Title Page

Title: Sero-prevalence of SARS-CoV-2 Antibodies among First Trimester Pregnant Women during the Second Wave of Pandemic in India'

Authors:

1. Dr Aparna Sharma (Corresponding Author)

Additional Professor

Department of Obstetrics and Gynecology

All India Institute of Medical Sciences, Ansari Nagar, 110029

Delhi, India

kaparnasharma@gmail.com

2. Dr Nilanchali Singh

Assitant Professor

Department of Obstetrics and Gynecology

All India Institute of Medical Sciences

Delhi, India

3. Dr Sara Hillman

Consultant

Department of Obstetrics and Gynecology

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University College of London

United Kingdom

4. Dr Purva Mathur

Professor

Department of Microbiology, Trauma Center

All India Institute of Medical Sciences

Delhi, India

5. Dr Kapil Yadav

Additional Professor

Department of Community Medicine

All India Institute of Medical Sciences

Delhi, India

6. Dr Anapti Garg

Senior Resident

Department of Obstetrics and Gynecology

All India Institute of Medical Sciences

Delhi, India

7. Dr Vatsla Dadhwal

Professor

Department of Obstetrics and Gynecology

All India Institute of Medical Sciences

Delhi, India

8. Dr Neerja Bhatla

Professor & HOD

Department of Obstetrics and Gynecology

All India Institute of Medical Sciences

Delhi, India

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Synopsis: First trimester sero-molecular screening suggests high prevalence of COVID antibodies in the study population of asymptomatic pregnant women in first trimester, during COVID wave.

Short Title: Sero-prevalence of SARS-CoV-2 Antibodies in Pregnancy

Keywords: Sero-prevalence, SARS-CoV-2, First Trimester, Pregnancy, Pandemic, COVID, Wave

Abstract:

Objective: Data on the immune response to SARS-CoV-2 during pregnancy are lacking and the potential role and effect of SARS-CoV-2 vaccination in pregnancy is yet to be completely investigated. **Method:** This is a cross-sectional observational study wherein, pregnant women were tested for SARS-CoV-2 immunoglobulin M and immunoglobulin G levels, irrespective of their infective status or presence or symptomatology. **Result:** Of the 220 pregnant women tested**,** 160 (72.7%) were SARS-CoV-2 IgG positive, 37 (16.8%) were SARS-CoV-2 IgM positive and 27 (16.9%) were both IgG and IgM positive. The average antibody titre found was 10.49 BAU/ml (±14.0) and 0.6 (±0.55) for anti-SARS-CoV-2 IgG and IgM non neutralizing antibodies respectively. ROC analysis for SARS-CoV-2 IgG positivity showed a cut-off value of 1.19 with a sensitivity of 99.3% (0.99 AUC, 95% CI) and specificity of 98.3% (0.99 AUC, 95% CI) respectively. Similarly for SARS-CoV-2 IgM positivity showed a cut-off value of 1 with a sensitivity of 97.3% (0.99 AUC, 95% CI) and specificity of 98.9% (0.99 AUC, 95% CI) respectively. **Conclusion:** First trimester sero-molecular screening suggests high prevalence of COVID antibodies in the study population of pregnant women in first trimester, without the patients being symptomatic.

Manuscript:

Introduction

The World Health Organization was informed of a cluster of pneumonia cases of unknown origin in Wuhan City, China in December 2019. Since then, and as of 26th September, 2021, about 33.6 million cases of COVID-19 with 4.5 lakhs deaths have been reported in India, and Delhi recorded 1.4 million cases and about 26,000

deaths [1]. All age groups are susceptible to COVID-19 infection, however, impact in pregnant women has drawn much attention because of the unique immunological state of pregnancy and the increased risk of respiratory infections [2,3].

