

## ORIGINAL ARTICLES

**HISTOPATHOLOGICAL CHANGES IN PLACENTAL TISSUE OF COVID-19 PREGNANT WOMEN DELIVERED AT PAK EMIRATE MILITARY HOSPITAL, RAWALPINDI****Samina Waqar, Aiza Saadia, Zainab Abbas\*, Farhan Akhtar, Abeera Choudry\*, Jehan Ara Rafiq Baig\***

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**ABSTRACT****Objective:** To document the histological changes in the placentas of COVID-19 pregnant women.**Study Design:** Case series.**Place and Duration of Study:** Gynaecology department of Pak Emirate Military Hospital, from Mar 2020 to Jul 2020.**Methodology:** Nine placentas of COVID-19 positive women were included in the study. Placentas of patients with other co-morbid conditions (diabetes, hypertension, pre eclampsia, eclampsia or any infection) were excluded from the study.**Results:** The most common lesions were avascular, hyalinised, infarcted villi, perivillous and intervillous fibrin deposition, thrombosis of umbilical cord artery and congestion. Thrombosis of chorionic vessels were seen in 5 cases (Mean 0.55, 55.55%). Intervillous thrombosis was seen in 5 cases (Mean 0.55, 55.55%) and chorangiomas were seen in three cases (Mean 0.33, 33.3%). One case of acute chorioamnionitis and intervillitis (Mean 0.11, 11.11%) with neutrophilic infiltrate was present, which was confirmed by applying immunohistochemistry with Myeloperoxidase (MPO) stain.**Conclusion:** In this study, histological changes are seen in nine placentas of COVID-19 affected women, which showed evidence of thrombosis and associated changes. Although new-borns did not show any abnormality, significant histological changes observed, warrant the risk to fetuses like still birth and miscarriages. Further studies with large sample size could possibly make these consequences more clear. Also introduction of treatment like anti thrombotic therapy may be considered to overcome placental changes to avoid their harmful effects.**Keyword:** COVID-19, Placenta, Thrombosis.

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**INTRODUCTION**

The scientific name of COVID-19 is SARS (Severe Acute Respiratory Syndrome)-CoV-2 or COVID-19<sup>1</sup>. Its first outbreak in Wuhan in 2019 in China has now turned out to be worldwide pandemic<sup>2,3</sup>. On March 11, 2020, The World Health Organization acknowledged the novel corona virus (COVID-19) or SARS CoV-2-19, a single stranded RNA virus as the causative agent of this pandemic<sup>4</sup>. This virus can be isolated from different animal species. For unknown reasons, the virus can cross species barrier and cause human illness. Usually COVID-19 viruses do not cause

serious problems, but elderly people and immunocompromised population can have high mortality<sup>5</sup>. Five percent of COVID-19 patients present with critical condition requiring intensive care<sup>6</sup>. Almost 14.6 million cases by 20th July 2020 have been confirmed worldwide and 608,978 deaths have occurred. Only in Pakistan total number of cases until 20th July 2020 were 265083 and total deaths were 5599<sup>7</sup>. Some previous outbreaks of COVID viruses with SARS COVID 2 already occurred in 2002 in China and Middle East Respiratory Syndrome (MERS) COVID in Middle East in 2012. The major source of spread of this virus is thought to be respiratory droplet<sup>4</sup>. The viral load associated with this virus is found to be low and transient, suggesting doubtful transmission across the placenta. The new borns of

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COVID-19 women are usually not affected by the disease<sup>8</sup>. From the studies that have been conducted so far, it is known that corona virus results in high rates of pre-term births before 34 or 37 weeks of pregnancy. Also the incidence of pre-eclampsia and caesarean deliveries are also more common in pregnant females affected with COVID-19 than in normal population<sup>9</sup>. We have little information about the placental pathologies of females affected with COVID-19. Few studies have shown, that the placentas of patients suffering from COVID-19 display fetal vascular malperfusion (FVM), which is linked with bad perinatal outcome<sup>10,11</sup>.

