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1 **Title Page**

2 Title : **Association of poor virus specific immunoglobulin G antibody**
3 **responses with higher viral load is seen in Bangladeshi pregnant**
4 **women having acute Hepatitis E Genotype 1 infection**

5 Short Title: **Poor IgG response and high viral load in pregnancy with Hepatitis E**

6

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40

41 ## Abbreviations

42 CI, Confidence Interval; ELISA, Enzyme Linked Immunosorbent Assay; IFN-γ, Interferon-
43 gama; IL-4, Interleukine-4; ORF2, Open Reading Frame 2; RT-PCR, Reverse Transcription-
44 Polymerase Chain Reaction; SI, Sample Index; Th1, T helper 1; Th2, T helper 2

45

46 **Disclosures**

47 All the authors declare that they have no competing interests.

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48 # Abstract

49 Although Hepatitis E viral illness is usually self-limiting, higher rates of morbidity and
50 mortality are frequently observed during pregnancy in South Asian countries including
51 Bangladesh. Of the four common variants, hepatitis E virus genotype 1 is mainly prevalent in
52 South Asian countries. Pregnant women usually suffer from a state of immunosuppression. It
53 is yet to be known whether virus specific immunoglobulin G (IgG) immune responses have
54 any association with the vulnerability of pregnant women to acute hepatitis with E virus. The
55 study aimed to compare the viral load and IgG responses of hepatitis E-infected pregnant
56 women with that of non-pregnant women with same infection. Real Time –quantitative reverse
57 transcription Polymerase Chain Reaction and Sanger sequencing were performed to determine
58 the viral load and genotype, respectively, whereas Enzyme Linked Immunosorbent Assay
59 method was used to determine hepatitis E virus specific serum IgG antibody index along with
60 IgG avidity index. Although significant negative correlations were observed between log viral
61 copy number and log IgG antibody index in the late acute phases of jaundice for both pregnant
62 ($r= -0.7971$, $p=0.0002$) and non-pregnant women ($r= -0.9117$, $p=0.0002$), serum log viral copy
63 number of pregnant women was significantly higher than that of the non-pregnant counterpart
64 ($p=0.0196$) in the late acute stage of jaundice. In addition, log hepatitis E virus IgG antibody
65 index of pregnant women was significantly lower than the non-pregnant women in the late
66 phase of jaundice induced by hepatitis E virus ($p=0.0303$). Moreover, pregnant women with
67 acute hepatitis E had higher cross-reactive IgG than in the non-pregnant women ($p=0.0017$).
68 All the patients got infected with hepatitis E virus were in Genotype 1 variety. The study
69 demonstrates that virus-specific poor IgG responses might be responsible for vulnerability of
70 pregnant women to acute hepatitis with hepatitis E virus.

71 **Keywords:** Hepatitis E virus, IgG, pregnant women, antibody index, avidity index.

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72 # Author Summary

73 Acute hepatitis caused by hepatitis E virus (HEV) Genotype 1 is a public health problem in Asian
74 countries and especially it poses a potential health threat to pregnant women causing 19% to 25%
75 mortality, particularly in South Asian countries including Bangladesh. The study aimed to explore
76 whether HEV IgG immune responses were compromised during pregnancy, which might
77 contribute to higher viral load and disease severity. Accordingly, pregnant and non-pregnant
78 women with acute hepatitis (clinically presented with nausea, loss of appetite and /or jaundice)
79 were enrolled from different tertiary care hospitals in Dhaka city. All these patients were screened
80 and hepatitis E were differentiated from other hepatitis (caused by A, B, C) using Enzyme Linked
81 Immunosorbent Assay (ELISA) methods. HEV IgG antibody/avidity indices and viral loads were
82 measured using ELISA and real time quantitative polymerase chain reaction (RT- qPCR),
83 respectively. The study showed that pregnant women with acute hepatitis E had lower IgG indices
84 with higher viral load than their non-pregnant counterpart. Overall, the study revealed that virus-
85 specific poor IgG responses might render pregnant women vulnerable to acute hepatitis E of
86 varying degree of severity which might be associated with higher viral load.

87

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88 # Introduction

89 Acute hepatitis caused by HEV is a global public health concern as it affects 20 million
90 individuals with an annual mortality of 44,000 worldwide [1]. The disease is usually self-
91 limiting in men and non-pregnant women with a case-fatality rate of <0.1% [2]. However,
92 severity is documented among pregnant women in Bangladesh, often leading to deaths in up to
93 19–25% of the cases [3]. During the last decades, an improved understanding of the natural
94 history of HEV infection, reservoirs, and transmissions modes has been achieved [4].
95 Moreover, studies have reported significant changes in immunological and hormonal responses
96 in pregnant women with fulminant hepatic failure caused by HEV, explaining high morbidity
97 and mortality rate in this group of patients [2]. However, it is not well understood why there
98 are disparities in terms of disease severity and outcomes in some geographical locations in
99 developing countries and in pregnant women with HEV Genotype 1 hepatitis. There are some
100 reports demonstrating high viral load in pregnant women with acute hepatitis compared to that
101 of non-pregnant [5, 6].

102 The determination of anti-HEV specific antibodies (IgM and IgG) and/or detection of viral
103 RNA in serum are the main diagnostic methods for HEV diagnosis. HEV-RNA appears in the
104 blood shortly before the onset of symptoms and generally peaks during early acute phase of
105 illness with persistent viremia for approximately 3-6 weeks [4, 7]. While anti-HEV IgM
106 response is an indicator of acute infection, peak of anti-HEV IgG predicts convalescent phase
107 ensuring recovery of the patients [8], although acute and ongoing infection is often
108 accompanied by both anti-HEV IgM and IgG [9]. Currently, no kits are commercially available
109 for quantitative measurement of anti-HEV IgG antibody level. However, HEV IgG SI (sample
110 index) value, antibody avidity index and their correlation with the viral load can be used as an
111 important tool for prediction of viral elimination and assessment of immune response against
112 the virus [10]. Moreover, studies on immune responses among individuals with HEV hepatitis

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113 as well as various animal models on HEV infections reported a protective role of IgG antibody
114 [11]. In humans, HEV infections had been reported to promote Th1 immunity, whereas Th2
115 immune responses are induced during the pregnancy to ensure the safety of the fetus [2]. An
116 imbalance between Th1 and Th2 responses was expected when pregnant women are infected
117 with HEV, which may suppress anti-HEV IgG immune responses in pregnancy. This study
118 was carried out to investigate whether there were any differences in IgG immune responses
119 imparted by anti-HEV IgG between pregnant and non-pregnant Bangladeshi women with
120 icteric symptoms due to acute HEV hepatitis.