Recent data from the United Kingdom has confirmed that pregnant women are at more risk of severe illness from SARS-CoV-2 infection, compared with non-pregnant women. Furthermore, infection is associated with increased risk of stillbirth, growth restriction and preterm birth. [4]

Data on the immune response to SARS-CoV-2 during pregnancy are lacking and the potential role and effect of SARS-CoV-2 vaccination in pregnancy is yet to be completely investigated [5]. The Indian Council of Medical Research has validated and approved IgG kits for SARS-CoV-2 to be used to conduct serosurveys in India [6]. Reports of cases of SARS-CoV-2 infection in pregnancy have been documented but are concentrated mainly in the second and third trimester of pregnancy [7-10]. However, viral infections can be harmful to the foetus during the first trimester of pregnancy as well; and whether, SARS-CoV-2 is one of these serious infections is creating concerns for obstetricians [11-13] and pregnant women. Screening pregnant women has gained importance because of the high proportion of asymptomatic cases and because of the increasing evidence of adverse maternal and foetal outcomes related to COVID-19 [14]. Data on the immune response to SARS-CoV-2 during pregnancy are lacking and the potential role and effect of SARS-CoV-2 vaccination in pregnancy is yet to be completely investigated. The aim of this study was to evaluate the seropositivity among pregnant women in their ??trimester during the pandemic. This data will be further helpful, when the pregnancy outcomes are evaluated.

Methods

We report epidemiologic data from a study investigating a cohort of women who became pregnant just before or during the COVID-19 pandemic during the second peak, from April, 2021 to August, 2021. Ethical approval was taken from the institutional ethical committee. 298 pregnant women in trimester (11-13 weeks of gestation) were recruited at the rural centre (Ballabhgarh, Haryana, India) of the All India Institute of Medical Sciences, New Delhi. Data on demographic characteristics and COVID-19-related symptoms were collected using a structured questionnaire. Patients were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin M and immunoglobulin G levels. Only asymptomatic women, who have not been diagnosed with COVID-19 in the past three months, were recruited. Written, informed consent was obtained from all participants.

VIDAS® (Biomerieux, France) SARS-CoV-2 IgM (qualitative) and VIDAS® SARS-CoV-2 IgG II (semi-quantitative) assay was used with automated VIDAS® system for detection of IgM and IgG respectively. Both are specific for the SARS-CoV-2 receptor binding domain of the spike protein in human serum which is based on Enzyme linked Fluorescent immunoassay (ELFA) technique.

Data analysis was carried out using STATA version 16.0. Quantitative variables were expressed as the mean ± standard deviation and qualitative categorical variables were expressed as frequency and percentages. Mean values of normally distributed data were compared using the Student's *t*- test Qualitative variables were compared using the χ^2 test or Fisher's exact test, as appropriate. To decide the cut off values of IgG and IgM markers for an optimum level of sensitivity and specificity ROC analysis

was carried out. Area under curve (AUC) with 95% was presented. A two-sided probability of $P<0.05$ considered to be statistically significant.

Results

A total of 298 women in the first trimester of pregnancy (11-13 weeks of pregnancy), were included in the study. Participants had an average age of 24.0 ± 4.1 years and a body-mass index of $22.51 \pm 4.3\ kg/m^2$. Of the 298 women 94 (31.5%) were primigravidae, 61 (20.5%) have given birth once, 143 (47.9%) have been pregnant more than once. All women were homemakers and none were smokers. One woman (0.3 %) had essential hypertension. No women had associated medical disorders like type 1 or type 2 diabetes mellitus, chronic kidney disease or any other autoimmune disease. Other demographic details are presented in Table 1.

