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Research Article

Comparison of APOBEC3 C levels in pregnant women infected with hMPV

Zainab Mohammed Madfoon[1], Musa Nima Mezher[2]

College of Science Biology Department, University of Kufa, Iraq
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I N F O

Corresponding Author:
Zainab Mohammed Madfoon College of Science Biology Department, University of Kufa, Iraq
E-mail Id:
zainabm.algeubori@uokufa.edu.iq
Orcid Id:
https://orcid.org/0000-0003-2246-2903
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A B S T R A C T

Introduction: Human metapneumovirus (hMPV) is a life-threatening respiratory disease with a high risk in pregnancy. Our study aimed to ascertain the APOBEC3C protein levels and IgM antibody levels in hMPV-positive pregnant women and relate them to obstetric and demographic variables. Methods: We tested 250 pregnant women's samples with symptoms of a cold, and 36 of them were found to be positive for the virus infection. We contrasted these women with 36 normal pregnant women. Clinical and laboratory findings were compared based on age, residence, second pregnancy trimester, and history of miscarriage.

Result: The findings were higher in both IgM and APOBEC3C in hMPV-infected women than in uninfected women ($p < 0.001$). High APOBEC3C expression was most prominent in the second trimester and in rural-dwelling women, with significant correlation with a history of miscarriage.

Conclusion: These findings suggest the APOBEC3C gene as a potential biomarker for predicting complicated outcomes of pregnancy following respiratory viral infections. Further studies are recommended to explore the mechanistic role of APOBEC3C in maternal immunity and pregnancy complications.

Keywords: Human metapneumovirus (hMPV), Respiratory viral infection, Obstetric outcomes, APOBEC3C

Introduction

The human metapneumovirus (hMPV) is an enveloped RNA virus belonging to the Pneumoviridae family. The virus exhibits a global distribution, with infections occurring in both developed and developing countries, and shows a seasonal pattern, peaking during winter and spring months in temperate regions.[1]

Transmission of hMPV occurs primarily through respiratory droplets expelled when an infected person coughs or sneezes, as well as through direct contact with contaminated surfaces.[2] The clinical manifestations of hMPV infection range from mild upper respiratory symptoms, such as the common cold, to severe lower respiratory tract diseases, including bronchiolitis and pneumonia.[3]

Pregnant women represent a uniquely vulnerable population due to the physiological and immunological changes that occur during pregnancy. These changes, which include a shift toward T-helper 2 (Th2) immune responses and increased regulatory T cell (Treg) activity, are essential for maintaining foetal tolerance but may also impair the mother's ability to mount effective antiviral immune responses.[4]

Human APOBEC3 (apolipoprotein B mRNA-editing catalytic polypeptide-like 3) enzymes are capable of inhibiting a wide range of endogenous and exogenous viruses using deaminase and deaminase-independent mechanisms.[5]

During pregnancy, studies suggest that the expression of this protein may be influenced by the immunological changes of pregnancy, including suppressing certain innate immune mechanisms to protect the foetus.[6]

This study aimed to evaluate APOBEC3C protein levels in pregnant women infected with human metapneumovirusand determine their association with IgM antibody levels, demographic characteristics, and obstetric history, in order to evaluate their potential role as biomarkers for predicting pregnancy complications.

Materials and method

Our study included 250 pregnant females with respiratory symptoms at private maternity centres in Najaf Governorate and at Al-Zahraa Teaching Hospital and Al-Furat Al-Awsat Hospital. Out of the sample size, 36 samples tested positive for human metapneumovirus (HMPV) by serological screening. Thirty-six healthy pregnant females of comparable age were taken as controls, age range of participants was 16–40 years

Exclusion criteria were

- Coexisting Chronic Systemic Diseases (such as Diabetes Mellitus, Autoimmune Diseases, Chronic Respiratory Diseases)
- Known immunodeficiency and the use of immunosuppressive therapy
- Co-infection with other viruses and/or bacteria that infect the respiratory tract
- Pregnancy-related complications that are not infections (like preeclampsia, gestational diabetes) Inability or refusal to grant informed consent.

Ethical Approval: The research methodology has been scrutinized and approved by the Ethics Committee of the College of Science, University of Kufa, Najaf, Iraq, with the Approval No. [428/2024].

Patients were classified according to age: <20 years, 20-30 years, and 31-40 years.

