Intrahepatic Cholestasis of Pregnancy - time to redefine the reference range of total serum bile acids: a cross-sectional study

Mor Huri[1], Viola Seravalli[1], Camilla Lippi[1], Lorenzo Tofani[1], Andrea Galli[1], Felice Petraglia[1], and Mariarosaria Di Tommaso[2]

[1]University of Florence
[2]Careggi Hospital University of Florence

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Abstract

Objective To establish pregnancy-specific reference ranges for fasting and postprandial total serum bile acids (TSBA) levels. Design Cross-sectional study. Setting Tertiary care university hospital. Population Healthy pregnant women at term admitted to the Obstetrics Department over one year. Exclusion criteria were an established diagnosis of intrahepatic cholestasis of pregnancy (ICP) or any co-existing condition of increased risk for ICP. Methods and Main Outcome Measures Both fasting and postprandial TSBA levels were measured in 612 women (528 fasting and 377 postprandial samples). Results Reference intervals of 4.4-14.1 μmol/L for fasting TSBA, and 4.7-20.2 μmol/L for postprandial TSBA were established. The postprandial values were significantly higher than the fasting measurements, with a mean increase of 1.77 μmol/L (22%). A correlation between fasting TSBA levels and postprandial levels was found, as well as with fetal gender, parity, and the use of assisted reproductive technologies. A seasonal pattern was noticed for both fasting and postprandial TSBA, with the highest values in the winter season ($p < 0.01$ and 0.02, respectively). Conclusions Normal pregnancy is a sub-cholestatic state and is associated with a physiological elevation of TSBA levels, therefore a higher threshold should be considered for the diagnosis of ICP. We suggest using the upper reference limit observed in our healthy pregnant population (fasting [?]14 μmol/L and postprandial [?]20 μmol/L). As the fasting measurement is more specific for the diagnosis, and the postprandial is essential for severity assessment, it is recommended to measure both values, rather than use random samplings. Funding No funding to declare.

Huri Mor MD[1], Seravalli Viola MD[1], Lippi Camilla MD[1], Tofani Lorenzo MA[2], Galli Andrea MD PhD[3], Petraglia Felice MD[4], Di Tommaso Mariarosaria MD PhD[1]

[1]Department of Health Sciences, Obstetrics and Gynecology Unit, University of Florence, Florence, Italy.

[2]Department of Statistics, Computer Science, Applications, University of Florence, Florence, Italy.

[3]Department of Experimental and Clinical Biochemical Sciences, Gastroenterology Unit, University of Florence, Florence, Italy.

[4]Department of Experimental and Clinical Biomedical Sciences, Obstetrics and Gynecology Unit, University of Florence, Florence, Italy.

Corresponding author

Di Tommaso Mariarosaria, MD, PhD.

Department of Health Sciences, Obstetrics and Gynecology Unit, University of Florence.

Largo Brambilla, 3, 50134, Florence, Italy, Email: mariarosaria.ditommaso@unifi.it.

Tel 0039-335332709

Abstract

Objective

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Design

Cross-sectional study.

Setting

Tertiary care university hospital.

Population

Healthy pregnant women at term admitted to the Obstetrics Department over one year. Exclusion criteria were an established diagnosis of intrahepatic cholestasis of pregnancy (ICP) or any co-existing condition of increased risk for ICP.

Methods and Main Outcome Measures

Both fasting and postprandial TSBA levels were measured in 612 women (528 fasting and 377 postprandial samples).

Results

Reference intervals of 4.4-14.1 μmol/L for fasting TSBA, and 4.7-20.2 μmol/L for postprandial TSBA were established. The postprandial values were significantly higher than the fasting measurements, with a mean increase of 1.77 μmol/L (22%). A correlation between fasting TSBA levels and postprandial levels was found, as well as with fetal gender, parity, and the use of assisted reproductive technologies. A seasonal pattern was noticed for both fasting and postprandial TSBA, with the highest values in the winter season ($p < 0.01$ and 0.02, respectively).

Conclusions

Normal pregnancy is a sub-cholestatic state and is associated with a physiological elevation of TSBA levels, therefore a higher threshold should be considered for the diagnosis of ICP. We suggest using the upper reference limit observed in our healthy pregnant population (fasting [?]14 μmol/L and postprandial [?]20 μmol/L). As the fasting measurement is more specific for the diagnosis, and the postprandial is essential for severity assessment, it is recommended to measure both values, rather than use random samplings.

Funding

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Keywords: Intrahepatic cholestasis of pregnancy; total serum bile acids; reference ranges; diagnostic thresholds

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-associated liver disease. It classically presents in the third trimester, the cardinal clinical symptom is pruritus, and the specific laboratory examination is an elevated total serum bile acids (TSBA) level[1,2].

The prevalence of the condition is 0.2%-2%, but varies greatly among ethnic groups and geographic regions. The pathogenesis is multifactorial: increased reproductive hormones synthesis, environmental factors, genetic predisposition and underlying liver disease are all considered contributions to disease development

and severity[1,3-5]. ICP is a relatively nonthreatening condition to the mother, but it is associated with fetal complications such as spontaneous preterm delivery, meconium passage, and, in severe cases, intrauterine death[7]. The increased risk of stillbirth is possibly due to bile acids toxicity on fetal cardiomyocytes or to vasoconstriction of chorionic vessels[22-24].

