Prevalence, Resistance Profile and Virulence Genes of *Streptococcus agalactiae* Colonizing Near-term Pregnant Women Attending Ain Shams University Hospital

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**Abstract**

Group B streptococcus (GBS) is a common cause of infections in pregnant females and non-pregnant adults with chronic diseases (such as diabetes and cancer), also it is the main reason of septicaemia and meningitis in infants. The aim of this study was to figure out how common GBS is in pregnant women, the antimicrobial sensitivity pattern of the isolated GBS colonies and check the presence of *scpB* and *rib* virulence genes in these isolates. We screened 203 pregnant women attending the maternity Hospital of Ain Shams University using vaginal sampling. Isolation was done on CHROMagar\textsuperscript{TM} Strep B and sheep blood agar plates then identified via colony characters, Gram stain, test for catalase production, Christie–Atkins–Munch–Petersen (CAMP) test, test for hippurate hydrolysis and latex agglutination test. This was followed by an antibiotic susceptibility test. Finally, Detection of *scpB* and *rib* virulence genes by conventional PCR was done. Our study detected that the prevalence rate of GBS in involved pregnant women was 11.33%. A statistically significant association between colonization and history of spontaneous abortion and preterm labor was observed. CHROMagar\textsuperscript{TM} Strep B showed the same sensitivity of sheep blood agar with extensive effort to isolate suspected GBS colonies from blood agar. GBS was 100% sensitive to levofloxacin, linezolid, cefepime, ceftaroline and ceftriaxone. Also, it was highly sensitive to vancomycin (91.3%). Sensitivity to clindamycin, azithromycin, penicillin and ampicillin was (21.70%, 21.70%, 47.80%, 47.80%) respectively. The least sensitivity of GBS was to erythromycin (8.7%). All isolates possessed the *scpB* gene (100%) while only 18 isolates (78.26%) had the *rib* gene.

**Keywords:** Group B streptococcus (GBS), CHROMagar\textsuperscript{TM} Strep B, *scpB*, *rib*, virulence genes

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INTRODUCTION
GBS is detected in 10-30% of pregnant women as a colonizing agent in the vagina and/or rectum. The infants of these women may be at high risk of developing disease if they exposed to this bacteria before or after birth\(^1\),\(^2\).

Early-onset infection, the most prevalent kind of newborn GBS disease, and late-onset infection are the two types of GBS infections in neonates. The early-onset infection occurs in neonates under the age of seven days, while the late-onset infection occurs in those aged seven to ninety days\(^3\),\(^4\).

By preventing GBS transmission from mother to infant, intrapartum antibiotic prophylaxis (IAP) can minimize the risk of GBS neonatal illness. The Centers for Disease Control and Prevention (CDC) recommended two methods for detecting pregnant women, which were first published in 1996 and subsequently revised in 2002 and 2010: either a risk-based method of detecting clinical risk factors for newborn illness and administering IAP to all cases demonstrating any of these risk factors, or a universal screening for GBS by obtaining rectovaginal cultures between 35 and 37 weeks of pregnancy\(^5\),\(^6\).

GBS is still responsive to penicillin and most beta lactam antibiotics, but some investigations have found that GBS susceptibility to penicillin has decreased\(^7\).

**Alternative antibiotics such as** vancomycin, clindamycin, and erythromycin are given to pregnant women who are allergic to penicillin. Emerging clindamycin and erythromycin resistance strains have been found in several parts of the world, including Egypt\(^8\).

However, available data suggests that erythromycin and clindamycin given to pregnant women may not reliably reach foetal tissues, and cefazolin may be a suitable alternative for patients who do not have a severe penicillin allergy\(^9\).

GBS strains are able to cause infections not only because of the development of resistance but also due to their virulence traits. The most important virulence factor is capsule, but other virulence factors include; surface protein *Rib* & CSa peptidase\(^9\).

A substantial percentage of GBS strains, that have caused invasive infections in newborns, have the invasin *rib* protein, which is generated by the *rib* gene\(^9\),\(^10\).

CSa peptidase is a surface enzyme that can deactivate the human complement component CSa. The *scpB* (Streptococcal CSa Peptidase) gene encodes it, and it may be horizontally transmitted between pyogenic streptococci. CSa peptidase also makes GBS strains adhere to epithelial cells and extracellular matrix proteins easier\(^11\).

*scpB* gene is used as a standard gene for checking prevalence rate of GBS in pregnant women via polymerase chain reaction (PCR) due to its high prevalence among GBS human isolates\(^12\). Only strains with the *scpB* gene are thought to be infective to humans\(^11\).