Location of pregnancy (urban, rural)

Trimester (third, second, first)

Date of miscarriage (The interview definition of a history of miscarriage was having one or more pregnancy losses before 20 weeks of gestation. This data was initially gathered through structured patient interviews and later validated using existing medical records whenever possible. The date of miscarriage was determined from hospital and antenatal care program records.)

Laboratory Tests

Detection of IgM antibody to hMPV was done by using enzyme-linked immunosorbent assay (ELISA) kits as per the manufacturer's manual. China , SUNLONG

Levels of APOBEC3C protein in serum samples were assessed by using a commercially available APOBEC3C-specific ELISA kit. China , BT

Statistical Analysis

Statistical tests were conducted using the GraphPad Prism® computer software package version 9.3.1. Normality was checked against data distribution with the Shapiro Wilk normality test, while equality of variances was determined by Levene's test.

Comparisons between groups were performed by independent samples t-tests for normally distributed variables. Logistic regression analysis was used to test the relationship between APOBEC3C expression, the presence of hMPV IgM antibodies, and certain clinical variables.

The effect sizes were determined using Cohen's d value to establish how big the differences are between the groups. Although multiple comparisons were few and guided by hypotheses, a correction test was not necessary. The level of statistical significance used was a p-value of <0.05.

Results

Patient's Distribution

We randomly collected approximately 250 samples of pregnant women with cold symptoms. Upon diagnosis, we found 36 samples positive for human metapneumovirus (HMPV). We also identified 36 controls in pregnant women who were negative for the virus. We classified the positive samples into three age groups (under 20 years, 20-30 years, and 31-40 years) and residential areas (urban and rural). The positive samples were also separated according to obstetric history into the first, second, and third trimesters of pregnancyand presence or absence of miscarriage in the patient's history, the control group was divided likewise as the patient group, as seen in Table 1 and figure 1:

Detection of hMPV IgM antibody in pregnant patients according to demographic characteristics and obstetric history

Our results demonstrated highly statistically significant differences in the concentrations of IgM ($p < 0.001$) across demographic characteristics. According to age groups, the

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highest concentrations of IgM (3.13 ± 0.64 pg/ml) were observed in the 30-40 age group, with an odds ratio of 48.7 (95% confidence interval: 16.4–144.5), indicating a high association of age with elevated concentrations of IgM. While rural women also possessed significantly higher IgM levels (3.24 ± 0.83 pg/ml) compared to urban women (2.82 ± 0.89 pg/ml), with a large effect size and adjusted odds ratio (AOR) of 42.1 (95% CI: 26.3–67.5). Results further revealed a consistent positive increase in the levels of IgM during pregnancy periods, which was (3.07 ± 0.78 pg/ml) in the third trimester, with an odds ratio of 41.3 (95% confidence interval: 14.7–116.2). Miscarriage history was also associated with higher levels of IgM (3.15 ± 0.83 pg/ml) and higher odds (OR = 52.3, 95% CI: 12.8–213.5). Women who delivered preterm also had higher levels of IgM (3.07 ± 0.81 pg/ml) compared to term-delivering women (2.87 ± 0.89 pg/ml), with an adjusted odd ratio (OR) of 38.9 (95% CI: 18.6–81.4).

The effect sizes (3.8 to 5.4 Cohen's d) indicated substantial contrasts between controls and patients. These findings suggest a large immune response in pregnant women, particularly those at higher clinical risk, as demonstrated with logistic regression and effect sizes that are shown in Tables 2 and 3

The results indicated that the concentration of APOBEC C3 protein in subjects younger than 20 years was 19.76 ± 4.66 pg/ml, compared to 4.30 ± 1.81 pg/ml for controls. The very large difference (Cohen's d = 4.12, $p < 0.001$) with an odds ratio (OR) of 32.7 indicates that infection significantly increases APOBEC C3 levels in this group and may be associated with increased immune sensitivity at an early age.

