 12	Negative	Positive	Negative	Negative	Negative	NO	CS	Lack of progress in labor	Term
Patient 13	Negative	Positive	Negative	Negative	Negative	NO	NVD	–	Term
Patient 14	Negative	Positive	Negative	Negative	Positive	NO	CS	Twin	Preterm
Patient 15	Positive	Positive	Positive	Negative	Positive	YES	NVD	–	IUFD
Patient 16	Positive	Positive	Positive	Remdesivir	Positive	YES	CS	Breech	Preterm

Table 4 Outcome of neonatal

	Gender	Gestational age (weeks)	Pregnancy	Birth weight (g)	Apgar score			Complications	*SARS-CoV-2 RT-PCR
					10 min	5 min	1 min		
Patient 1	Male	40.1	Single	3850	10	10	9	Negative	Not sent
Patient 2	Female	39	Single	3000	10	10	9	*Meconium staining	Not sent
Patient 3		41	Single	3250	10	10	9	Negative	Negative
Patient 4	Female	35	Single	2700	10	10	9	Negative	Not sent
Patient 5	Female	36	Single	3030	10	10	9	Negative	Negative
Patient 6	Female	38	Single	3440	10	9	5	Negative	Positive
Patient 7	Female	38	Single	1970	10	10	9	Negative	Positive
Patient 8	Male	37	Single	2570	10	9	8	Negative	Negative
Patient 9	Female	38	Single	3020	10	10	9	Negative	Negative
Patient 10	Male	37	Single	3640	10	10	9	Negative	Not sent
Patient 11	Male	38	Single	3800	10	10	9	Negative	Not sent
Patient 12	Male	40	Single	3615	10	10	9	Negative	Negative
Patient 13	Male	41	Single	3950	10	10	9	Negative	Negative
Patient 14	Female	34	Twin	2500	10	10	9	Preterm	Negative
	Male			2700	9	8	7		Negative
Patient 15	–	22.5	Twin	–	0	0	0	IUFD	Not sent
Patient 16	Male	33	Single	2200	7	5	2	Preterm	Negative

*SARS-CoV-2 RT-PCR: Neonate nasopharyngeal swab samples for SARS-CoV-2

*Meconium Staining: Meconium stained Amniotic fluid

Table 5 SARS-CoV-2 RT-PCR of placenta

	PowerChek	Pishtaz Teb
Patient 1	E positive	Negative
Patient 2	Negative	Negative
Patient 3	Negative	Negative
Patient 4	Negative	Negative
Patient 5	Negative	Negative
Patient 6	RdRp/E positive	RdRp/N Positive
Patient 7	E Positive	RdRp/N Positive
Patient 8	Negative	Negative
Patient 9	E Positive	Negative
Patient 10	E Positive	Negative
Patient 11	E Positive	Negative
Patient 12	Negative	Negative
Patient 13	Negative	Negative
Patient 14	Negative	Negative
Patient 15	E Positive	Negative
Patient 16	RdRp/E Positive	RdRp/N Positive

a case with twin pregnancy at 22 weeks and died after one week of hospitalization in the ICU.

Results of a recent review study by Yin Ping Wong et al., showed that 9 studies had identified the SARS-CoV-2 viral RNA by RT-PCR test in the placenta, and totally 10 placenta samples were investigated in these studies. Only 2 neonate nasopharyngeal swab samples were positive for SARS-CoV-2, which is in line with our results indicating that although in 8 out of 16 cases, SARS-CoV-2 RNA was passed to the fetus, none of the infants had any signs and symptom of the virus [35]. Thus, the question is how the vertical transmission to fetus is very rare despite positive test result in the placenta sample.

Pique-Regi et al., in a study explained that transcription of ACE2 receptor and TMPRSS2 was extremely low for SARS-CoV-2 in the placenta, whereas receptor is highly expressed for other viruses resulting in pregnancy complication, such as Zika or cytomegalovirus, thus due to these differences in the level of activity of genes in the placenta, perinatal transmission of SARS-CoV-2 is very unusual [36].

Moreover, some studies have also proposed that rate of preterm delivery is increased in the patients with COVID-19 compared to the general population [37–39]. In our study, 6 out of 17 patients developed preterm labor and in 3 of them, the placenta samples were positive for SARS-CoV-2.

A strong point of our case series was that the number of the studied patients was much higher than the mentioned previous studies and also, result of testing was confirmed by two experienced people.

Two kits from two manufacturers were used to improve the quality of tests. The purpose was not to compare the two kits and use multiple genetic targets has been to identify the virus.

Nevertheless, this study had several limitations; first, tests were not performed for COVID-19 infection in all fetal samples, such as umbilical cord blood and amniotic fluid. Also, vaginal swab testing was not done in a sense of contamination for vaginally delivered placenta. Nasopharyngeal swab test did not perform for detection of SRAS-CoV-2 in all the neonates. Second, it is recommended to perform in-situ hybridization to directly visualize SARS-CoV-2 spike proteins in the syncytiotrophoblast samples as the molecular target since it retains the tissue morphology while it may be lost in other methods like PCR test. Finally, we could not rule out other reason for IUFD in one case.

In conclusion, results of the present study revealed that infection of placenta with SARS-CoV-2 may occur in pregnancy. However, whether this infection leads to neonatal infection and serious complication in pregnancy remains unclear. Thus, further studies with multiple testing methods for detection of SARS-CoV-2 are needed to confirm vertical transmission of COVID-19 to the neonates.

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Author contributions MM, NJ, AA, EZ: data collection. AA, SH: performing lab tests. MM, SD: writing the manuscript with support from ATS. MM, AA, SAM: design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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Data availability The datasets generated for this study are available on request to the corresponding author.

Declarations

Conflict of interest There are no conflicts of interest. All authors have consented to the publication of the paper "Placental Infection with SARS-CoV-2, Analysis of 16 case and literature review" in Archives of Gynecology and Obstetrics.

Informed consent All subjects were informed and consented to their enrollment. The ethics committee of Mashhad University of Medical Sciences approved the study with the approval code of IR.MUMS.REC.1399.214.

Consent for publication Not applicable.

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