Pregnant women were asked regarding symptioms related to covid -19 infection during their first trimester . Symptom profile showed that 31 (10.4%) had fever, 12 (4%) had cough, 8 (2.7%) had shortness of breath, 3 (1%) had headache, 2(0.9 %) had lethargy and 1 (0.3 %) had vomiting during their first trimester. None had joint pains, loss of smell/taste, rhinorrhoea or diarrhoea. Nasopharyngeal and throat swabs for COVID-19 RT PCR for 5 symptomatic women (who presented with current symptoms and not just history of symptoms in first trimester) included in study were negative. None had exposure to a case of Covid -19 infection at home, community or hospital, nor did anyone had history of travelling to abroad destination. Of the 298 women eligible women, who were recruited, 78 were unwilling to participate in serological prevalence study. Around 20% of these women had symptoms suggestive of COVID. As shown in table 2 and 3, the presence or absence of symptomatology in their first trimester is not related to IgG or IgM positivity.

Of the 220 patients tested for IgG and IgM 160 (72.7%; 95% CI: 66.8-78.6%) were SARS-CoV-2 IgG positive, 37 (16.8%; 95% CI: 11.8-21.8%) were SARS-CoV-2 IgM positive and 27 (16.9%; 95% CI: 7.9-1.6%) were both IgG and IgM positive. The temporal association of the antibodies prevalence in shown in figure 1. The average (Sd) antibody titre found was 10.49 BAU/ml (±14.0) and 0.6 (±0.55) for anti-SARS-CoV-2 IgG and IgM non neutralizing antibodies, respectively. ROC analysis for SARS-CoV-2 IgG positivity showed a cut-off value of 1.19 with a sensitivity of 99.3% (0.9949 AUC, 95% CI) and specificity of 98.3% (0.9949 AUC, 95% CI) respectively. (Figure 2) Similarly for SARS-CoV-2 IgM positivity showed a cut-off value of 1 with a sensitiity of 97.3% (0.9935 AUC, 95% CI) and specificity of 98.9% (0.9935 AUC, 95% CI). (Figure 3) ROC analysis for SARS-CoV-2 IgG positivity showed a cut-off value of 1.19 with a sensitivity of 99.3% and specificity of 98.3% contributing AUC with 0.995. Similarly for SARS-CoV-2 IgM positivity showed a cut-off value of 1 with a sensitivity of 97.3% and specificity of 98.9% yielding AUC with 0.993 Even though the IgG and IgM positivity was determined based on manufacturer cut-off value, the cut off value derived from the data may have implication for Indian population to correctly classify the true positivity and true negatives.

Discussion:

Principal Findings: In this study of 220 patients, 160 (72.7%; 95% CI: 66.8-78.6%) were SARS-CoV-2 IgG positive, 37 (16.8%; 95% CI: 11.8-21.8%) were SARS-CoV-2 IgM positive and 27 (16.9%; 95% CI: 7.9-1.6%) were both IgG and IgM positive.

Results: A study evaluated the progression of seroprevalance of COVID antibodies in pregnant population of the south of Madrid, Spain during the first wave of the COVID-19 pandemic. They reported that seropositivity increased from 0% to 21.4%

(95% CI 11.8–31.0) during the study period, of which 27.9% had an asymptomatic course. They tested 769 serum samples during the first and third trimesters of pregnancy for specific IgG anti SARS-CoV-2 RBD and S proteins.[17] In another study from New York city, 19/47 (40.4%) tested positive for antibodies.[18] Of the 19 women with antibodies detected, 3 noted symptoms of COVID-19 prior to enrollment and four developed symptoms after study enrollment. Our study showed a high prevalence of 72.7% of IgG antibodies in the study population, as the data was collected during the second peak of pandemic. The ICMR data during this time period also showed similar sero-positivity in general population.[6]