These virulence proteins have been studied as possible vaccine candidates due to their capacity to generate a strong protective immunity against GBS infections\(^13\).

The sensitivity of cultures in identifying GBS colonisation ranges from 54-87%, results are acquired in 36 to 72 hours, and identification of colonies, which are not necessarily beta-hemolytic, requires an experienced technician\(^14\).

Rapid methods of identifying GBS colonisation in pregnant women have been available in recent years, such as DNA probes and nucleic acid amplification tests (NAAT) like PCR, and they have become the primary method of research. PCR is said to be highly sensitive and specific, with results appearing in 30 to 45 minutes\(^14\).

The aim of this study was to investigate how common GBS was in pregnant women, as well as the antibiotic sensitivity pattern of isolated GBS colonies and if the *scpB* and *rib* virulence genes were present.

SUBJECTS AND METHODOLOGY

**Subjects**

The current study was a cross-sectional observational study done on 203 pregnant women who attended the Maternity Hospital outpatient clinic at the Faculty of Medicine, Ain Shams University Hospital in Cairo, Egypt, From September 2020 to February 2021. The mean age among cases was 26.2±5.9 and the mean gestational age was 36.1 ±0.8. Before taking samples, all subjects gave their informed consent,
as required by the “Ethical Committee of Scientific Research of the Faculty of Medicine, Ain Shams University, Cairo, Egypt,” and in accordance with “The World Medical Association’s Code of Ethics (Declaration of Helsinki) for human experiments.”

**Inclusion criteria**

Women between 35 and 37 weeks gestation, women with a history of complications in past pregnancies, such as termination, preterm birth, prolonged rupture of membranes for 18 hours or more, intrapartum fever of 38°C or more, and women with a previous GBS infected baby were all included in the study.

**Exclusion criteria**

Taking antibiotics within the previous month for any reason, a woman in labour (“The candidate has regular and painful uterine contractions causing cervical dilatation and effacement”), a woman with infection involving urinary tract or vagina in the current pregnancy, vaginal douche 24 hours before screening, or vaginal bleeding were all reasons for exclusion from the study.

**Methods**

The steps in the research process were as follows:

1. Obtaining a complete medical history as well as clinical data
2. Specimen collection, transport and processing
3. Detection of antimicrobial susceptibility pattern in GBS strains
4. Molecular test (PCR) for the detection of scpB & rib genes
5. Analyzing data

The following procedures were performed on all of the enrolled patients:

**Obtaining a complete medical history as well as clinical data**

Patient’s age, parity, gestational age, obstetric complications, any current medical or surgical diseases and any current medications.

**Specimen collection, transport and processing**

A sterile cotton swab was rotated against the vaginal wall for vaginal sample then inoculated directly onto CHROMagar™ Strep B (CHROMagar microbiology, France) as well as a sheep blood agar. CHROMagar™ Strep B medium selectively inhibits most of normal flora bacteria and yeasts. On this medium, GBS in aerobic conditions produce characteristic pink mauve colonies.

The plates were immediately transported at room temperature to laboratory, Microbiology department, Faculty of medecine, ASU to be processed according to the recommendations of the American Society for Microbiology (ASM). Incubation was done aerobically at 37°C for 24 hours.

The following conventional techniques were used to identify the isolates: colonial morphology on CHROMagar™ Strep B, haemolysis on blood agar, Gram staining, catalase test, CAMP test, hippurate hydrolysis and latex agglutination test with specific antisera.

**Detection of antimicrobial susceptibility pattern in GBS strains**

Antibiotic susceptibility testing was done for the 23 GBS isolates by disk diffusion technique on Mueller–Hinton agar containing 5% sheep blood according to Clinical Laboratory Standards Institute (CLSI) guidelines.

The used antibiotics disks were Penicillin (10 units), Ampicillin (10µg), Cefepime (30µg), Ceftriaxone (30µg), Vancomycin (30µg), Clindamycin (2µg) and Erythromycin (15µg). The control strain used was S. pneumoniae ATCC 49619.

Double-disk diffusion method (D-zone test) to detect erythromycin resistance phenotype was performed.