Therefore it is important to establish histological changes in placenta of COVID-19 women to predict the possibilities of mortality and morbidity associated with prothrombotic phenomena.

## METHODOLOGY

The placentas of full term delivered patients were retrieved from Gynaecology department of Pak Emirate Military Hospital (PEMH), a tertiary care hospital, in 10% formalin from March, 2020 till July 2020. All those placentas delivered by COVID-19 pregnant women were included in this study. Placentas of women with comorbidities like diabetes mellitus, hypertension, pre-eclampsia, eclampsia or any infection were excluded from this study. Nine control cases of placentas delivered by healthy females were also included. Ethical review board (ERC/ID/37) approval and written consent were obtained and confidentiality was kept by maintaining secrecy. Coding was not done as the sample size was small. Information about the patient was also retrieved from medical records. Due to infectious nature of the specimen fixation for 48 hours was done. The patients and the new-borns were tested for COVID-19 with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) on nasopharyngeal (NP) swabs. Representative sections of placenta (two sections from the chorionic side, two sections from maternal side, two through and through section of placentas and two sections of umbilical cord were taken)<sup>12</sup>. Sections were placed in cassettes and

processed in automatic tissue processor. After fixation and processing the blocks were cut with microtome set at 5µm thickness. Hematoxylin (Merk) and Eosin (Sharlan) staining were done. Immunohistochemistry with Myeloperoxidase (MPO) by Dako was done on paraffin embedded sections by automatic immunostainer (Leica-Bond Max). Histopathological examination of the slides were done by two histopathology consultants independently in the department. All histopathological parameters were according to Amsterdam criteria<sup>12</sup>.