Clinical Implications: The present work highlights the crucial role of serum antibodies for early diagnosis of SARS-CoV-2 among asymptomatic pregnant patients. The specificity of real-time reverse transcription polymerase chain reaction (RT-PCR) for the detection of COVID-19 is remarkable, but its accuracy depends on sampling quality [15]. Advantages of testing pregnant women for antibody response to COVID-19 are, to identify possibly “healed” women (e.g., IgG positive) who were never tested with RT-PCR assay using nasopharyngeal (NP) swab specimens and also detect women who are still at risk for COVID-19 infection (e.g., IgM and IgG negative). Women who do not know their infective status represent a potential threat to others, including healthcare workers (HCWs) and other patients. Antibodies to SARS-CoV-2 could serve as the basis for an “immunity passport” or “risk-free certificate” (digital or physical documents that certify an individual has been infected and is purportedly immune to SARS-CoV-2) [16]. This statement is yet not verified. Also, while evaluating the effect of COVID on pregnancy outcomes, the antibody evaluation might be useful. However, as seen from the data analysis, there was a

high prevalence of COVID like symptoms in seronegative women and vice-versa, that is, no symptoms in women with positive IgG or IgM antibodies (Table 2,3).

Research Implications: According to the Indian Council of Medical Research, IgG antibody test for COVID-19 may be useful in serosurveys among asymptomatic individuals and high risk or vulnerable population to understand the proportion of population exposed to infection with SARS-CoV2 and hence, appropriate public health interventions for prevention and control of disease can be planned and implemented accordingly [6]. As our study clearly shows, a high percentage of seropositivity in asymptomatic woman, any research on maternal and neonatal outcomes, only on the basis of nasopharyngeal or oral testing in symptomatic woman, may be flawed.

Strengths and Limitations: This study may serve as a basic framework to detect vertical transmission of SARS-CoV-2 from mothers to foetuses and later to detect neonatal outcomes. A further follow-up of these pregnant woman may enlighten with the impact of COVID seropositivity on materno-fetal outcomes, which our study is yet lacking.

Conclusions:

We report epidemiologic data from this study investigating a cohort of women who became pregnant just before or during the COVID-19 pandemic during the second peak. First trimester sero-molecular screening suggests high prevalence of COVID antibodies in the study population of pregnant women in first trimester during COVID wave. Thus, this fact needs to be taken into account while evaluating the effect of COVID in pregnancy.

Legends to Figure 1: Distribution of IgG and IgM levels in pregnant women in their first trimester, during the second wave of pandemic in Delhi, India

Legends to Figure 2: ROC analysis for serum IgG levels among pregnant women in their first trimester

Legends to Figure 3: ROC analysis for serum IgM levels among pregnant women in their first trimester

Author contributions:

Authors:

AS- Planning of study, conception of idea, data compilation, manuscript drafting

NS- Data compilation, manuscript preparation, data Collection

SH- Planning of study, conception of idea, final manuscript drafting

PM- Data Collection, data analysis, final manuscript drafting

KY- Data Collection, final manuscript drafting

AG- Data Collection, manuscript preparation

VD- Planning of study, conception of idea, final manuscript drafting

NB- Planning of study, final manuscript drafting

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

1. Johns Hopkins Coronavirus resource center [Internet]. Jhu.edu. [cited 2021 Oct 11]. Available from: https://coronavirus.jhu.edu/map.html.
2. Cosma S, Borella F, Carosso A, Sciarrone A, Cusato J, Corcione S, Mengozzi G, Preti M, Katsaros D, Di Perri G, Benedetto C. The “scar” of a pandemic: Cumulative incidence of COVID- 19 during the first trimester of pregnancy. Journal of medical virology. 2021 Jan;93(1):537-40.
3. Cosma S., Carosso A.R., Cusato J., Borella F., Carosso M., Bovetti M., Filippini C., D’Avolio A., Ghisetti V., Di Perri G., Benedetto C. Coronavirus disease 2019 and first-trimester spontaneous abortion: a case-control study of 225 pregnant patients. *Am. J. Obstet. Gynecol.* 2020 doi: 10.1016/j.ajog.2020.10.005.
4. RCOG Guidelines on coronavirus (COVID) infection in pregnancy. https://www.rcog.org.uk/globalassets/documents/guidelines/2021-08-25-coronavirus-covid-19-infection-in-pregnancy-v14.pdf.
5. Benedetto C., Carosso A., Corezzi M., Zotti C.M., EBCOG EBCOG position statement: vaccination in pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2019;240:375–376. doi: 10.1016/j.ejogrb.2019.04.022.
6. Gov.in. [cited 2021 Oct 11]. Available from: https://www.icmr.gov.in/pdf/covid/strategy/New_additional_Advisory_23062020_3
7. Jiao J. Under the epidemic situation of COVID-19, should special attention to pregnant women be given? J Med Virol. 2020;92(9):1371–2.

8. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. The lancet. 2020 Mar 7;395(10226):809-15.
9. Carosso A, Cosma S, Borella F, Marozio L, Coscia A, Ghisetti V, Di Perri G, Benedetto C. Pre-labor anorectal swab for SARS-CoV-2 in COVID-19 patients: is it time to think about it? European Journal of Obstetrics & Gynecology and Reproductive Biology, Forthcoming. 2020 Apr 11.
10. Chen S, Liao E, Cao D, Gao Y, Sun G, Shao Y. Clinical analysis of pregnant women with 2019 novel coronavirus pneumonia. Journal of medical virology. 2020 Sep;92(9):1556-61.
11. Yan J, Guo J, Fan C, Juan J, Yu X, Li J, Feng L, Li C, Chen H, Qiao Y, Lei D. Coronavirus disease 2019 in pregnant women: a report based on 116 cases. American journal of obstetrics and gynecology. 2020 Jul 1;223(1):111-e1.
12. Ferrazzi E, Frigerio L, Savasi V, Vergani P, Prefumo F, Barresi S, Bianchi S, Ciriello E, Facchinetti F, Gervasi MT, Iurlaro E. Vaginal delivery in SARS-CoV- 2- infected pregnant women in Northern Italy: a retrospective analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 2020 Aug;127(9):1116-21.
13. Liao J, He X, Gong Q, Yang L, Zhou C, Li J. Analysis of vaginal delivery outcomes among pregnant women in Wuhan, China during the COVID- 19 pandemic. International Journal of Gynaecology & Obstetrics. 2020 Jul;150(1):53-7.
14. Breslin N, Baptiste C, Gyamfi-Bannerman C, Miller R, Martinez R, Bernstein K, Ring L, Landau R, Purisch S, Friedman AM, Fuchs K. COVID-19 infection

among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. Am J Obstet Gynecol MFM. 2020 Apr 9: 100118. doi: 10.1016/j. ajogmf. 2020.100118. Epub ahead of print. PMID: 32292903; PMCID: PMC7144599. 2020.

15. Ali A, Rashid Z, Zhou J, Zubair Yousaf M, Galani S, Ashraf A, et al. Evaluating antibody response pattern in asymptomatic virus infected pregnant females: Human well-being study. J King Saud Univ Sci. 2021;33(1):101255.
16. Zullo F, Di Mascio D, Saccone G. Coronavirus disease 2019 antibody testing in pregnancy. American journal of obstetrics & gynecology MFM. 2020 Aug;2(3):100142.
17. Villalaín C, Herraiz I, Luczkowiak J, Pérez-Rivilla A, Folgueira MD, Mejía I, et al. (2020) Seroprevalence analysis of SARS-CoV-2 in pregnant women along the first pandemic outbreak and perinatal outcome. PLoS ONE 15(11): e0243029. https://doi.org/10.1371/journal.pone.0243029
18. Baptiste C, Breslin N, Chong A, et al. 919 Rates of seroprevalance of COVID-19 among pregnant patients in New York City. *Am J Obstet Gynecol*. 2021;224(2):S571. doi:10.1016/j.ajog.2020.12.944

