Abstract: The criterion of total bile acid (TBA) concentration for the diagnosis of intrahepatic cholestasis in pregnant women remains debated. The purpose of this study was to establish a reference range of TBA concentration in Chinese pregnant women. Clinical data of Chinese women aged >18 years with singleton pregnancy were retrospectively analyzed. The blood samples were collected during pregnancy and postpartum, respectively. The concentration of TBA was quantitatively measured by using enzymatic electrochemiluminescence. In total, 11022 pregnant women who attended for antenatal care in our hospital were recruited in this clinical trial. The serum bile acid levels were assayed at the 23rd to 25th, 27th to 29th and 35th to 37th weeks of pregnancy and at 1-3 days postpartum. Different reference ranges for the TBA concentration differs were determined for each trimester. The TBA level was steadily elevated from the 24th (1.54±0.89 µmol/L) to 36th week (2.25±1.38 µmol/L) of pregnancy and reached the peak level at the 36th week (2.97±1.28 µmol/L) of pregnancy, and subsequently tended to decline after birth delivery (2.31±1.87 µmol/L). The serum levels of TBA significantly fluctuate during each trimester in pregnant women. The TBA levels increase over the gestational age and then dramatically decline after birth delivery. The reference threshold of TBA in Chinese pregnant women was investigated, which might be refered to the diagnose of liver disorders for pregnant women.

Keywords: Bile acid, reference range, intrahepatic cholestasis, pregnancy

Introduction

Pregnancy can provoke physiological, hormonal and physical changes and exert effect upon all organs for the pregnant women. The blood supply by the liver remains unchanged or tends to decline reported by several publications, accounting for 35% of the cardiac output in non-pregnant females and 28% in pregnant counterparts. Liver dysfunction has been commonly encountered in pregnant women, which affects the physical and physiological health of both mother and fetus. Liver disease occurring during pregnancy can be divided into three categories including pre-existing disease, pregnancy specific disease and coincidental acute liver or biliary tree disease [1]. Although the physiological changes in liver function during pregnancy are constantly transient, minor liver dysfunction may be a harbinger of life-threatening processes. Early and accurate diagnosis of liver dysfunction in conjunction with correct identification of relevant symptoms and signs such as pruritus, upper abdominal pain, and jaundice at an early stage can lead to timely management, thereby improving the clinical prognosis of both mother and fetus.

Intrahepatic cholestasis of pregnancy (ICP), known as obstetric cholestasis, is a rare but most common pregnancy-specific liver disorder, primarily manifesting during the third trimester and associated with increased perinatal morbidity and mortality [2]. The prevalence of ICP, influenced by genetic and environmental factors, significantly varies among different populations. In China, the estimated prevalence of ICP considerably differs among varying geographic locations, with the highest incidence of ICP in Sichuan and Zhejiang provinces. ICP is typically a transient and benign process for pregnant women. However, ICP has
been consistently associated with a high risk of fetal complications, such as an increased risk of fetal distress, spontaneous preterm labor and unexplained stillbirth.

Although multiple laboratory parameters, such as elevated transaminases or serum bile acid ratios, have also been described to diagnose ICP, the elevated serum concentration of total bile acid (TBA) remains the most reliable diagnostic marker for ICP [3]. The risk of ICP-induced complications for the fetus is associated with the serum level of maternal TBA, especially higher risk for those women with more severe ICP. In spite of potential impact of TBA on the fetal morbidity and mortality, there is a paucity of information regarding the temporal roles of TBA plays in the diagnosis and assessment of ICP. First, the TBA changes during pregnancy are confounded by not only a wide range for the diagnosis of ICP in the current literature, but also by the fact that current reference ranges are generally for fasting values of TBA and exclude the TBA levels of pregnant women. Second, the definition of ICP based on laboratory criteria is further complicated by various methodologies used to measure the TBA concentration including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), enzymatic-spectrophotometric assay, enzymatic colorimetric assay, enzymatic cycling assay and enzymatic electrochemiluminescence.