## RESULTS

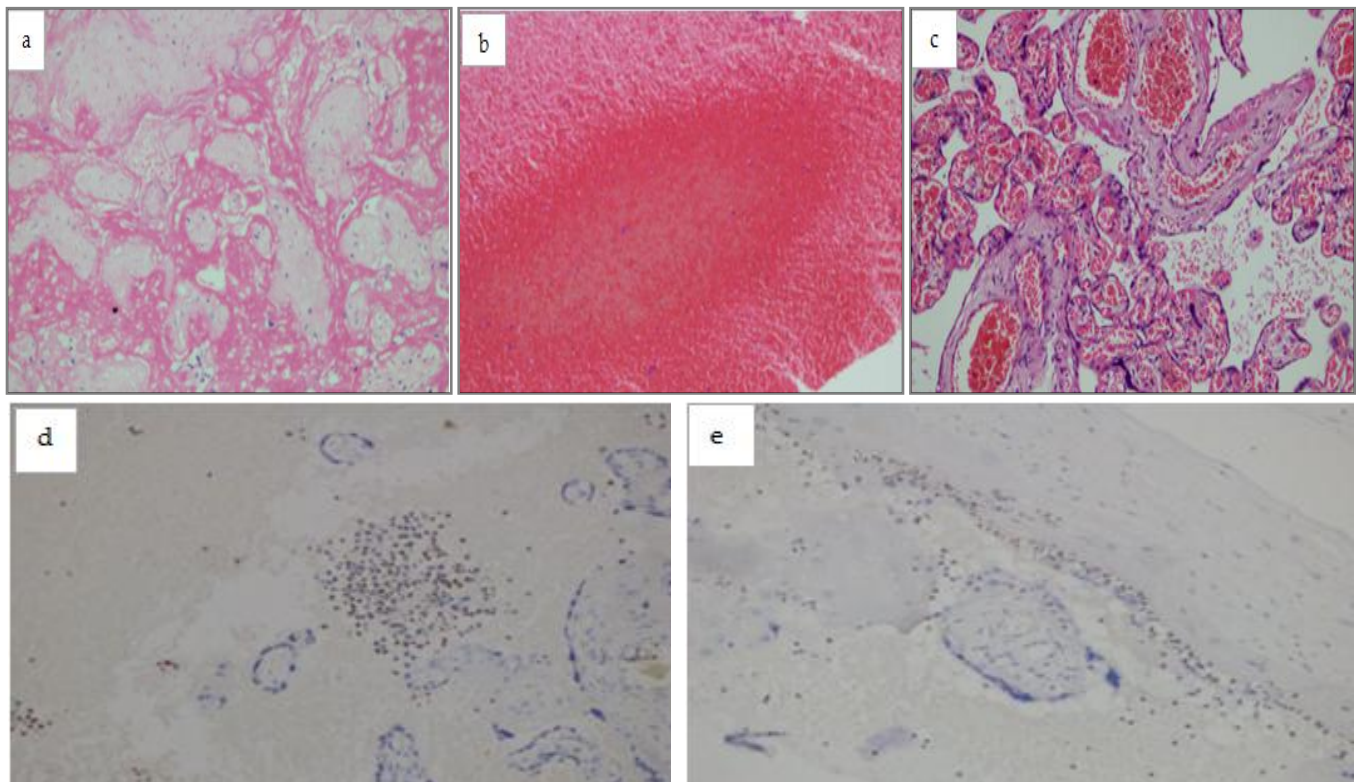
Between the periods of 20th March 2020 to 20th July 2020, nine COVID-19 pregnant women gave birth to new-borns at Pak Emirates Military Hospital, Rawalpindi. These pregnant patients were confirmed as COVID-19 positive by RT-PCR from NP swabs. The mean age of the pregnant women was 25.22 years  $\pm$  SD 1.99) and mean gestational age of new-borns was 37.78 weeks  $\pm$  SD 0.83). Four patients were delivered by caesarean sections (c-section) while five had spontaneous vaginal deliveries. The placentas of all the patients were of normal size. Patients were found to be COVID-19 positive at the time of delivery. RT-PCR from the placental tissue was not done in our patients. Eight patients were asymptomatic whereas one patient presented with cough. All investigations were within normal limits, including Haemoglobin level, platelet count, lactate dehydrogenase, C reactive proteins and X-ray chest. Serum fibrinogen level and serum ferritin of only one patient were done and were found to be normal. There was no history of diabetes, hypertension, pre-eclampsia or eclampsia in any patient in both control and experimental groups. Mean Apgar score of the new-borns at birth was 8/10 (9/9) and at five minutes 10/10 (9/9). Mean birth weight of new-borns was 2.86kg  $\pm$  SD 0.07). All of them were discharged at 2nd or 3rd day of delivery. RT-PCR of NP and throat swabs (TS) were negative in all the new-borns and Immunoglobulin, including IgG and IgM were also negative, revealing no vertical transmission of the disease.

On microscopic examination of placenta, the most common lesions were avascular, hyalinised, infarcted villi (fig-1a), perivillous and intervillous fibrin deposition, and congestion, which were seen in all the cases (100%). Thrombosis of umbilical cord artery (fig-1b) was present in 7 cases (Mean 0.77, 77.77%). In control group, congestion and perivillous and intervillous fibrin deposition were present in all the cases (100%), while avascular hyalinised villi were seen in two cases only

intervillositis (Mean 0.11, 11.11%) with neutrophilic infiltrate was present, which was confirmed by applying immunohistochemistry with Myeloperoxidase (MPO) (fig-1 d,e).

## DISCUSSION

Literature on placental changes in COVID-19 pregnant women is limited. In a study by Mascio *et al*, it was revealed that COVID-19 infection in pregnant women is associated with higher rates



**Figure-1:** Photomicrograph showing a) Avascular, Infarcted villi (H&E x 20). b). Thrombus in umbilical cord (H&E x 10), c). Chorangiomas (H&E x 20), d). Expression of MPO, granular positivity in neutrophils in intervillous spaces x 20. e). Expression of MPO, granular positivity in neutrophils in chorionic plate x 20).