Infected females between the ages of 20-30 recorded the highest value, 27.06 ± 2.39, compared to 5.88 ± 2.36 among the controls. The significant difference (OR = 8.86, OR = 148.5) showed peak immune activity at this age. In the age group of 30-40 years, the rates were 18.61 ± 2.12 in infected women versus 5.79 ± 1.05 in the control group (d = 7.89, OR = 105.3), and hence, the profound effect was observed even at older ages. The other results showed the first pregnancy rate to be 22.82 ± 1.72 in infected females versus 4.80 ± 1.93 for controls (d = 9.87, OR = 420.5), and this represented an active early immune response. The second pregnancy rate was the highest, 26.73 ± 2.82 versus 5.46 ± 1.45 (d = 10.12, OR = 582.1), which represented the period when the most active APOBEC C3 stimulation takes place. Third, the rates were relatively low but elevated, 16.68 ± 0.75 versus 6.52 ± 2.01 (d = 6.92, p = 112.8), possibly due to immunological adaptations close to delivery. For the rural locations, the incidence rate in infected women was 26.73 ± 2.44 versus 4.74 ± 1.23 in controls (d = 11.24, p = 896.3), which suggests an environmental factor or greater exposure to infectious organisms in the rural locations. In urban populations, the prevalence of infected women was more at 18.36 ± 2.22 versus 5.54 ± 2.03 (d = 6.15, p = 142.7), although the difference was still significant. In pregnancy loss history, APOBEC C3 risk was 22.62 ± 3.11 versus 5.12 ± 1.84 (d = 6.87, OR = 315.2), indicating that APOBEC C3 could play a part in miscarriage as a result of chronic inflammation or infection. As is evident in Tables 4 and 5 , figures 4 and 5:

Table 1.Sample Distribution

Category		Subdivisions	hMPV+ (n=36)	%	Control (n=36)	%
Demographic characteristics	Age Group	<20 years	10	27.7%	13	36.1%
		20–30 years	17	47%	11	30.6%
		31–40 years	9	25%	12	33,3%
	Residence	Rural	12	33%	18	50%
		Urban	24	66.6%	18	50%
obstetric history	Pregnancy Trimester	First trimester	10	27.7%	11	30.6%
		Second trimester	12	33%	13	36.1%
		Third trimester	14	38.8%	12	33,3%
	Pregnant loss history	Yes	13	36%	14	38.9%
		No	23	63.8%	22	61.1%

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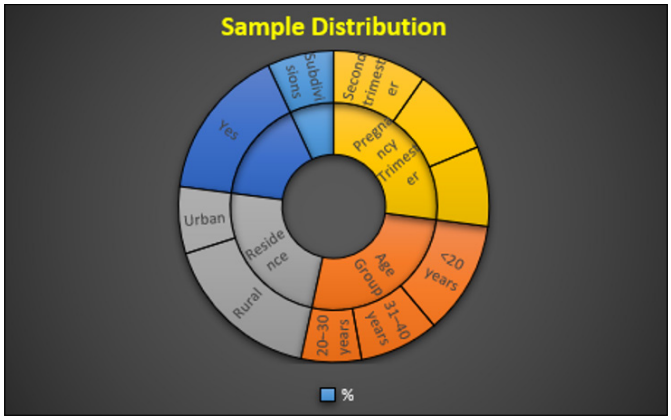

Figure 1.sample distribution

Table 2.Comparative of IgM pg/ ml Levels Between pregnant women Patients and Controls According to Demographic characteristics

Category	Subgroup	Patients (n=36) Mean ± SD IgM pg/ ml Levels	n	Controls (n=36) Mean ± SD IgM pg/ ml Levels	n	Effect Size (Cohen's d)	p-value	Logistic Regression Results OR (95% CI)
Age Group	<20 years	2.14 ± 0.71	10	0.05 ± 0.02	13	4.1	<0.001	36.2 (12.8–102.1)
	20-30 years	2.96 ± 0.82	17	0.04 ± 0.01	11	4.6	<0.001	42.5 (15.3–118.0)
	30-40 years	3.13 ± 0.64	9	0.06 ± 0.03	12	5.2	<0.001	48.7(16.4–144.5)
SUM			36		36	*Adjusted OR = 39.8 (95% CI: 25.6–61.9)		
Residence Area	Rural	3.24 ± 0.83	12	0.04 ± 0.01	18	5.42	<0.001	48.6 (18.2-129.8)
	Urban	2.82 ± 0.89	24	0.07 ± 0.03	18	4.15	<0.001	39.4 (15.7-98.9)
SUM			36		36	*Adjusted OR=42.1 (95%CI:26.3-67.5)		