**Molecular test (PCR)**

Conventional PCR was done for the detection of scpB and rib genes. DNA extraction was performed using Qiagen DNeasy (Qiagen, USA) according to the manufacturer instructions. Amplification of scpB gene was carried out using the Forward primer: ACAACGGAA GGCCTACTGTTC and the Reverse primer: ACTGGTGTGGACCTGAACTA (Invitrogen by Life Technologies, Thermo Fisher Scientific Inc., USA), While amplification of rib gene was carried out using the Forward primer: CAGGAAGTGCTGTTACGTTAC and the Reverse primer: CTGCACCATTAGGTTCTTCC (Invitrogen by Life Technologies, Thermo Fisher Scientific Inc., USA). An initial denaturation phase at 95°C for 2 minutes was followed by 35 amplification cycles of denaturation at 95°C for 30 s, annealing at 55°C for
60 s, extension at 72°C for 60 s, and a final cycle of extension at 72°C for 10 minutes. Every reaction includes negative extraction and master mix controls. The size of the amplicons was confirmed by electrophoresis on 1% agarose gel at 255 bp for scpB gene and 369 for rib gene18.

**Data assessment**

The IBM Statistical Program for Social Science (SPSS) version 25.0 was used to analyse the data. The mean and standard deviation were used to convey quantitative data. Frequency and percentage were used to convey qualitative data.

**RESULTS**

Positive GBS result was present in 11.3% of case (23 cases out of 203 total candidates). The prevalence of GBS among cases as regard age and gestational age

The mean age in negative GBS cases was 26.23±5.98 years while it was 26.70±5.6 in positive cases. The mean gestational age was 36.11±0.87 in negative cases while it was 36.43±0.79 in positive cases. There was no significant difference between negative and positive GBS cases as regard age and gestational age (P value > 0.05) as shown in Table 1.

The prevalence of GBS among cases as regard Obstetric characteristics of the participants

There was no significant distinction among cases with different parity as regard the GBS result (P value > 0.05); 7%, 13% and 11.1% of P0, P1-2 and P3-4 cases were positive for GBS result respectively as shown in Table 2.

On the other hand, there was a highly significant difference between cases with and without history of previous abortion as regard the GBS result (P value < 0.05); 29.6% of cases with

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**Table 1. Comparison between GBS positive and negative as regard age and gestational age**

<table>
<thead>
<tr>
<th></th>
<th>GBS</th>
<th></th>
<th>P-value*</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>26.23 a 5.98</td>
<td>26.70 b 5.60</td>
<td>0.72* NS</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>36.11 c 0.87</td>
<td>36.43 d 0.79</td>
<td>0.09* NS</td>
<td></td>
</tr>
</tbody>
</table>

*Student t test; Sig: significance, NS: non-significant

**Table 2. Relationship between parity and GBS result among cases**

<table>
<thead>
<tr>
<th>Parity</th>
<th>GBS</th>
<th>P-value*</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>40</td>
<td>93.0%</td>
<td>100</td>
<td>87.0%</td>
</tr>
<tr>
<td>3</td>
<td>7.0%</td>
<td>15</td>
<td>13.0%</td>
</tr>
</tbody>
</table>

*Chi-Square Tests; N: Number, Sig: significance, NS: non-significant

**Table 3. Relationship between Previous abortion and GBS result among cases**

<table>
<thead>
<tr>
<th>Previous abortion</th>
<th>GBS</th>
<th>P-value*</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>161</td>
<td>91.5%</td>
<td>19</td>
<td>70.4%</td>
</tr>
<tr>
<td>15</td>
<td>8.5%</td>
<td>8</td>
<td>29.6%</td>
</tr>
</tbody>
</table>

*Fisher exact test; N: Number, Sig: significance, HS: Highly-significant
previous abortion had positive GBS compared to 8.5% only of cases without previous abortion as shown in Table 3. Moreover, there was a highly significant difference between cases with and without history of previous preterm birth as regard the GBS result (P value < 0.05); 43.8% of cases with previous preterm birth had positive GBS compared to 8.6% only of cases without previous preterm birth as shown in Table 4.

**Culture and biochemical reaction results**

Direct plating of GBS from vaginal specimens onto CHROMagar strep B or sheep blood agar was similarly sensitive in detecting GBS. All pink mauve colonies on CHROMagar strep B were proven to be GBS. On the other hand, not all beta hemolytic colonies or tiny nonhemolytic catalase negative colonies on sheep blood agar proved to be GBS, and the isolation of the suspected GBS colonies took greater work.

The CAMP test, Hippurate test, and latex agglutination assay (Oxoid, UK) were used to confirm the presence of GBS in all gram positive and catalase negative cocci isolates.

**Antimicrobial susceptibility testing results**

GBS isolates were 100% sensitive to penicillin, ampicillin, cefepime, ceftriaxone, cefotaxime, vancomycin, levofloxacin and linezolid. Sensitivity to clindamycin and erythromycin was (73.91%, 60.86%) respectively. Antimicrobial susceptibility results are shown in Table 5.