121

122 Materials and Methods

123 Study design, patient selection and data collection

124 This cross-sectional study spanned between August 2016 to September 2019 which recruited
125 a total 153 (67 pregnant, 86 non-pregnant) consecutively admitted suspected acute hepatitis
126 female patients, age range 18-50 years, from Female Medicine and Antenatal wards of three
127 tertiary care hospitals of Dhaka, namely Dhaka Medical College Hospital, Bangabandhu
128 Sheikh Mujib Medical University Hospital and Bangladesh Institute of Health Sciences and
129 General Hospital. Recruitment criteria included, diagnosis of acute viral hepatitis was
130 confirmed by the presence of constellation of clinical symptoms (nausea, anorexia, upper
131 abdominal pain, low grade fever and yellowish urine), presence of jaundice for <6 weeks, and
132 biochemical evidence of hepatocellular damage demonstrated by high levels of alanine
133 transaminase (ALT) and aspartate transaminase (AST) or both. An informed written consent
134 was taken from each patient. A structured questionnaire was filled out which included
135 information on each patient's socio-demographic particulars (age, residence, annual family
136 income, education level etc.), potential risk factors for viral transmission (source of drinking

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137 water, living arrangements, food habits etc.), and previous history of jaundice. The study was
138 approved by the Institutional Research Review Boards of Bangladesh University of Health
139 Sciences (BUHS) and Dhaka Medical College (DMC), Dhaka, Bangladesh.

140 **Specimen collection and serum preparation**

141 For the study, 4.0 ml blood sample was collected in a plain vacutainer tube from each
142 participant and transported to the institute for developing Science and Health initiatives
143 (ideSHi) laboratory in a cool (4-8ºC) box within an earliest possible convenience. Serum was
144 prepared after centrifugation of the sample tube for 10 minutes at 3000 r.p.m. in a refrigerated
145 Centrifuge machine. Serum was aliquoted (800 microliter in each of the three microcentrifuge
146 tubes). One aliquot was used for biochemical liver function tests, another for serological
147 screening of viral hepatitis markers on the same day of collection and third aliquot was
148 preserved at -80 ºC for molecular analysis at later period.

149 **Liver Function Tests, anti-HEV IgM and anti-HEV IgG Detection**

150 Liver function tests; serum bilirubin, ALT and AST were performed using a biochemistry
151 autoanalyzer (Dimension, Siemens, Germany). Patients with serum bilirubin level ≥1.2 mg/dl
152 of blood and ALT level ≥ 40 IU/L were subjected to screening for viral hepatitis markers, such
153 as Hepatitis B surface antigens (HBsAg) and Hepatitis C antibodies (anti-HCV) using ELISA
154 kits (BioKits, Spain), IgM antibodies for Hepatitis A (anti-HAV IgM) and anti-HEV IgM and
155 IgG using ELISA kits (Wantai, China). To determine anti-HEV IgG index, a minor
156 modification of the manufacturer's protocol was adopted. The IgG sample index (SI) value was
157 calculated as follows: [(Sample absorbance-Negative Control absorbance)/(Positive control
158 absorbance-Negative control absorbance)] *dilution factor *100.

159

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160 **Avidity ELISA**

161 Avidity ELISA was performed using the commercially available anti-HEV IgG antibody
162 (Wantai, China) kit with minor modifications. The samples were incubated before addition of
163 conjugate with 7M urea solution in some wells of the HEV antigen coated plates, while the
164 same samples in another set of wells were treated with buffer following the protocol provided
165 with the kit. Finally, the avidity index was calculated using the following formula: Avidity
166 Index (AI) = (Sample OD of untreated well-sample OD of urea treated well)*100/ Sample OD
167 of untreated well.

168 **Categorization of the Study participants**

169 The hepatitis A, B and C positive samples were excluded and finally the study analyzed 121
170 (57 pregnant, 64 non-pregnant) patients with acute HEV hepatitis. On the basis of pregnancy
171 status and duration of jaundice, the patients were divided into (1) early acute phase, ≤14 days
172 (2) late acute phase≥14 days) [12] of jaundice.

173 **Determination of Viral Genotype and Copy Number**

174 To calculate the viral load, seven synthetic viral nucleic acid standards containing the
175 conserved region of HEV genome of four common genotypes with known copy number were
176 used. A total of 78 serum samples (positive for HEV IgM in ELISA) were subjected to RNA
177 extraction using QIAamp Viral RNA Mini Kit (Qiagen, Germany). Using SuperScript™ III
178 One-Step RT-PCR System with Platinum™ Taq (Invitrogen, USA) and specific primers
179 targeting the conserved regions of the ORF2 of all HEV genotypes, real time RT-PCR was run
180 on CFX96 Touch™ Real-Time PCR detection system (Bio-Rad). Viral load was calculated
181 using the cycle of threshold **(Ct)** values. For genotype determination of the purified PCR
182 products, Sanger nucleotide sequencing was carried out using the ABI PRISM 310 Automated

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183 Sequencer (Applied Biosystems, USA) and the sequences were analyzed using BLAST tool to
184 compare the query sequence with the reference sequence retrieved from NCBI database.

185 Statistical Analysis

186 The IgG sample index, standard curve for viral copy number and avidity index were calculated
187 using Microsoft Excel v2016. Chi-square test with 95% confidence interval (CI) using SPSS
188 v17.0 was performed to find the association among different socio-demographic parameters.
189 Pearson correlation analysis was done for HEV copy number and HEV IgG SI values of
190 pregnant and non-pregnant women distributed in early and late acute phases of jaundice. Age,
191 duration of jaundice, serum bilirubin, ALT, AST, viral load and IgG SI values were compared
192 between different group stratifications using unpaired Student's t text and Mann–Whitney test,
193 as appropriate. The analyses were performed using GraphPad Prism v7.0 and a p-value of less
194 than 0.05 was considered statistically significant.

195 Results

196 Sociodemographic and biochemical profiles

197 A total of 121 women with acute HEV infection (57 pregnant and 64 non pregnant) were
198 included for data generation and analysis. Of the 121 patients, 78 were positive for anti-HEV
199 IgM antibody, which is an early marker of acute hepatitis E, whereas the other 43 were negative
200 for anti-HEV IgM but positive for HEV IgG antibody. Distribution of these patients on the
201 basis of residence, housing type, habit of eating street food, education and per-capita income
202 did not show statistically significant association with HEV IgM positivity (p=0.302, 0.186,
203 0.434, 0.093 and 0.474, respectively). Conversely, shared facilities for living (p=0.006, OR=
204 4.47, 95% CI: 1.53 – 13.06) and unsafe drinking water (p=0.017, OR= 3.26, 95% CI: 1.23 –
205 8.62) (Table 1) showed statistically significant association with HEV IgM positivity.