Table 1: Baseline Characteristics in the Study Population

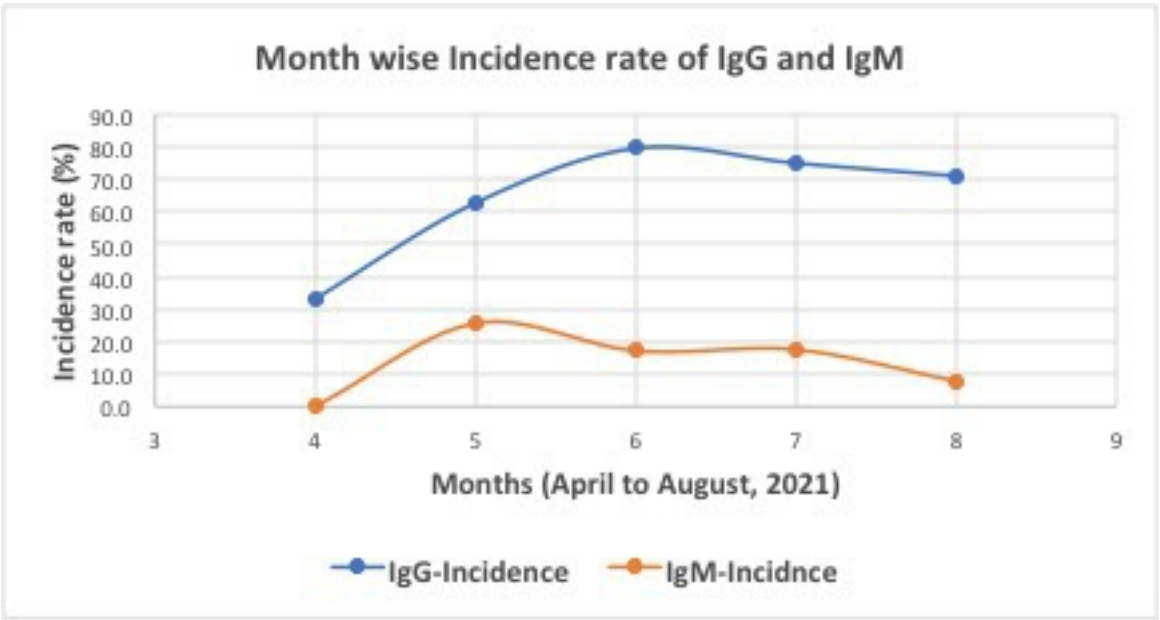
Charecteristics	IgG Positive (n=160)	IgG negative (n=60)	p-Value	IgM Positive (n=38)	IgM Negative (n=182)	p-Value
Mean Age (in years)	24.27	23.28	0.10	23.97	24.14	0.82
Mean Gestation (in weeks)	13.3	13.6	0.61	13.3	14.04	0.37
BMI (Kg/m2)	22.44	22.54	0.95	22.8	20.8	0.30
Multiparity	147	53	0.56	33	176	0.28

Table 2: Correlation of Symptomatology with IgG positivity

	IgG Positive (%)	IgG Negative	Exact Significance (2-sided)
Symptoms Present	**33 (20.6%)**	**13 (21.7%)**	**0.854**
Symptoms Absent	**127 (79.3%)**	**47 (78.3%)**	
Total	**160**	**60**	

Table 3: Correlation of Symptomatology with IgM positivity

	IgM Positive (%)	IgM Negative	Exact Significance (2-sided)
Symptoms Present	**5 (13.5%)**	**41 (22.4%)**	**0.273**
Symptoms Absent	**32 (86.5%)**	**142 (77.6%)**	
Total	**37**	**183**	