In recent years, sensitive biochemical assays have been advanced and the understanding of gestation-dependent trends of liver hormones has been deepened. Nevertheless, clinically useful gestation-specific reference ranges of TBA have been scarcely studied. Dramatic physiological changes in maternal hormones and their binding proteins complicate the assessment of normal levels of most hormones and the interpretation of the testing results during gestation. Moreover, no established gestation specific reference intervals have been determined in the Chinese population. Current findings are inconsistent throughout different literatures and should not be extrapolated due to differences in ethnicity, laboratory assay method and selection of reference population, sample size and assessment of outliers, etc. Therefore, a reliable gestation-specific reference value is urgently required for the diagnosis of liver disorders in pregnant women.

Currently used references ranges have been established for male adults and non-pregnant females. The biochemical threshold of TBA to diagnose ICP is uncertain for pregnant women. In this study, the reference threshold of TBA in Chinese pregnant women was investigated for the first time, aiming to analyze the changing pattern of TBA over pregnancy in Chinese pregnant females.

Materials and methods

Study subjects

Clinical data of 11022 Chinese women aged > 18 years with singleton pregnancy who were admitted to regular outpatient prenatal clinics from January 2005 to December 2014 at Women’s Hospital, School of Medicine, Zhejiang University, China, were retrospectively analyzed from an electronic database. Our institution was rated as a tertiary referral center in obstetrics and gynecology with a large fertility clinic. Pregnant women primarily attended prenatal clinics in a community hospital before 20 weeks of pregnancy and then were regularly followed up until 1 week post-delivery. Venous blood samples were collected for quantitatively detection of TBA concentration after an overnight fasting at the 23rd-25th, 27th-29th and 35th-37th weeks of pregnancy and at 1-3 days after delivery.

Inclusion and exclusion criteria

The inclusion criteria included: pregnant women at or over the age of 18, singleton gestation with a viable fetus, and the absence of any other risk factors for ICP. Exclusion criteria included: <18 y or > 41 y; the presence of pruritus, multiple gestation, diabetes, pre-eclampsia, known history of hypercholesterolemia or dyslipidemia, ICP in a previous pregnancy, current history of ICP, known liver disease (e.g. hepatitis, cirrhosis), current use of corticosteroids

Ultrasound analysis

Gestational age of most women was confirmed by ultrasound based upon the first day of the last menstrual period, and dependent upon the difference of the biparietal diameter between approximately the 20th week and the menstrual date added by or minus 14 days in several cases. If the pregnant woman was uncertain
Total bile acid measurement

about the date of the last menstrual period or had used oral contraceptives within 6 months prior to gestation, ultrasound examination was performed to further determine the gestational age. Demographic data including maternal age, parity, delivery time, and anthropometric measurements such as height, weight at the time of delivery were collected from each subject.

Measurement of TBA concentration

Fasting blood samples with a portion of approximately 6 ml were prepared for subsequent quantitative detection of serum TBA levels by using enzymatic electrochemiluminescence methods at the 23rd to 25th, 27th to 29th and 35th to 37th weeks of pregnancy and at 1-3 days postpartum, respectively. All test procedures were approved by the clinical ethics committee of Women’s Hospital affiliated to Zhejiang University. Written informed consents were obtained from 11022 pregnant women enrolled in this investigation.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) with extremes. Analysis of repeated measurements was performed to obtain the estimated mean ± SD of the main outcome and the outcome among different time points were analyzed by pairwise comparison performed with Tukey’s method. This modeling approach can accommodate an arbitrary pattern of missing data, assuming the data was missing at random. In this model, time was used as a predictor. A P value of less than 0.05 was considered as statistical significance. Statistical analyses were performed using version 9.3 SAS statistical software.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Results

Patient demographics and characteristics

A total of 15462 pregnant women without any complications, aged from 18 to 41 years, were initially included in this investigation. After preliminary screening by inclusion and exclusion criteria described above, 11022 women were eventually eligible for subsequent detection. Venous blood samples were collected for serum TBA concentration at the 23rd to 25th, 27th to 29th and 35th to 37th weeks of pregnancy and at 1-3 days postpartum, respectively (Figure 1).