(Mean 0.22, 22.22%). Thrombosis of umbilical cord artery was not seen in any of the control group. In experimental group, thrombosis of chorionic vessels were seen in 5 cases (Mean 0.55, 55.55%) out of 9, whereas it was not seen among control cases. Intervillous thrombosis was seen in 5 cases (Mean 0.55, 55.55%) and chorangiomas (fig-1c) was seen in three cases (Mean 0.33, 33.33%). These findings were also not seen in control group (0%). One case of acute chorioamnionitis and

of preterm births, pre eclampsia, caesarean and perinatal deaths<sup>9</sup>. In our study none of these findings were present except increased rate of caesarean section (44.44%).

Our findings are somewhat similar to that of Mulvey *et al*<sup>13</sup>. They examined 5 placentas of COVID-19 patients. They found 3 cases of thrombosis in chorionic vessels and 2 cases of thrombosis in stem villi. Two cases revealed avascular villi and one case showed villous stromal and



vascular karyorrhexis. They also found complement deposition (C3d, C4d, C5b-9) in villous and perivillous areas and decidua similar to normal placenta controls.

In a study of 20 cases conducted by Rebecca *et al*, fetal vascular malperfusion was found, according to Amsterdam criteria. In most cases there was intra mural fibrin deposition. In 2 cases there was villous stromal and vascular karyo-hexis, while the remaining cases showed multiple lesions like non occlusive thrombi. Five cases of perivillous fibrin deposition were also seen. One case of chorioamnionitis and fucinitis was there. Four cases showed chronic villitis<sup>14</sup>. Similar findings were also seen in our study.

David Baud *et al*, worked on a single case of second trimester miscarriage and found mixed inflammatory infiltrate in subchorionic space comprising neutrophils and monocytes with increased intervillous fibrin deposition<sup>15</sup>. In present study, in one case, neutrophils were seen in intervillous spaces and subchorionic space which was confirmed by immunostain, MPO.

Luisa Pantane *et al* worked on 22 placentas delivered by COVID-19 mothers and found two placentas showing chronic intervillitis with the presence of macrophages in the intervillous spaces which were anti CD 68 positive<sup>16</sup>. While in this study, the changes were of acute intervillitis and acute sub chorionitis in one case.

Chen *et al*, conducted a study on 3 placentas and found perivillous and intervillous fibrin deposition and villous infarct similar to our study. There was no case of villitis or chorioamnionitis but in our study, 1 out of 9 cases showed intervillitis and chorioamnionitis<sup>17</sup>. In a case report published by Kuhrt and McMicking revealed accelerated villous maturation without any evidence of inflammation, whereas in our study one case showed inflammation<sup>18</sup>. Chen *et al*, reported one new born positive by quantitative RT-PCR, delivered by COVID-19 mother<sup>19</sup>. In our study, no new born was found positive with the same test.

This study has shown thrombosis in the arteries resulting in avascular, infarcted villi, which were not seen in normal controls. These histological changes may be part of the generalized thrombotic activity associated with SARS-Cov-2-19 infection, and associated with subsequent fetal mortality and morbidity. Though in our study all the new-borns had uneventful outcome, the study probes into the fact that placental changes were part of systemic prothrombotic activity of SARS Cov-2-19 infection, which causes pathologies in multiple organs, including placentas.

## CONCLUSION

In this study, COVID-19 pregnant patients were detected just prior to their deliveries. Had they contacted the disease earlier in their pregnancies, the chances of developing miscarriages and stillbirths could not be excluded. Therefore we would suggest perinatal counselling of the patients along with early anti thrombotic treatment in the course of pregnancy to prevent the possibility of fetal miscarriages and still birth in COVID pregnant women. The histopathological changes established in this study could be further authenticated by increasing sample size in upcoming studies.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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