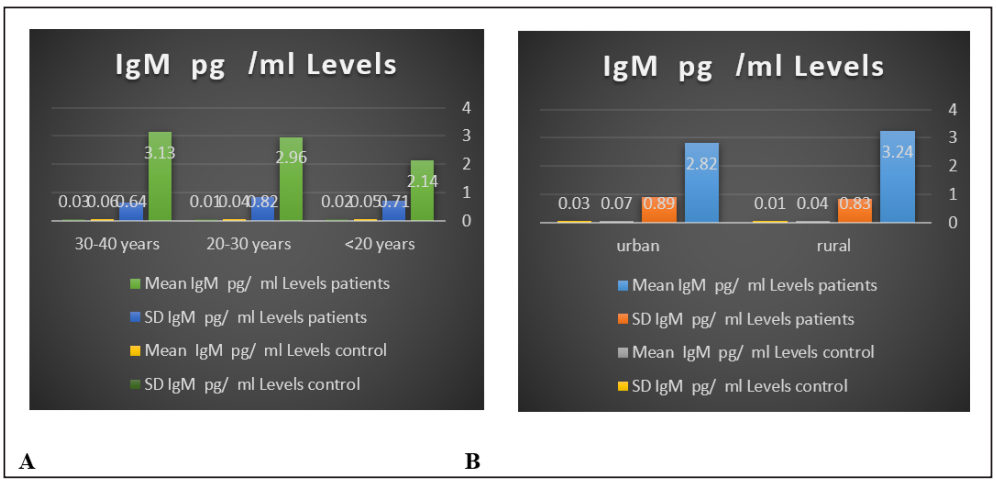

Figure 2.Comparative of IgM pg/ ml Levels Between pregnant women Patients and Controls According to Demographic characteristics (A: age groups : B: Residence Area)

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Table 3.Comparative of IgM pg/ ml Levels Between pregnant women Patients and Controls According to obstetric history

Category	Subgroup	Patients (n=36) Mean ± SD IgM pg/ ml Levels	n	Controls (n=36) Mean ± SD IgM pg/ ml Levels	n	Effect Size (Cohen's d)	p-value	Logistic Regression Results OR (95% CI)
Trimester	First Trimester	2.17 ± 0.71	10	0.05 ± 0.03	11	4.02	< 0.001	35.8(12.1-105.9)
	Second Trimester	2.99 ± 0.84	12	0.04 ± 0.02	13	4.87	< 0.001	44.2(15.8-123.6)
	Third Trimester	3.07 ± 0.78	14	0.06 ± 0.03	12	4.65	< 0.001	41.3(14.7-116.2)
SUM						*AdjustedOR=38.2(95%CI:24.5.3)		
Pregnancy Loss History	With History	3.15 ± 0.83*	13	0.06 ± 0.04	14	5.07	<0.001	52.3(12.8–213.5)
	Without History	2.94 ± 0.89	23	0.07 ± 0.04	22	3.91	<0.001	44.1 (10.2–190.1)
SUM			36		36	*Adjusted OR=47.2 (20.3–109.8)		
	Odds Ratio – OR , Confidence Interval – CI							