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206 **Table 1. Socio-demographic status of the HEV IgM positive and negative hepatitis**
207 **patients (N=121)**

208

Variables	**HEVIgM+ve**	**HEVIgM-ve**				
	(n=78)	**(n=43)**	***P* value**	***Odds Ratio***	***95%***	***CI***
	N (%)	**N (%)**			**Lower**	**Upper**
Residence						
Semi-urban (29)	16 (55.2)	13 (44.8)	0.302	1.67	0.63	4.41
Urban (92)	62 (67.4)	30 (32.6)				
Housing						
Tin shade (62)	39 (62.9)	23 (37.1)	0.186	1.98	0.72	5.48
Building (59)	39 (66.1)	20 (33.9)				
Living arrangements						
Individual facilities (86)	49 (57.0)	37 (43.0)	0.006	4.47	1.53	13.06
Shared facilities (35)	29 (82.9)	6 (17.1)				
Drinking water						
*Safe drinking (38)	19 (50.0)	19 (50.0)	0.017	3.26	1.23	8.62
**Unsafe drinking (83)	59 (71.1)	24 (28.9)				
Street food habit						
Yes (102)	65 (63.7)	37 (36.3)	0.434	0.63	0.19	2.03
No (19)	13 (68.4)	6 (31.6)				
Educational status						
Up to twelve grade (102)	66 (64.7)	36 (35.3)	0.093	2.18	0.88	5.39
Graduate and higher (19)	12 (63.2)	7 (36.8)				
Per-capita income (USD)$						
Below cut-off group (110)	70 (63.6)	40 (36.4)	0.474	0.58	0.13	2.56
Above cut-off (11)	8 (72.7)	3 (27.3)				

209 *Safe water: filtered/boiled/ deep tube-well water; **Unsafe drinking water: Tap water/ municipal
210 supply/ pond water; $Per capita income USD 1751/- to date

211

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212 Mean (±SD) age (years) of HEV IgM positive women were 26.03±6.8, whereas it was
213 29.09±9.9 for the negative cases. When HEV IgM positive patients were further divided based
214 on pregnancy status, a comparable distribution (pregnant, n=37 and non-pregnant women,
215 n=41) was found. The average duration of jaundice for HEV IgM positive cases was
216 13.68±9.27 days and it was significantly lower than that of the negative cases (21.12±16.03
217 days) (p=0.008), which further supports the acute status of the patients (Table 1b). Regarding
218 biochemical parameters, serum ALT level, a potential liver damage marker was significantly
219 higher in the HEV IgM positive cases than in the negative cases (p<0.0001). Similarly, the
220 serum bilirubin and AST levels were also significantly higher in the positive group than the
221 negative group, with the p-values of 0.027 and <0.0001, respectively (Table 2).

222 **Table 2. Pregnancy status, age, duration of jaundice and biochemical variables of the**
223 **study subjects in respect to HEV IgM antibody status**

Variable	HEV IgM +ve (n=78)	HEV IgM-ve (n=43)	*P* value
Pregnant/ Non-pregnant	37/ 41	20/23	0.922
Age (yrs)	26.03±6.8	29.09±9.9	0.036
Duration jaundice (Days)	13.68±9.27	21.12±16.03	0.008
Serum bilirubin (mg/dl)	8.75±3.94	6.60±5.45	0.027
Serum ALT (IU/L)	501±469	176±188	<0.0001
Serum AST (IU/l)	407±391	161±126	<0.0001
AST-ALT Ratio	1.19±1.30	1.42±1.09	0.329

224 Results were expressed as mean±SD. Unpaired Student's t-test was performed to calculate statistical
225 difference between the two groups. P<0.05 was considered significant.

226

227 The HEV IgM positive cases were further categorized into 2 groups: early acute (duration of
228 jaundice ≤14 days) and late acute phases (duration >14 days) of hepatitis [12]. Accordingly,
229 among the 78 HEV IgM positive acute hepatitis cases, 48 (20 pregnant and 28 non-pregnant)

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230 were in early acute and 30 (17 pregnant and 13 non-pregnant) were in late acute phase of
231 disease. Next, we wanted to compare whether there were any differences in ALT and AST
232 levels, log HEV-RNA copy numbers and log HEV IgG sample index (SI) values between early
233 and late acute phases of jaundice. As expected, serum ALT, AST and Log HEV viral copy
234 number were significantly higher in early acute phase patients than in the late acute phase
235 patients ($p<0.0001$, $p=0.0036$ and $p<0.0001$, respectively) (Fig 1A, 1B, 1C). Conversely,
236 serum log HEV IgG SI value was significantly higher in late acute phase compared to early
237 acute phase of jaundice ($p<0.0001$) (Fig 1D). The findings therefore suggest that an increase
238 in IgG antibody levels at the late acute phase of HEV-induced jaundice might contribute to
239 reduced hepatocellular damage resulting in production of lower levels of ALT, AST and log
240 viral copy number, and thus demonstrating the protective role of IgG during HEV infection.
241

242 **Fig 1. Comparison of (mean±SD) (A) serum ALT, (B) serum AST, and (C) log HEV**
243 **copy number and (D) log HEV IgG SI values between early acute and late acute phases**
244 **of HEV jaundice due to HEV infections.** A p value <0.05 was considered significant.

245

246 ## Correlation analysis between log viral copy number and log HEV IgG SI

247 Irrespective of pregnancy status, HEV-RNA log viral copy numbers and log HEV IgG SI value
248 were inversely correlated in early and late acute phases of hepatitis. Although these two
249 parameters did not show any significant correlation in early acute stage in pregnant women (r=
250 -0.2278, p=0.3793) (Fig 2A), there was a significant negative correlation in non-pregnant
251 counterpart (r=-0.5065, p=0.0320) (Fig 2B). On the other hand, significant negative
252 correlations were observed between log viral copy number and log IgG SI values in the late
253 acute phases of jaundice for both pregnant (r= -0.7971, p=0.0002) (Fig 2C) and non-pregnant

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254 women (r= -0.9117, p=0.0002) (Fig 2D). The result, therefore, suggests that correlation
255 between HEV copy number and HEV IgG SI value depends on the intensity of the IgG immune
256 responses during early and late acute phases of jaundice.
257
258 **Fig 2. Pearson's Correlation analysis between log HEV copy number and log HEV IgG SI**
259 **value for pregnant and non-pregnant women in early and late acute phases of jaundice.**
260 (A) Log HEV copy number vs log HEV IgG SI of pregnant women in early acute phase; (B)
261 Log HEV copy number vs log HEV IgG SI of non-pregnant women in early acute phase; (C)
262 Log HEV copy number vs log HEV IgG SI of pregnant women in late acute phase; (D) Log
263 HEV copy number vs log HEV IgG SI of non-pregnant women in late acute phase. A p value
264 <0.05 was considered significant.
265

266 Log viral copy and log IgG SI in pregnant vs non-pregnant

267 The HEV log viral copy number and log IgG SI value were compared between pregnant and
268 non-pregnant women. As it is seen in Fig 3A and 3C, there were no differences in log viral
269 copy number and log IgG SI value in early acute phase of jaundice between the pregnant and
270 non-pregnant women (p=0.7905, p=0.6279, respectively). On the other hand, although log viral
271 copy number in pregnant women was significantly higher than that of the non-pregnant
272 counterpart (p=0.0196) (Fig 3B) in the late acute stage of jaundice, log HEV IgG SI value was
273 significantly lower in the pregnant than in the non-pregnant women (p=0.0303) (Fig 3 D). The
274 result, therefore, suggests that the increase in HEV copy number in the late acute phase of
275 jaundice in pregnant women compared to the non-pregnant women might be due to a weak IgG
276 immune response during pregnancy (Fig 3B, 3D).