Median maternal age was (29.36±3.73) years. Mean height was (160.97±4.72) cm, and mean weight at the time of delivery was (67.58±9.18) kg. Mean pregnancy duration was (38.76±1.91) weeks and mean parity was (1.84±1.09). Ninety women (0.58%) received in vitro fertilization as illustrated in Table 1 in details.

Reference threshold of TBA concentration

As shown in Table 2, the reference standards or the (mean ± SD) ranges for the serum TBA

![Figure 1. TBA concentration in healthy pregnant women in the 23rd to 25th (n=2427), 27th to 29th (n=964), and 35th to 37th (n=5005) weeks of pregnancy and 1-3 days after delivery (n=4478), expressed as mean ± SD, paired-sample t-test. * denotes statistical significance compared to 27-29 weeks of pregnancy; **represents P < 0.01; ***stands for P < 0.001. † denotes statistical significance compared to 35-37 weeks of pregnancy; ††represents P < 0.05; †††represents P < 0.01. ††††represents P < 0.001. # denotes statistical significance compared to post-delivery; # stands for P < 0.05, ##represents P < 0.01, ###stands for P < 0.001.](image-url)
Total bile acid measurement

Table 1. Maternal demographics

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>Number of women</th>
<th>Age (year)</th>
<th>Height (cm)</th>
<th>Parity</th>
<th>Gestational age at delivery (week)</th>
<th>Weight at delivery (kg)</th>
<th>IVF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (23-25)</td>
<td>2427</td>
<td>29 (21-44)</td>
<td>161 (140-178)</td>
<td>2 (1-7)</td>
<td>39 (25-42)</td>
<td>68 (42-167)</td>
<td>0.91</td>
</tr>
<tr>
<td>28 (27-29)</td>
<td>964</td>
<td>29 (18-42)</td>
<td>161 (145-179)</td>
<td>2 (1-8)</td>
<td>38 (28-42)</td>
<td>67 (42-145)</td>
<td>0.41</td>
</tr>
<tr>
<td>36 (35-37)</td>
<td>5005</td>
<td>30 (18-45)</td>
<td>161 (140-180)</td>
<td>2 (1-11)</td>
<td>38 (27-42)</td>
<td>68 (42-170)</td>
<td>0.8</td>
</tr>
<tr>
<td>Postnatal (days 1-3)</td>
<td>4478</td>
<td>30 (18-46)</td>
<td>161 (140-179)</td>
<td>2 (1-8)</td>
<td>39 (27-42)</td>
<td>68 (42-163)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 2. Serum concentration of TBA in 11022 healthy pregnant women at 23th to 25th, 27th to 29th and 35th to 37th weeks of pregnancy and 1-3 days after delivery

<table>
<thead>
<tr>
<th>Gestation (week)</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA (µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 (23-25)</td>
<td>2427</td>
<td>1.54</td>
<td>0.89</td>
<td>0.3-8.6</td>
</tr>
<tr>
<td>28 (27-29)</td>
<td>964</td>
<td>2.25</td>
<td>1.38</td>
<td>1.0-13.2</td>
</tr>
<tr>
<td>36 (35-37)</td>
<td>5005</td>
<td>2.97</td>
<td>1.28</td>
<td>0.5-12.1</td>
</tr>
<tr>
<td>Postnatal (days 1-3)</td>
<td>4478</td>
<td>2.31</td>
<td>1.87</td>
<td>0.5-10.0</td>
</tr>
</tbody>
</table>

concentrations in each trimester were described. The serum TBA level at 24 weeks of pregnancy was measured as (1.54±0.89) µmol/L with a reference range from 0.3 to 8.6 µmol/L. The TBA level was considerably increased up to (2.25±1.38) µmol/l with a reference range of 1.0-13.2 µmol/l (P < 0.05). The peak TBA level was observed at 36 weeks of pregnancy up to (2.97±1.28) µmol/l (P < 0.05), and then gradually declined to (2.31±1.87) µmol/l after delivery (P < 0.05) with a reference range of 0.5-10.0 µmol/l.