Concerning D-zone test result, 44.4% (4/9) of erythromycin resistant isolates were found to have cMLSB (constitutive macrolide lincosamide Streptogramin B resistance), while iMLSB resistance (inducible macrolide lincosamide Streptogramin B resistance) was detected in three isolates (3/9; 33.3%). M phenotype (erythromycin resistant and clindamycin susceptible) was reported in two isolates (2/9; 22.2%).

**PCR results**

scpB gene was found in all GBS isolates (100%) while only 18 isolates tested positive for the rib gene (78.26%) as shown in Table 6.

**DISCUSSION**

Neonatal infections are linked to GBS rectal or vaginal colonisation during pregnancy. Thus, screening pregnant women for GBS colonisation and determining the isolates’ antibiotic susceptibility are critical for the prevention and treatment of streptococcal infections in both mothers and their newborns.20.

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### Table 4. Relationship between Previous preterm birth and GBS result among cases

<table>
<thead>
<tr>
<th>Previous preterm birth</th>
<th>P-value*</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>N: 171</td>
<td>%: 91.4%</td>
</tr>
<tr>
<td>Yes</td>
<td>N: 16</td>
<td>%: 8.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GBS Negative</th>
<th>9</th>
<th>56.3%</th>
<th>0.001*</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>43.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test; N: Number, Sig: significance, HS: Highly-significant

### Table 5. Description of Antibiotic sensitivity for positive GBS cases

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Cefepime</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Linezolid</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>17</td>
<td>73.91%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>14</td>
<td>60.86%</td>
</tr>
</tbody>
</table>

**SCP**

scpB Negative 0 0.0%
scpB Positive 23 100.0%

**Rib**

rib Negative 5 21.7%
rib Positive 18 78.3%

N: Number
The aim of this study was to investigate how common GBS was in pregnant women, as well as the antibiotic sensitivity pattern of isolated GBS colonies and if the scpB and rib virulence genes were present.

The study included 203 pregnant females. Vaginal swab samples from the involved pregnant women were collected for culture. The mean age among cases was 26.2±5.9; the mean gestational age was 36.1 ±0.8. About 57% of cases were P1-2; the majority of cases (86.7%) had no previous abortion, while about 8% had previous preterm birth.

The prevalence rate of GBS detected among pregnant women was 11.33%. Our findings agreed with those of many countries that also detected a prevalence rate ranging between (10-30%) as Blantyre (16.5%)\textsuperscript{21}, Egypt (17.89%)\textsuperscript{22}, Kuwait (16.4%)\textsuperscript{23}, Zimbabwe (21%)\textsuperscript{24}, Tanzania (23%)\textsuperscript{25}, Belgium (22%)\textsuperscript{26}, Netherlands (21%)\textsuperscript{27}, Germany (23%)\textsuperscript{28} and Sweden (25.4%)\textsuperscript{29}.

However, when compared to other countries, such as Mozambique (1.8%)\textsuperscript{30}, Iran (4.8%)\textsuperscript{31}, the Philippines (7.5%)\textsuperscript{32}, and France (8%)\textsuperscript{33}, the prevalence rate of this study is greater. Many factors, such as gestational age at culturing, variations in culture sites, sensitivity of culture methods, various demographics, personal cleanliness, or intensive use of antiseptics or antibiotics, might explain the varied prevalence rates\textsuperscript{27}.

Our findings revealed that the majority of GBS positive pregnant women were between the ages of 20 and 30, and that GBS colonisation is more common among pregnant women in the 37th week of pregnancy, but the frequency of GBS colonisation among different age and gestational age groups was not statistically significant.

Vaginal flora contains abundant microorganisms, so the isolation of GBS won’t be easy and the overgrowth of other organisms should be avoided. This mission can be aided by chromogenic media that discriminate GBS colonies. The CDC recommendations published in 2002 encouraged the development of these media in order to increase the accuracy of culture results and make culture processing easier\textsuperscript{6}.

In our study, a selective chromogenic medium (CHROMagar™ Strep B) was used. It enabled the identification of GBS as characteristic pink mauve colonies by direct visual inspection within 24 hours. Other bacterial species may be inhibited, or colonies of a different colour may form (e.g. blue, colorless).
It also allowed all GBS strains to grow in an aerobic environment, regardless of their haemolytic characteristics.

Both CHROMagar™ Strep B and sheep blood agar performed similarly, yielding the same results. The work to isolate suspected GBS colonies from blood agar, on the other hand, was quite intensive. Similar findings were reported in studies accomplished by Sadaka et al. 18, El Aila et al. 26 and Tazi et al. 49,50.