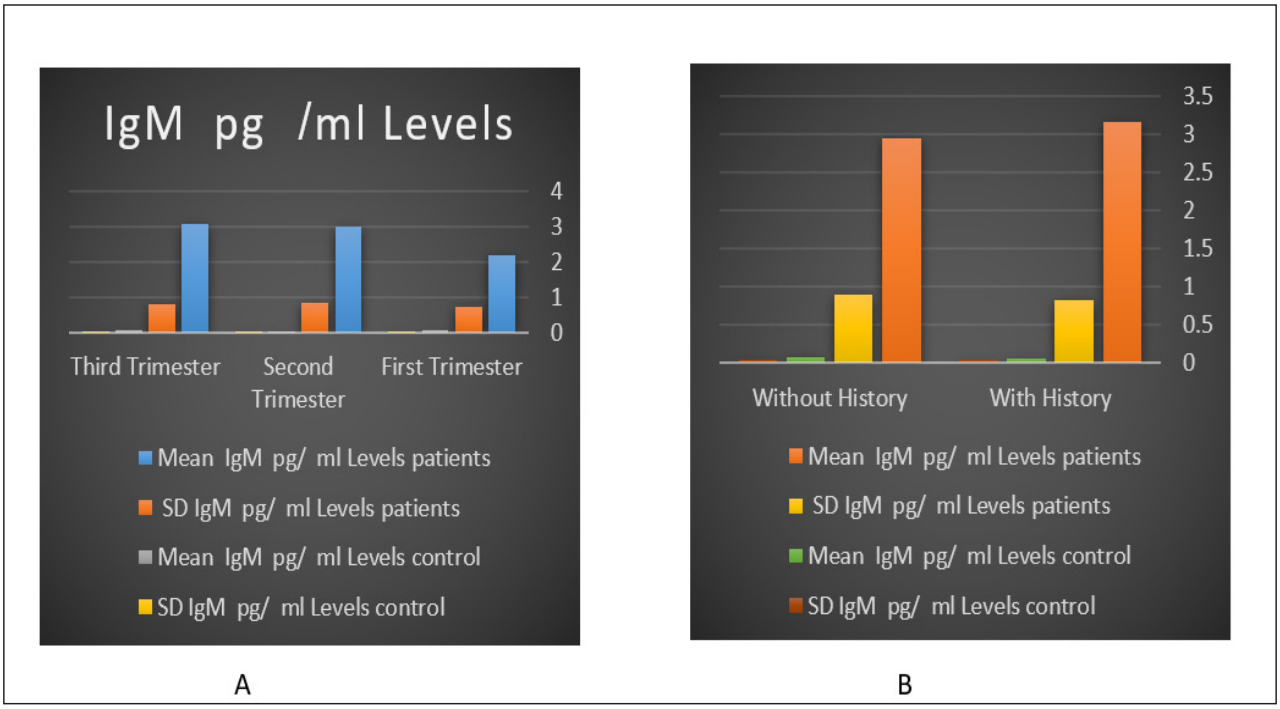

Figure 3.Comparative of IgM pg/ ml Levels Between pregnant women Patients and Controls According to obstetric history (A ; Trimester ; B: Pregnancy Loss History)

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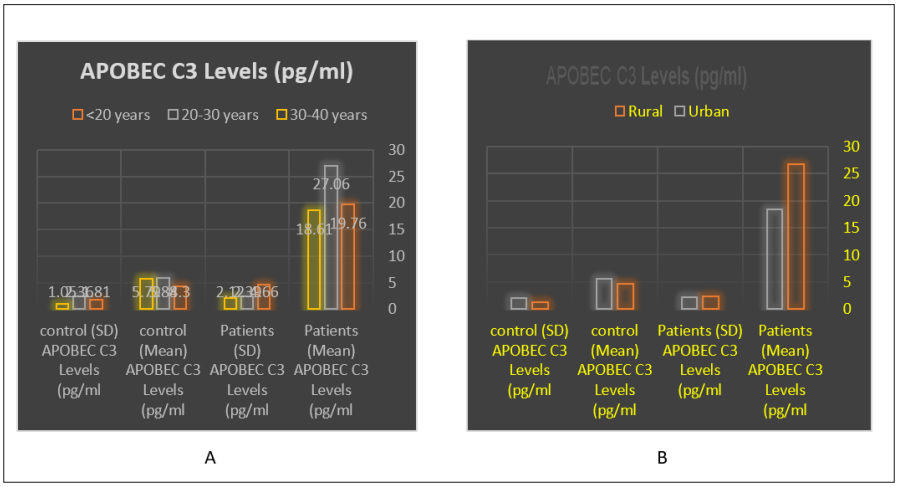

Figure 4.Comparison APOBEC3 C of Hmpv patient according to Demographic characteristics (A; age groups B; Residence Area)