Discussion

Bile acids are synthesized from cholesterol in the liver. Individual serum bile acids are endogenous markers reflecting the transport and synthesis function of the liver. The rate of fetal complications increases when maternal serum bile acid levels become abnormal in women who develop ICP. Thus, the clinical importance of ICP lies in the potential fetal risks, especially sudden fetal death. Although most experts generally agree that the diagnosis of ICP is contingent on the presence of pruritus, the absence of a rash and the presence of an elevated serum TBA concentration, the reference standard of TBA concentration threshold remains debated. The diagnosis of ICP depends upon serum TBA concentration diagnostic test, which provides population and method-specific reference ranges. It has been demonstrated that the reference ranges of TBA concentration are impacted by pregnancy. Adams also reported that fasting TBA levels may be the most sensitive indicator of severe liver disease for pregnant patients as postprandial levels may be elevated at baseline [4]. However, the pregnancy-specific reference ranges for TBA concentration in ICP pregnant women is urgently required. Otherwise, women with subclinical high TBA levels might be misdiagnosed as normal subjects.

To establish the reference intervals for TBA concentration, multiple factors, such as ethnic background, laboratory measurement techniques, definition of reference population, exclusion criteria, and method of statistical analysis need to be considered, which may justify the inconsistency in the results among different investigations. The literatures related to ICP have reported several different reference thresholds for the TBA concentration using different testing methodologies. Previous investigations reported varying cutoff points for TBA levels ranging from > 2 to > 20 µmol/L [5-9]. Different approaches, such as radioimmunoassay, enzymatic and colorimetric method were employed to quantitative detection of TBA levels. Lee et al. have suggested that the upper TBA concentration threshold for healthy women from Latina is measured as 8.5 µmol/L based on LC-MS/MS measurement [10]. Although the guideline of ICP by Obstetrics Branch of Chinese Medical Association and other international organizations propose 10 µmol/L as the cutoff standard to diagnose ICP, the laboratory testing methods used to attain those values are still not defined.

In present investigation, the reference ranges for serum liver enzymes in each trimester were
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample/detection methods</th>
<th>Sample size and design</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; trimester</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; trimester</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; trimester</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacq et al., 1996*</td>
<td>France</td>
<td>Serum/Enzymic fluorimetric method</td>
<td>103 cross-sectional n=34</td>
<td>2.3±2.6 (0.5-13.0)</td>
<td>1.9±1.8 (0.6-6.5)</td>
<td>1.8±2.2 (0.5-12.0)</td>
<td>-</td>
</tr>
<tr>
<td>Egan et al., 2012#</td>
<td>Ireland</td>
<td>Serum/Randox Total Bile Acids Assay</td>
<td>219 cross-sectional n=48</td>
<td>1.68, 9.05 (1.2-9.8)</td>
<td>1.29, 6.72 (0.3-8.7)</td>
<td>1.80, 8.22 (1.3-12.0)</td>
<td>0.95, 7.24 (0.3-11.6)</td>
</tr>
<tr>
<td>Lee et al., 2013&amp;</td>
<td>California</td>
<td>Serum/Liquid chromatography-tandem mass spectrometry (LC-MS/MS)</td>
<td>211 cross-sectional n=112</td>
<td>5.6±1.5 (8.5)</td>
<td>5.4±2.3 (8.7)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chen et al., 2016*</td>
<td>China</td>
<td>Serum/Enzymatic electrochemiluminescence</td>
<td>11022 cross-sectional n=247</td>
<td>1.54±0.89 (0.3-8.6)</td>
<td>2.97±1.28 (0.5-12.1)</td>
<td>2.31±1.87 (0.5-10.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

*All data are expressed as mean ± SD with extremes in parentheses. #All data are expressed as the 25<sup>th</sup> and 75<sup>th</sup> percentiles of the samples with extremes in parentheses. &All data are expressed as mean ± SD with upper bound 95% reference ranges in parentheses.
separated investigated in 11022 pregnant women. Statistical significance was observed in terms of the reference intervals of TBA level among different gestational ages, which is consistent with the reports from other countries and regions, suggesting the importance of gestational age-specific reference values for TBA levels in a specific population to avoid misclassification of patients with liver dysfunction during pregnancy. As illustrated in Table 3, trimester-specific reference intervals of TBA concentration in pregnant women from different nations were summarized. In these studies, statistical comparison was performed based upon the nationality, sample size and study design and methods.