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277 The overall data suggest that HEV IgG has neutralizing effect on HEV-RNA and IgG immune
278 response to HEV hepatitis in immunocompromised women during pregnancy.
279
280 **Fig 3. Comparison of Log HEV copy number and log HEV IgG SI value between**
281 **pregnant and non-pregnant women with hepatitis in early (duration of jaundice ≤14 days)**
282 **and late (duration of jaundice >14 days) phases of the disease.** (A) Log HEV copy number
283 for pregnant vs non-pregnant women in early acute phase; (B) Log HEV copy number for
284 pregnant vs non-pregnant women in the late acute phase; (C) Log HEV IgG SI value for
285 pregnant vs non-pregnant women in early acute phase; (D) Log HEV IgG SI value for pregnant
286 vs non-pregnant women in the late acute phase. A p value <0.05 was considered significant.

287 ## Anti-HEV IgG Avidity Test

288 We further examined if there were differences in anti-HEV IgG avidity between pregnant and
289 non-pregnant women with HEV acute hepatitis. The anti-HEV IgG avidity test resulted in a
290 significantly lower level of avidity index in the pregnant group compared to the non-pregnant
291 patients (p=0.0017) (Fig 4). The findings indicate that more cross-reactive IgG antibodies are
292 produced in pregnant women with acute hepatitis E than in the non-pregnant women with acute
293 hepatitis E.
294 **Fig 4. Comparison of IgG avidity index between pregnant and non-pregnant patient.** A p
295 value <0.05 was considered significant.
296

297 ## Virial genotype determination

298 Sanger sequencing of HEV ORF2-specific PCR products and subsequent analysis by alignment
299 with reference sequence showed that all the study samples that had been analyzed were HEV
300 Genotype 1.

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301 # Discussion

302 This is the first study demonstrating that virus-specific IgG immune response against acute
303 HEV hepatitis are weaker in the pregnant women than in the non-pregnant women. Both animal
304 and human studies demonstrated HEV IgG as protective against HEV infections [11-13] Since
305 pregnant women had been reported to be vulnerable to HEV hepatitis, the present study
306 explored whether HEV IgG immune responses during pregnancy were immunocompromised,
307 which in turn, could contribute to vulnerability of pregnant women to acute HEV hepatitis.

308 All the commercially available HEV IgG ELISA kits are qualitative and as a result, it is not
309 feasible to measure IgG immune responses against HEV virus by quantitative measurement of
310 IgG using these commercially available IgG ELISA kits. However, to understand IgG immune
311 responses without its quantitative measurement in HEV hepatitis, IgG antibody indices were
312 measured to determine the fold-change of IgG HEV-infected sera by comparing its index with
313 that of positive control, which was provided with the kit. The calculation of antibody indices
314 could help to determine the role of HEV IgG in elimination of viruses and its association with
315 reduced HEV load. The findings of significant inverse association between IgG antibody
316 indices and HEV load in the late phase of jaundice in both pregnant and non-pregnant women
317 could be considered as an evidence that IgG antibody, which was produced during HEV
318 hepatitis, could render protection to HEV hepatitis. Furthermore, higher IgG indices, which
319 were associated with lower HEV load, had significantly lower levels of liver injury markers
320 including ALT, AST, and bilirubin, which indicates that IgG-mediated neutralization of HEV
321 could improve the HEV-induced liver injury and jaundice. The HEV IgG levels in the present
322 study was determined by using the kits from Beijing Wantai Biologicals, China, that was
323 directed against recombinant HEV ORF2 protein antigen which was immunogenic and seemed
324 to induce antibodies that possessed neutralizing effect on HEV and exert protective role.

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325 Pregnant women have been reported to be more vulnerable to HEV infections, demonstrating
326 58% of maternal mortality in HEV-induced acute hepatitis in hospital care settings in
327 Bangladesh [3]. So, we speculated that IgG production may be compromised in pregnant
328 women with acute HEV hepatitis as a result of the disturbance of of Th2 cytokine bias during
329 pregnancy [14] provided that IgG could possess protective function against HEV infections.
330 To our expectation, pregnant women in their late phase of jaundice did have significantly lower
331 levels of serum IgG against higher viral loads than their non-pregnant counterparts, which
332 demonstrates weak IgG immune responses to HEV hepatitis during pregnancy. Compromised
333 HEV IgG immune responses in pregnancy with HEV hepatitis was further supported by the
334 presence of low level of IgG antibodies in the sera of early acute phase of jaundice of pregnant
335 women and this phase of hepatitis was marked by higher levels of serum bilirubin, ALT, and
336 AST as well as log of viral copy number, further demonstrating the compromised responses of
337 humoral immunity due to lack of IgG antibodies during pregnancy with early acute HEV
338 hepatitis. Compromised immune responses during pregnancy with acute hepatitis E is
339 supported by a number of studies where they found generalized immune suppression with
340 Th1/Th2 imbalance compared to the non-pregnant women with HEV [2, 14]. A state of
341 maternal immune tolerance toward the fetus is characteristic in pregnancy [14]. Reduced T-
342 cell activity with a concomitant reduction in cytokine production results in a dominating Th2
343 immune response in pregnancy that is interrupted by HEV infection, which strongly supports
344 our finding that there was low level of IgG in late acute stage of HEV-induced jaundice [15].

345 Higher vulnerability to HEV infection during pregnancy may be associated with high levels of
346 steroid hormones (estrogen, progesterone and human chorionic gonadotrophin), which are
347 assumed to promote viral replication[2]. These steroid hormones have direct inhibitory effect on
348 hepatic cells that may predispose to hepatic dysfunction or failure resulting in increased
349 bilirubin, ALT, AST and high viral load upon exposure to infectious pathogens.

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350 In the present study IgG antibody response was observed against recombinant HEV ORF2
351 antigen. Reports suggest that E2s domain (a.a455–a.a.602) within the ORF2 exhibit high level
352 of immunogenicity and this peptide can be considered for manufacturing a candidate vaccine
353 [16]. Because of the immunological potential and protective role of IgG antibodies against
354 HEV, it can be passively transferred during outbreak of HEV with an aim to get neutralizing
355 effect on HEV.

356 The study had a number of limitations. Although initially 153 patients (both pregnant and non-
357 pregnant) with acute hepatitis were enrolled, we could not analyze all these samples because
358 we needed exclusion of HAV, HBV, HCV positive and negative HEV (both IgM and IgG)
359 cases. Finally, we could analyze only 121 samples with HEV acute hepatitis. Two pregnant
360 patients in their third trimester succumbed to death and they both had antibodies (both IgM and
361 IgG) to HEV. The possible reason of these deaths during third trimester may be due to interplay
362 between host immunity and HEV. During pregnancy, high cytokine levels secreted by the
363 HEV-specific antigen-stimulated peripheral blood mononuclear cells (PBMCs) coupled with
364 Th1/Th2 cytokine imbalance could cause fulminant hepatic failure in pregnant women of later
365 trimester [12]. However, we could not evaluate the cytokines levels (especially IFN-γ and IL-
366 4 etc.) because of the unavailability of the reagents and this was another limitation of this study.