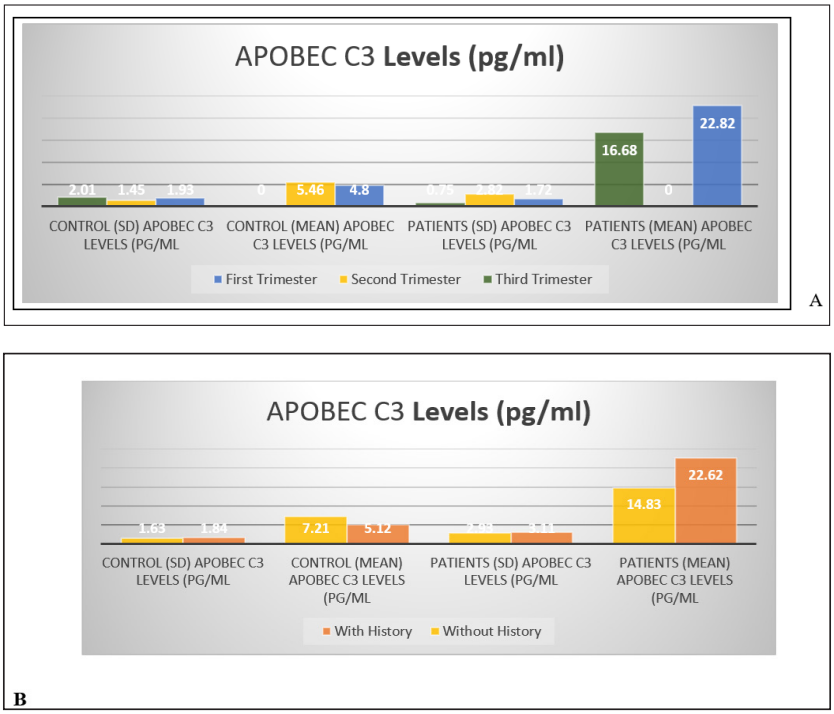

Figure 5.Comparative of IgM pg/ ml Levels Between pregnant women Patients and Controls According to obstetric history (A ; Trimester ; B: Pregnancy Loss History)

Table 4.Comparison APOBEC3 C of Hmpv patient according to Demographic characteristics

Category	Subgroup	Patients (Mean ± SD) APOBEC C3 Levels (pg/ml	n	(Mean ± SD) APOBEC C3 Levels (pg/ml control	n	Effect Size (Cohen's d)	p-value	Logistic Regression Results OR (95% CI)
Age Group	<20 years	19.76 ± 4.66	10	4.30 ± 1.81	13	4.12	<0.001*	32.7 (12.4-86.2)
	20-30 years	27.06 ± 2.39	17	5.88 ± 2.36	11	8.86	<0.001*	148.5 (45.3-486.9)
	30-40 years	18.61 ± 2.12	9	5.79 ± 1.05	12	7.89	<0.001*	105.3 (28.1-394.8)

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SUM			36		36	Adjusted OR=78.4 (35.2-174.6)		
Residence Area	Rural	26.73 ± 2.44	12	4.74 ± 1.23	18	11.24	<0.001	896.3(210.5-3815.7)
	Urban	18.36 ± 2.22	24	5.54 ± 2.03	18	6.15	<0.001	142.7 (52.3-389.4)
SUM			36		36	AdjustedOR= 318.4(698.3_145.2)		
Total	72					Odds Ratio – OR , Confidence Interval – CI		

Table 5.Compersion APOBEC3 C of Hmpv patient according to obstetric history

Category	Subgroup	Patients (Mean ± SD) APOBEC C3 Levels (pg/ml	n	(Mean ± SD) APOBEC C3 Levels (pg/ml control	n	Effect Size (Cohen's d)	p-value	Logistic Regression Results OR (95% CI)
Trimester	First Trimester	22.82 ± 1.72	10	4.80 ± 1.93	11	9.87	<0.001	420.5 (98.3-1798.2)
	Second Trimester	26.7 3± 2.82	12	5.46 ± 1.45	13	10.12	<0.001	582.1(135.6-2498.7)
	Third Trimester	16.68 ± 0.75	14	6.52 ± 2.01	12	6.92	<0.001	112.8 (34.7-366.4)
SUM						Adjusted OR=325.6 (142.1-745.9)		
Pregnancy Loss History	With History	22.62 ± 3.11	13	5.12± 1.84	14	6.87	<0.001	315.2 (78.4-1267.8)
	Without History	14.83 ± 2.93	23	7.21 ± 1.63	22	3.12	<0.001	42.7 (15.3-119.2)
						Adjust OR=148.6 (65.3_338.1)		
SUM			36		36			
Total	72					Odds Ratio – OR , Confidence Interval – CI		

Discussion

The results of our study are consistent with[7,8] stated that hormonal change in pregnant women exposes them to viral infections, subjecting them to more severe complications, such as death, caused by COVID-19. Elevated IgM levels in SARS-CoV-2-infected pregnant women reflect elevated immune activation. This concurs with our finding, where increased IgM is a marker of active viral infection irrespective of virus type.[9] Also presumed that overreaction of immunity to viruses during pregnancy is a trend. This may be due to inflammation with age, which may be a reason for increased levels of IgM as a marker of an out-of-balance immune system.

Our study showed a consistent and significant increase in the amount of IgM among pregnant women infected with hMPV, particularly in the third trimester. This is as noted by Lane [10] in terms of the capacity of the virus to elicit innate immunity, particularly where there is no adaptive immunity, and this is the condition most prevalent in pregnancy. This leads to a sustained increase in the level of IgM due to continuous antigenic stimulation, such that there is late immune switching from IgM to IgG. Such results also agree with findings by[10,11] where it was determined that the immune response to viral infection during pregnancy will most likely induce IgM and IgG antibodies.