The relationship between the serum TBA level and the gestational age in pregnant women remains debated. Longitudinal studies investigating serum TBA or postprandial cholyglycine throughout the period of pregnancy revealed that the serum TBA was significantly elevated from the 15th week to the 40th week of gestation [11, 12]. Subsequently, Barth et al. have demonstrated that TBA concentration remains unchanged during normal pregnancy and is lower than 3.0 μM in spite of increasing estrogen concentrations and showed an upward tendency postpartum [13]. These studies are in accordance with the results of Pascual et al. that (2.1±0.1) μM in the first trimester and increased to (3.1±0.1) μM in the third trimester, which was defined as asymptomatic hypercholanaemia of pregnancy with TBA values higher than 6.0 μM [14]. Our results have indicated that the derived reference intervals of TBA concentration for pregnant women are significantly different. The lower and upper reference thresholds of TBA concentration are the major discrepancy between present study and previous reports [15]. However, Bacq et al. [16] reported significantly lower upper reference threshold of TBA levels during the second trimester than that of the present study. In this study, the serum TBA concentration at 24 weeks of pregnancy was considerably increased at 36 weeks of pregnancy up to (2.97±1.28) μmol/L, and then considerably declined after delivery.

There are lines of evidence suggesting that bile acids may be responsible for the worst possible clinical prognosis associated with ICP, which is sudden intrauterine fetal death. However, the TBA level at which such outcome can be predicted is uncertain. Glantz et al. [7] demonstrated that the bile acid concentration ≥ 40 μmol/L was associated with an increased risk for adverse complications, such as preterm delivery, asphyxial events, meconium staining, etc. Alternatively, we have found that fetal death occurred in pregnant women with lower or normal bile acid concentrations [17]. Now that we have established population-specific reference ranges in the Chinese population in Zhejiang province, research is needed to elucidate the fetuses are at risk for spontaneous fetal death and whether or not the TBA concentration can be used to predict or classify the fetuses are at risk for this clinical outcome.

There are several limitations to be acknowledged in our study. Current survey is a cross-sectional study on different women of different gestational ages instead of longitudinal self-sequential survey. Gestational ages were calculated based on the last menstrual period and not confirmed by ultrasonography. A sample of non-pregnant women and longer time interval after delivery is required to enable us to more accurately compare the changes in these hormones. A control group is lacking. Next, we would compare our findings with non-pregnant matched women, but the purpose of our study was to compare the pregnant women with current reference ranges for TBA level obtained by laboratory testing.

Taken together, the reference range for TBA concentration in pregnant Chinese women diagnosed with ICP differs from the values obtained in the laboratory testing according to different trimesters. Further research is required to investigate the clinical utility of quantitative measurement of TBA and alternative biomarkers of liver dysfunction in relation to adverse pregnancy outcomes in ICP pregnant women.

Acknowledgements

The authors are grateful to Ms Chaonan Zhu, Le9 Healthcare Technologies, Inc. (Shanghai, China), for the assistance of clinical data extraction, query and statistical analysis. This study was supported by the foundation of
Health and family planning commission of Zhejiang Province, China [No. 2014KYA 253].

Disclosure of conflict of interest
None.

Address correspondence to: Jing He, Department of Obstetrics and Gynecology, Women's Hospital, Zhejiang University School of Medicine, 1 Xue Shi Road, Hangzhou 310006, China. Tel: 0086-57-187061501; Fax: 0086-57-187061501; E-mail: hej@zju.edu.cn

References