367 HEV IgG measurement in the present study was not an exact quantification, rather it was an
368 alternative approach to obtain information of fold increase of HEV IgG in respect of positive
369 control (when positive control absorbance was between 1.7 - 2.0) [10] that could help us to
370 observe the correlation of true IgG levels with HEV viral load and other liver damage markers
371 like serum ALT and AST.

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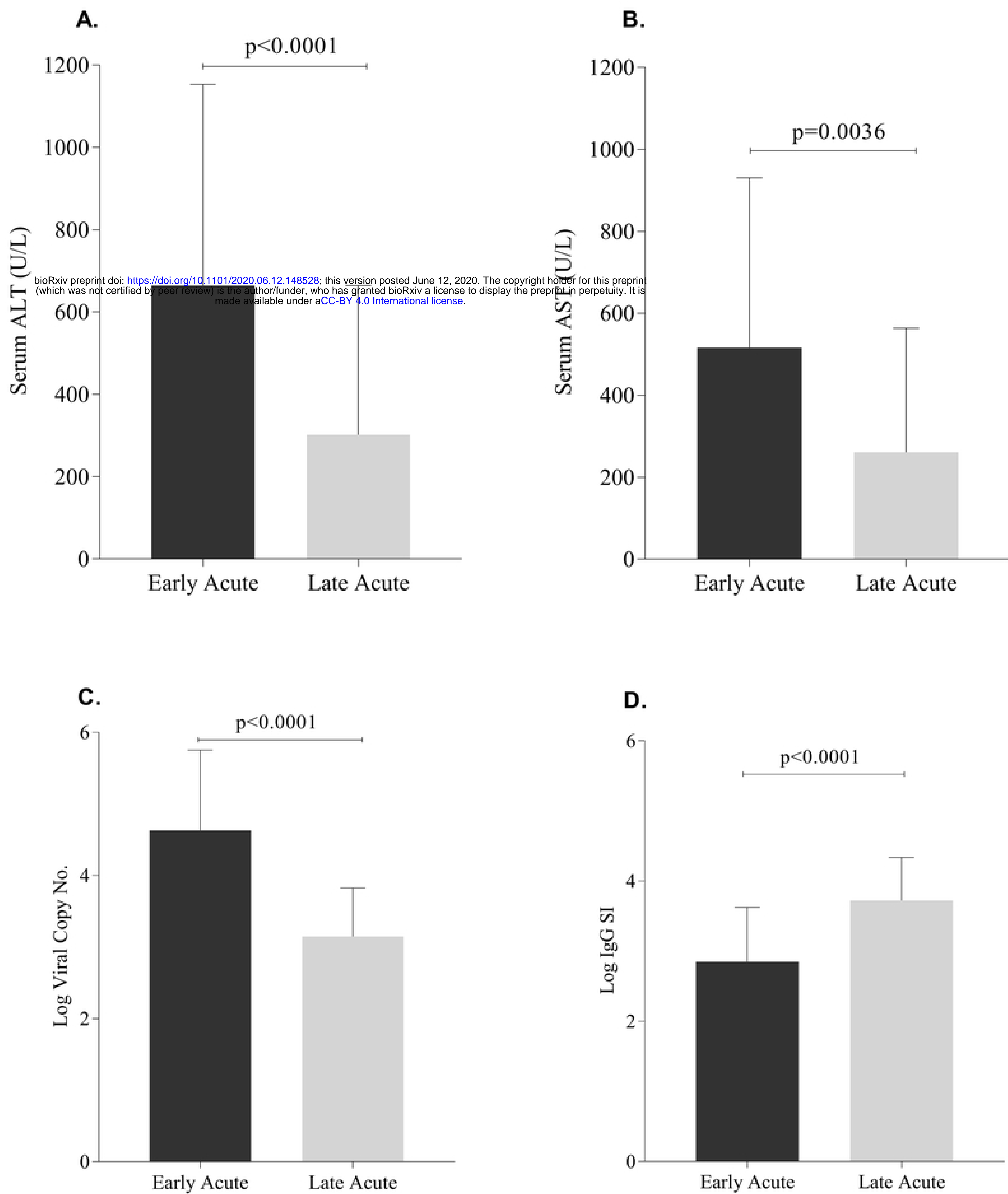
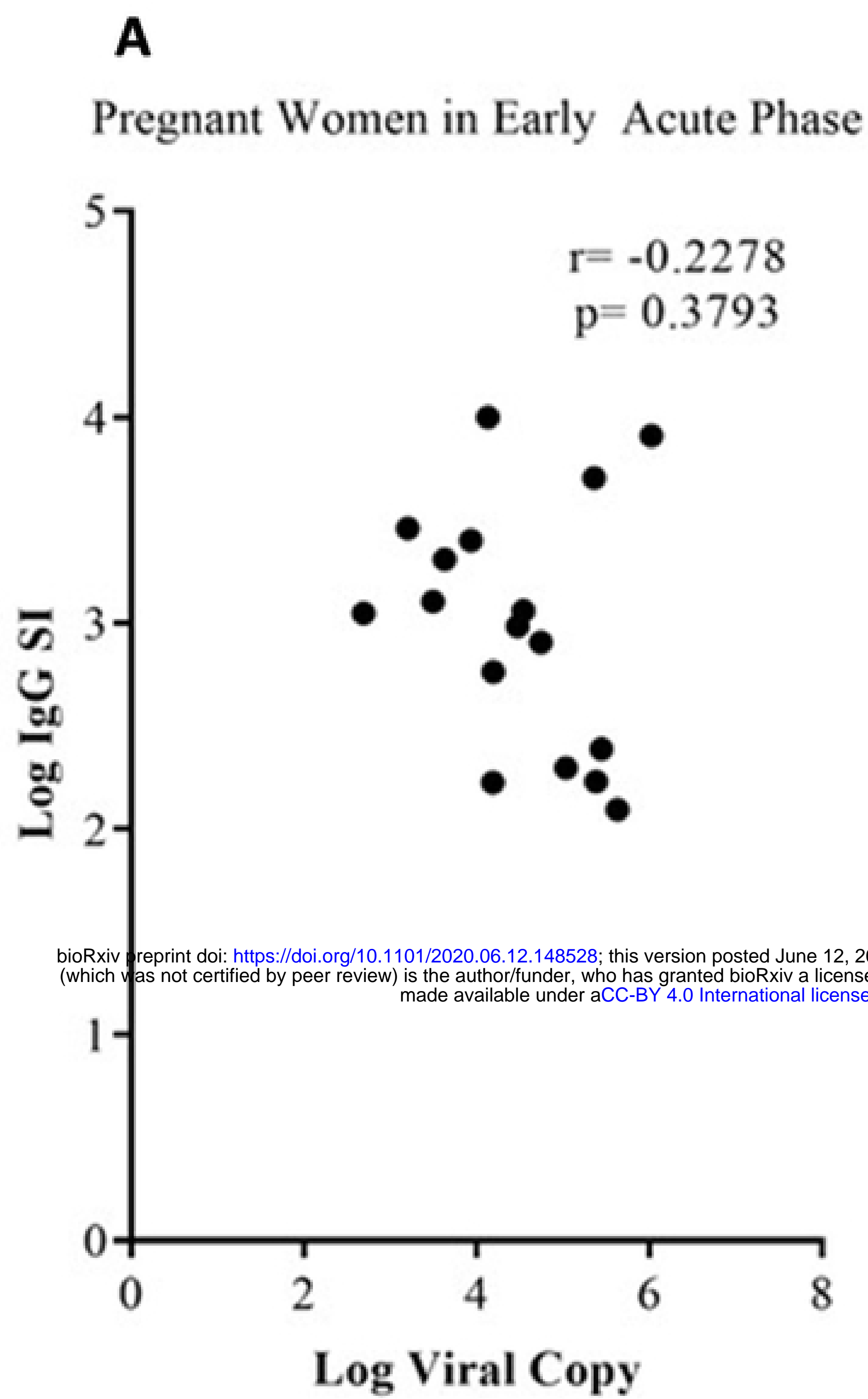
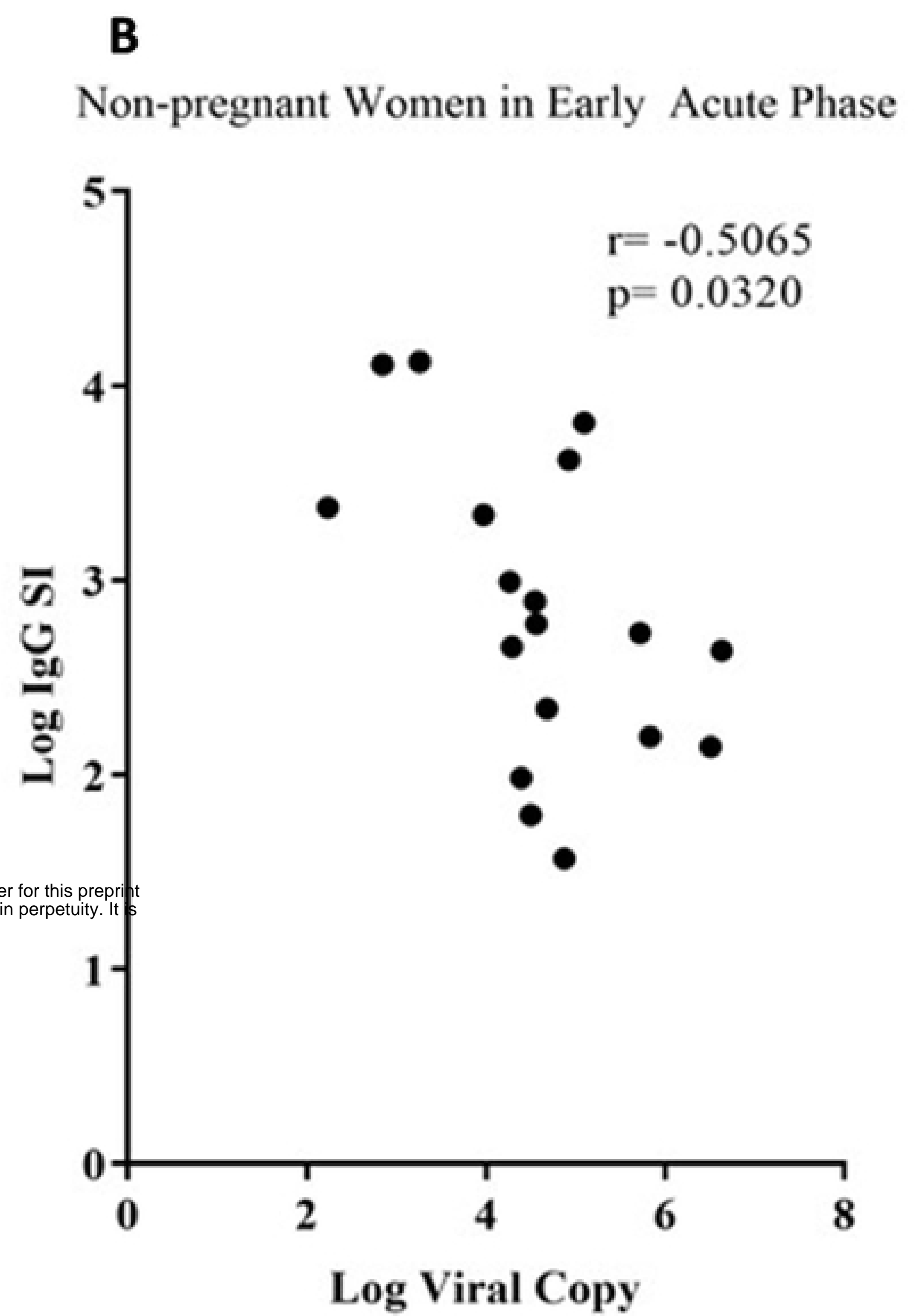