They also agree with studies by[12] , which revealed that women are more susceptible to respiratory viruses during

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the second half of pregnancy. This is also confirmed by our research, where a rise in the third trimester was confirmed in IgM levels. In terms of residential area[13] stated that in rural areas, there is poor awareness about healthcare and health facilities, so there is less immune response to viruses. Our results are opposite to this since there was a higher level of IgM in rural pregnant women than in urban pregnant women. This can be due to frequent exposure to infection without taking the patient to healthcare.[14] Clarified that the use of oil heaters during winters is responsible for respiratory infections in urban and rural societies, while [15] show that having infected children in nurseries is among the reasons responsible for the widespread incidence of respiratory viral infections in cities.

But our results oppose those of[16], who had explained the increase in severity of respiratory infections in cities as a result of industrial emissions and automobile exhaust, and assumed the countryside is cleaner.

Concerning pregnancy and pregnancy outcome, the current research concurs with[17,18], that viral infections adversely affect placental function and lead to complications.[19,20], Also has the importance of the discovery of IgM as a marker for recent infection and the association with pregnancy complications. [21, 22] Established by research that respiratory viruses cause severe infection leading to miscarriage or preterm birth, though in hMPV, this has not been directly established.

Our findings coincide with[23] whose work demonstrated that premature babies are influenced by respiratory viral infections to a greater degree. Furthermore, high IgM levels, even among full-term babies, suggest the involvement of other immune factors.[24] had given an alignment of preterm delivery with an increased inflammatory reaction, also evident in our findings. Finally, information from a study by[25] in Iraq reaffirmed the information of immunological alterations occurring among pregnant women with infection, like increased levels of antibodies, supporting the claim that increased IgM can act as a marker of ongoing exposure to the immune system during pregnancy and delivery.

The study shows an elevated level of APOBEC3C among pregnant women with hMPV infection, namely in the second trimester, possibly reflecting maximal innate immune activity in a balance between protecting the foetus and infection.[26] Placental blood supply, immune cell action, and gene activation through the release of interferon could be associated with this increase. Comparison between rural and urban populations was also found to be variable, for which reasons such as timing of sampling, severity of illness, lack of preventive care, and psychosocial factors influencing immune gene regulation are responsible.[27] The results show that APOBEC3C may be used as a biomarker to determine the risk of miscarriage or birth defects when there is respiratory viral infection during pregnancy, but further studies would be necessary to control other immune and genetic factors. The difference between preterm and term deliveries also suggests other factors linking immunity and delivery timing to the presence of viral infection.

Elevated APOBEC3C levels based on age point to induction as part of an immune reaction to infection, perhaps through immune signal transduction.[28] Immune alteration during pregnancy, cytokine dysregulation, and increased tolerance might explain APOBEC3C upregulation as protection or an anti-inflammatory response, as seen in APOBEC3G expression in immune cells.[29]

This accords with studies on APOBEC3 family function in immunity and antibody mutation[30] where APOBEC3C can regulate R-loop structure as in cancer cells[31] to inactivate RNA viruses.

Its second-trimester peak can be caused by interferon-inducible gene expression[32] (Uriu et al., 2023) by TLR3/RIG-I pathways.[33] Geographic variation, e.g., increased innate immunity in the countryside[34], could be the cause of the high titers. APOBEC3C is RNA virus-specific against influenza and RSV and has antiviral activity against RNA viruses in general.[35] High APOBEC3C in hMPV infection in both preterm and term delivery indicates overexpression of immunity during pregnancy that could also disrupt the immune balance required for stable gestation and delivery.[36,37]

Conclusion

The study demonstrated impressively higher APOBEC3C protein concentrations and IgM antibodies among HPV-infected pregnant women compared to healthy women. The study shows a strong association of APOBEC3C gene expression with obstetric and demographic characteristics, namely the second trimester of pregnancy, rural residence, miscarriage history, and preterm labour. These findings suggest that APOBEC3C will be an important aspect of maternal immune responses to viral infection and a promising prognostic biomarker for pregnancy complications. Further studies are needed to elucidate the molecular mechanisms through which overexpression of APOBEC3C is linked to viral illness and complications of pregnancy and to establish its value in guiding preventive and therapeutic strategies in susceptible pregnant women.

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