In contrast to our results, some studies reported lower sensitivity of blood agar in comparison to CHROMagar™ Strep B 15,51,52.

Sensitivity to penicillin and ampicillin is highly variable in different studies. In our study it was 100% to both antibiotics. This coincides with findings of Mohammed et al. 36, Brandon and Dowzicky 53, Adawaye et al. 54, Arif et al. 55 and Mengist et al. 56, who found that GBS isolates were 100% susceptible to both penicillin and ampicillin. Our results are different from those of El Aila et al. 45, Kimura et al. 57, Longtin et al. 58 and Banno et al. 59 who detected reduced susceptibility to both penicillin and ampicillin.

Non of the isolates was resistant to cefepime, ceftriaxone and cefotaxime. This coincides with results of Sadaka et al. 18, Elikwu et al. 60, Ji et al. 61 and Gomi et al. 62 who detected 100% sensitivity to these antibiotics.

On the other hand ceftriaxone and cefotaxime non-susceptible GBS isolates were found in studies by Simoes et al. 63, Kitamura et al. 64 and Mudzana et al. 65.

GBS isolates in our study were 100% sensitive to vancomycin. This agrees with many studies who also reported the same result 18,48,55.

In our study GBS isolates to vancomycin (76% and 80% respectively). This result is higher than that of El Aila et al. 49 who detected 31% sensitivity to clindamycin.

The same findings were detected by Hannoun et al. 10 and Eskandarian et al. 57 and. On the contrary Sadaka et al. 18 reported that only 56.6% of isolates were sensitive to levofloxacin.

Our isolates were also 100% sensitive to linezolid which is in consistent with results of Sadaka et al. 18 and Arif et al. 55.

Sensitivity to clindamycin was 73.91%. This was quite similar to Sadaka et al. 18, Arif et al. 55, Ghanbarzadeh et al. 68 and who reported the sensitivity to clindamycin as 75%, 76.92% and 85% respectively.

This result is higher than that of El Aila et al. 49 who detected 31% sensitivity to clindamycin. On the other hand, non of the isolates was sensitive to clindamycin.

In our study GBS sensitivity to erythromycin was (60.86%). This agrees with El Aila et al. 45, Arif et al. 55 and Ghanbarzadeh et al. 68 who detected reduced sensitivity to erythromycin.

On the other hand other studies detected 100% sensitivity to erythromycin 36,54,56.

The prevalence of virulence genes, namely scpB and rib among the 23 strains detected in our study is high. Other unknown virulence traits may be also present as the pathogenicity of GBS is related to multiple genes.

In our study, the scpB gene was found in all of the isolates. Some studies suggest that the presence of the scpB gene in GBS human isolates is required, and only strains carrying the scpB gene are infectious to humans 69.

This result agrees with Sadaka et al. 18, Dmitriev et al. 69, Dutra et al. 70 and Bobadilla 71 who also detected the presence of scpB gene in all isolates. However, other studies by Hannoun et al. 19, Mudzana et al. 65 and Lysakowska et al. 11 detected scpB in 94.7%, 90.1% and 92.5% of human isolates respectively.

On the other hand, the presence of rib gene was confirmed only in 18 isolates (78.26%). This result coincides with Sadaka et al. 18 and Mudzana et al. 65 who detected the presence of rib gene in 79.2% and 69.8% of isolates respectively, while Hannoun et al. 10 and Lysakowska et al. 11 detected this gene in only 35% and 33% of isolates respectively.

The inability to follow up the pregnant women who had a positive GBS culture to ascertain the rate of newborn infection or other complications was the most significant drawback of our study. Another disadvantage is that due to funding constraints, GBS serotyping was not done.

CONCLUSION

Screening is a quick and sensitive procedure that is both helpful and cost-effective,
particularly for individuals who present at term with unclear GBS colonisation status as well as those who are in preterm labour. Further research on the prevalence of virulence genes of GBS among pregnant women is required to obtain complete data about the pathogenicity of GBS bacteria and support vaccine development in the near future.

ACKNOWLEDGMENTS
None.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
All authors have made substantial contributions to conception and design of the study. AME and NG contributed to samples collection and acquisition of patients’ data. WAI and NG contributed to all laboratory work with analysis and interpretation of data. LA, WAI, AME and NG contributed to drafting the article. FM, LA and WAI and contributed to revising the draft critically for important intellectual and scientific content. AME revised the clinical content. All authors provided final approval of the version to be published.

FUNDING
None.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
The study was carried out in accordance to the recommendations of the Ethical Committee of Scientific Research of Faculty of Medicine, Ain Shams University, Cairo, Egypt (No. FMASU M D 215/2018) and according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

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