Fig 1





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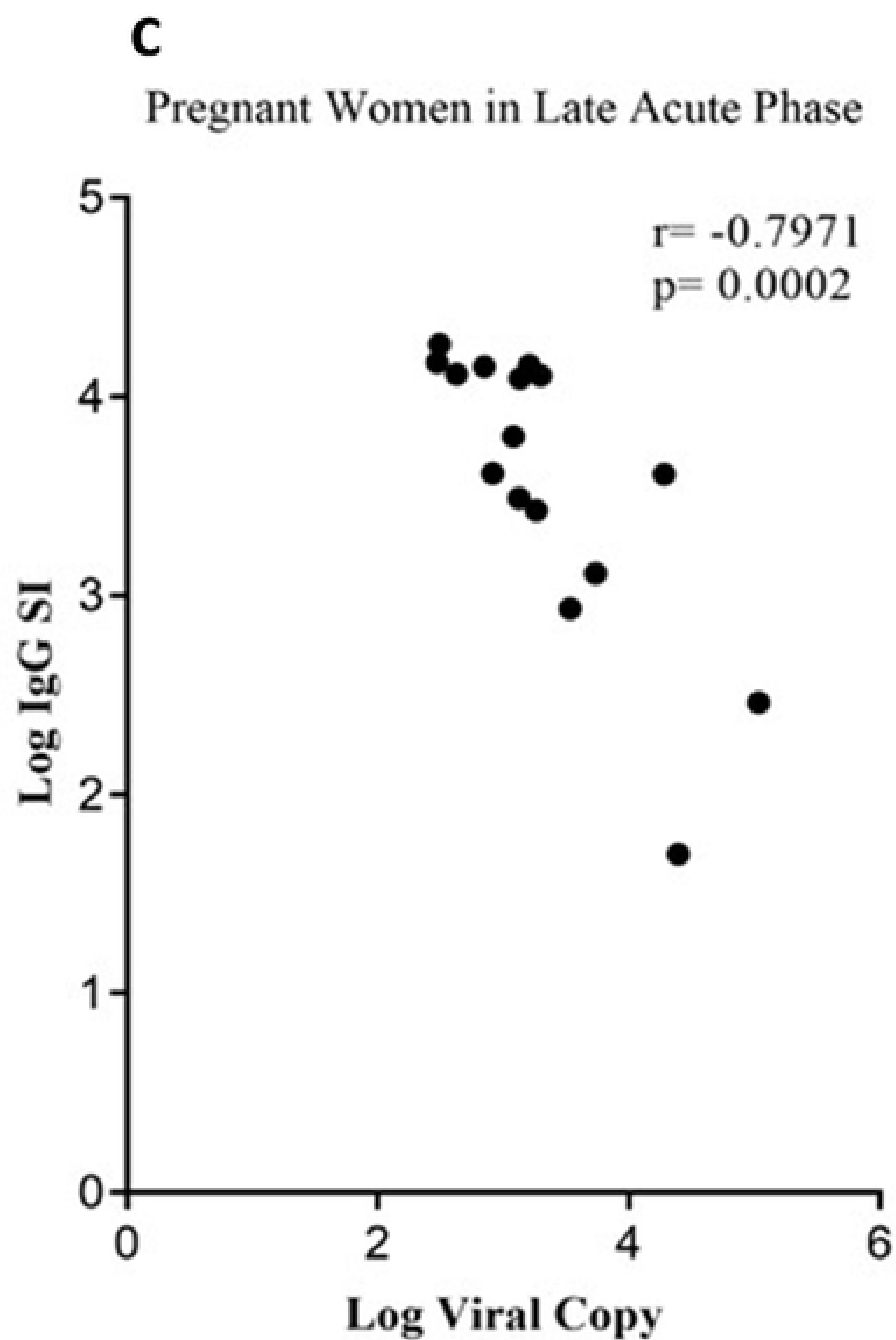