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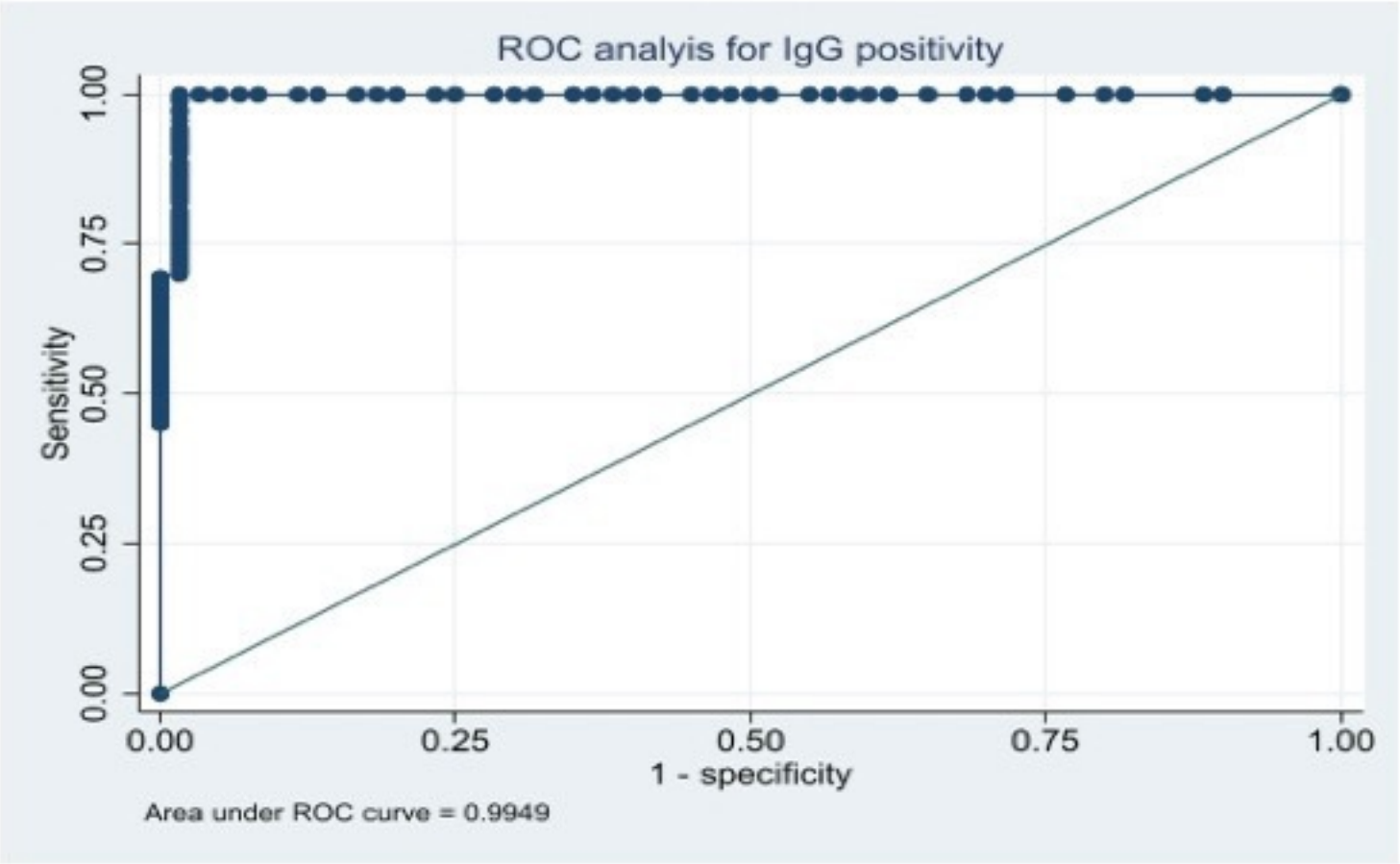
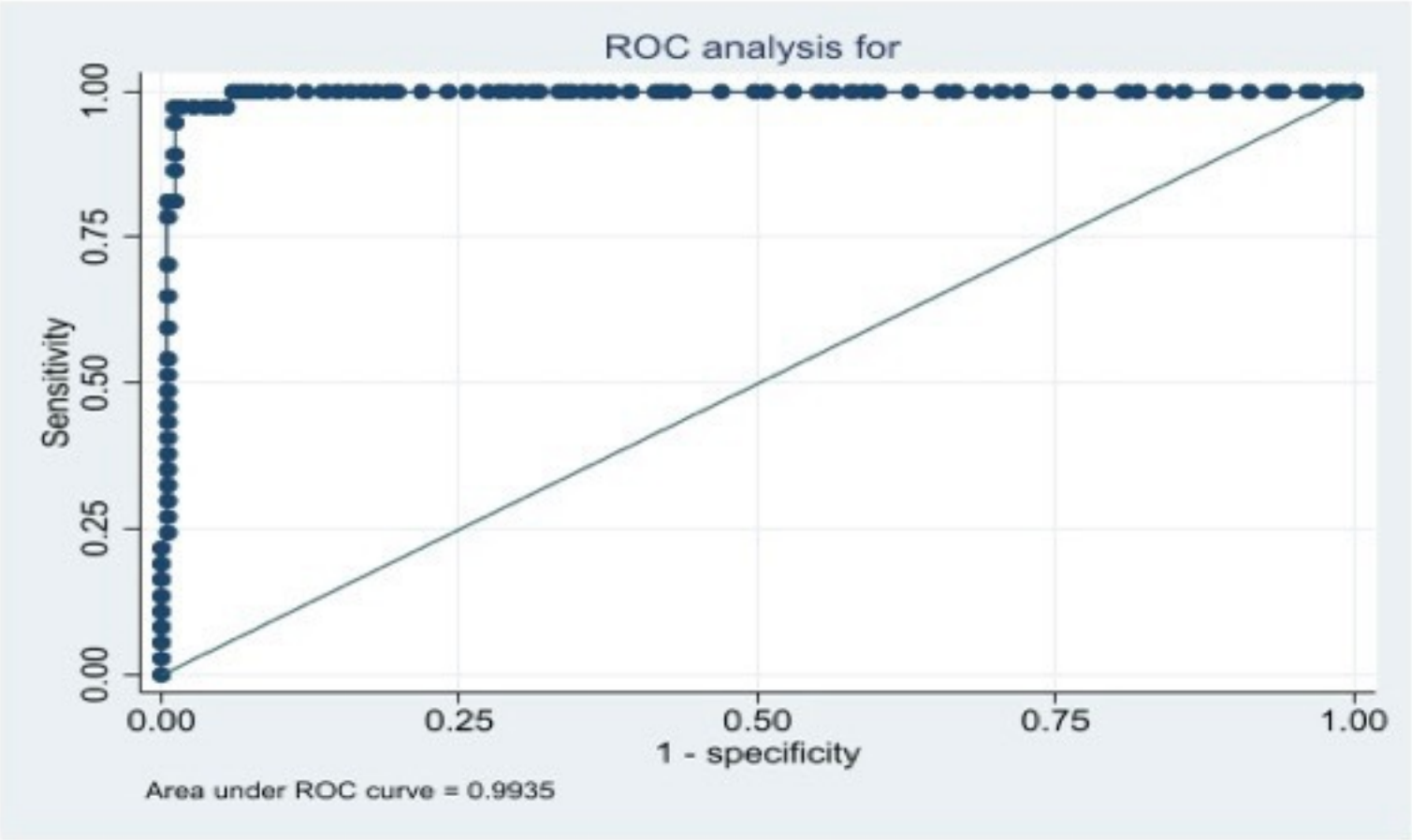


Cutpoint	Sensitivity	Correctly Specificity	classified	LR+	LR-
(>= 1.19)	98.33%	99.09%	59.6251	0.0064	99.38%

Obs	ROC area	Std. err.	Asymptotic normal [95% conf. interval]	
220	0.9949	0.0051	0.98498	1.00000

IJGO_14189_Figure 2.jpg



Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly classified	LR+	LR-
98.91%	98.64%	89.0271	0.0273	(>= 1)	97.30%

Obs	ROC area	Std. err.	Asymptotic normal [95% conf. interval]	
220	0.9935	0.0046	0.98449	1.00000

IJGO_14189_Figure 3.jpg