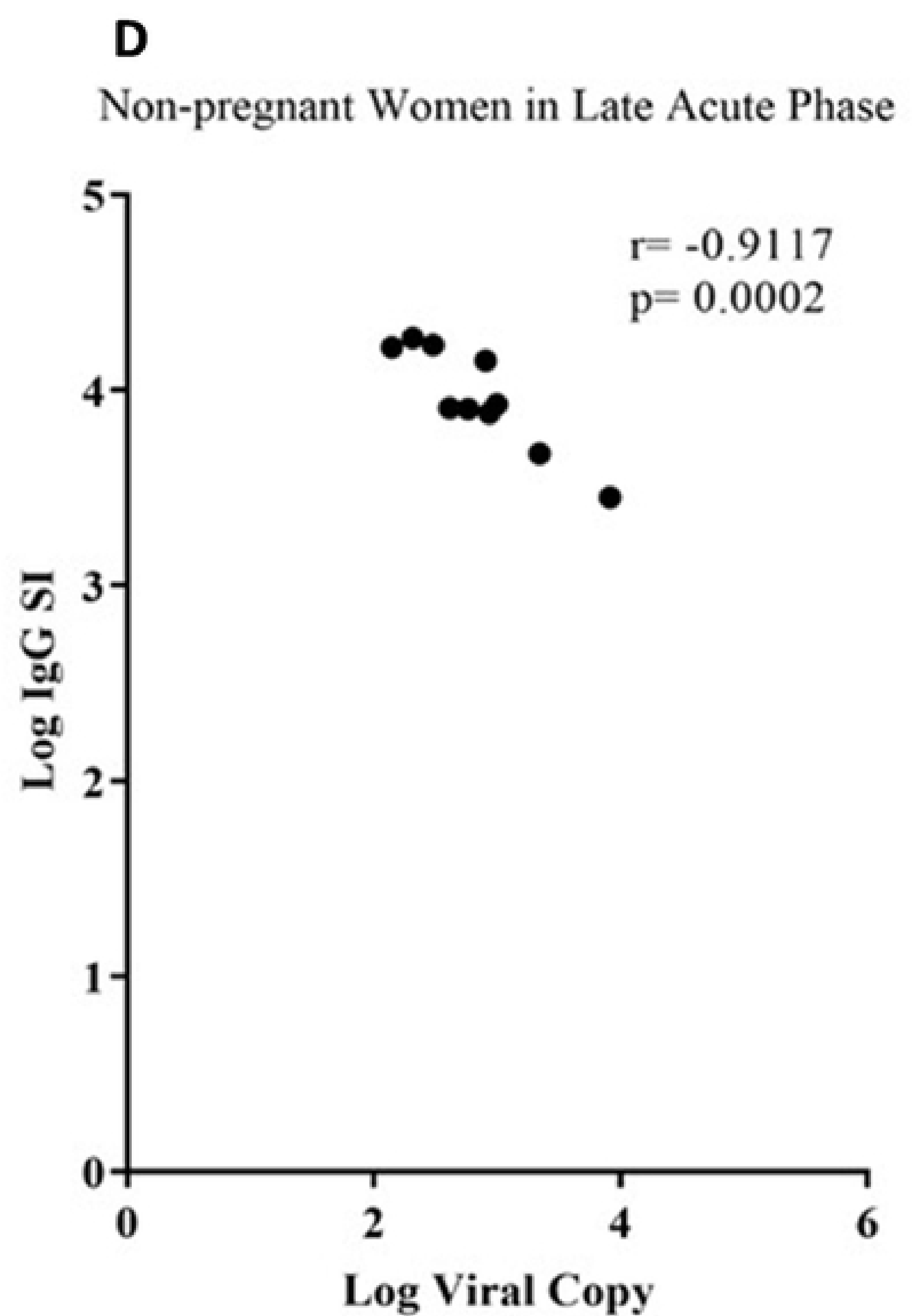

Fig 2

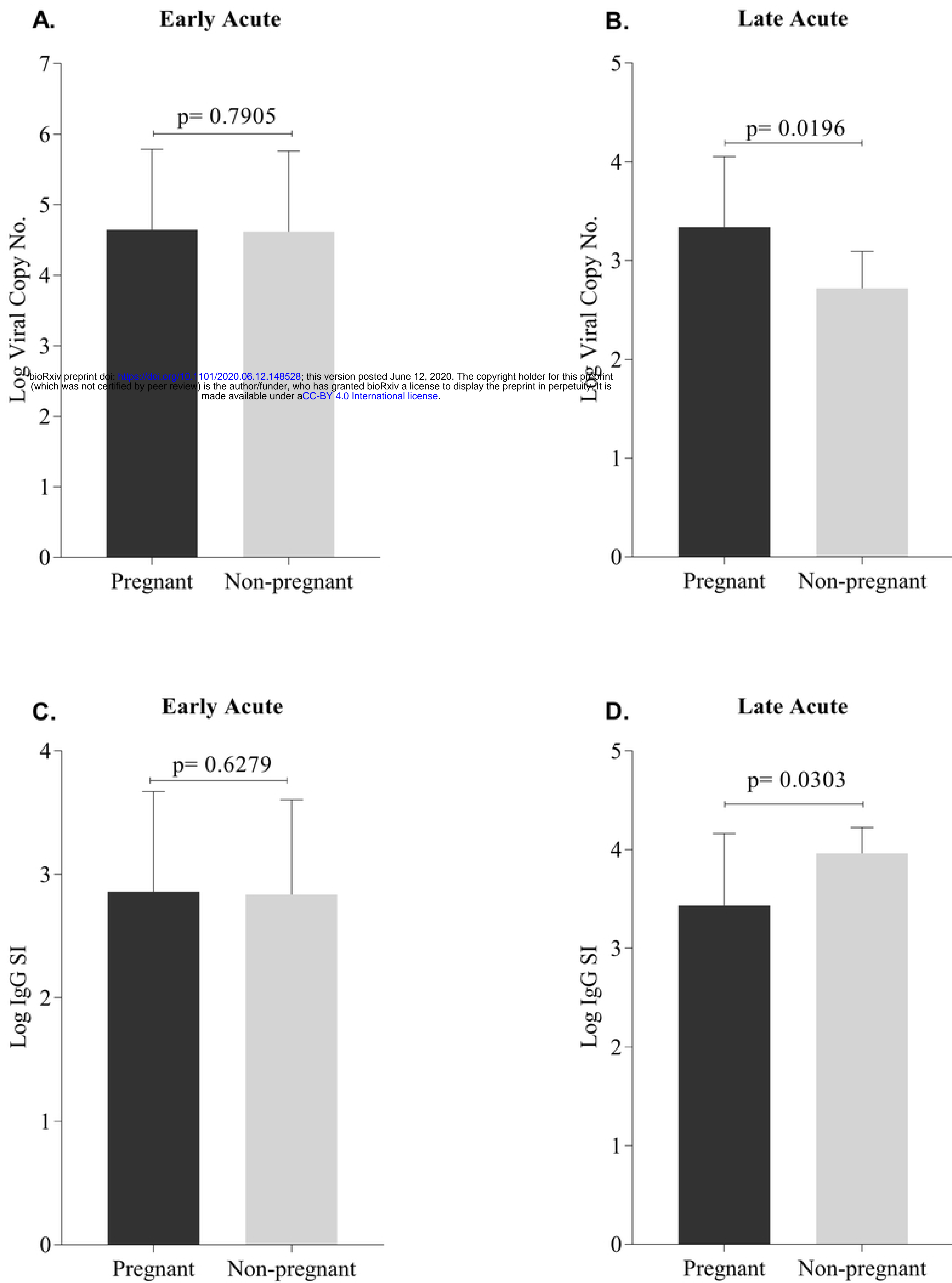

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Fig 3

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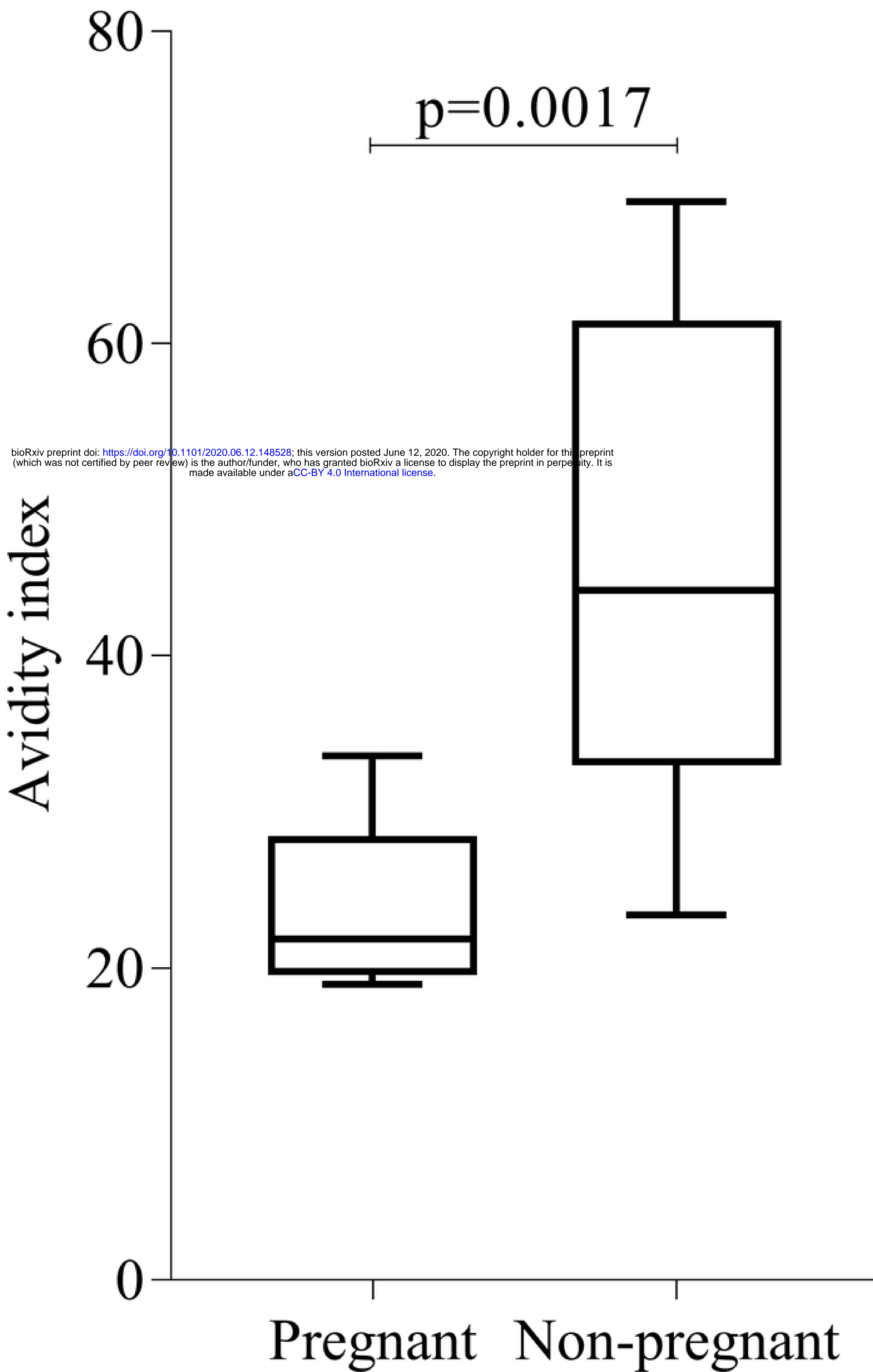

Fig 4