Internationally, there is lack of consensus regarding the diagnostic criteria for ICP. Most guidelines agree on the requirement of pruritus accompanied by otherwise unexplained abnormal liver function, both of which resolve rapidly after delivery. TSBA are the most often used biomarker for the diagnosis[9-11].

The Royal College of Obstetricians and Gynaecologists (RCOG) guideline recommends that the upper limit of pregnancy-specific ranges of TSBA should be used for diagnosis[25]. Nevertheless, reference ranges used in clinical laboratories are most often fasting values measured in nonpregnant subjects, a result of extremely limited data for pregnancy-specific reference ranges[4,11,17,29]. Consequently, there is a wide range of diagnostic criteria for ICP. Most guidelines recommend the use of TSBA values of 10 to 15 μmol/L as a diagnostic threshold, but this may be reduced to 6-10 μmol/L in fasting women[1,9,10]. In clinical practice, the most widely used threshold is non-fasting 10 μmol/L, despite not being supported by strong evidence[9,29].

Recently, only extremely high TSBA levels (100 μmol/L or higher) were shown to markedly increase the risk of stillbirth[8]. Nevertheless, since no pharmacological treatment has been shown to reduce adverse perinatal outcomes[12,27,30], early delivery is still often recommended to prevent the subsequent risk of stillbirth[2,7,8]. Policies of active management result, however, in increased intervention, caesarean-section rate and iatrogenic prematurity that must be balanced against possible reductions in perinatal mortality. The diagnosis of ICP has serious implications for maternal, and especially fetal and neonatal health[18], therefore a correct diagnosis is essential.

The aim of this study was to investigate values of fasting and postprandial TSBA in healthy pregnant women and to establish the reference standard in pregnancy.

Materials and methods

This is a cross-sectional study of TSBA levels in pregnant women attending Florence Careggi University Hospital, a tertiary referral Maternity hospital. Healthy pregnant women at term admitted to the Obstetrics Department between 2020 and 2021 were offered participation. The reference population was defined "healthy", after excluding women with a pathology for which there is an association with the measurement being considered.

Inclusion criteria were singleton pregnancy; gestational age at or above 37 weeks; body mass index (BMI) between 17 and 40 kg/m^2.

Exclusion criteria were the presence of an established diagnosis of ICP or abnormal liver function tests at any time throughout the pregnancy. We also excluded any co-existing condition of increased risk for ICP such as: multiple pregnancy; personal history of ICP; personal history of liver disease (such as history of hepatitis B and C); cholecystectomy; history of gastric bypass surgery; and the inability to provide informed consent.

Both fasting (after 8-14 hours of fasting at 8 A.M.) and postprandial (2 hours after meal at 2 P.M.) TSBA were measured. The limited time frame in which the blood samples could be sent to the laboratory, as well as the dynamic nature of the obstetrics department, was the main limit to patients' inclusion. Not all the potential candidates eligible for the study could participate or give an informed consent. In particular, pregnant women who were sent to the delivery room before the blood sample was taken could not participate.

For each patient, both venous blood samples were collected whenever possible compatibly with the needs of the laboratory (as specified above), otherwise only one of the two blood samples was taken.

TSBA levels correspond to the sum of more than 20 individual bile acids[21], and were estimated by enzymatic-spectrophotometric assay, based on microbial 3α hydroxysteroid dehydrogenase. Blood samples were analysed

using Total Bile Acids Assay Kit (Sentinel Diagnostics CH. SpA, Milan, Italy) at the Careggi hospital clinical laboratory.

TSBA values were included for reference interval calculation, according to the International Federation of Clinical Chemistry and Clinical and Laboratory Standards Institute C28-A3 recommendations. An abnormal level was defined as a value exceeding the upper reference limit (97.5^{th})[32,35], as there is no known clinical significance to low levels of TSBA. In our laboratory, the normal range of TSBA in the general population lies between 0 and 6 μmol/L.

The laboratory results were collected in a database along with maternal and pregnancy characteristics. This information was obtained upon admission, as part of the information routinely collected for hospitalization. Patients were then followed-up until delivery, and data regarding the delivery and neonatal outcome were collected.

Statistical analyses

Continuous variables were represented using mean, standard deviation, median, minimum, and maximum value while categorical ones using absolute and relative frequencies. Fasting and postprandial TSBA 2.5^{th} and 97.5^{th} percentiles and their 95% confidence intervals were reported.

In order to assess the association between fasting or postprandial TSBA and maternal and neonatal characteristics the Pearson's correlation coefficient was used. To evaluate the difference in fasting or postprandial TSBA between groups a T-test, Satterthwaite T-test or Mann-Whitney test were used according to Shapiro-Wilk test and F-Test for normality and homoschedasticity respectively.

The Kruskal-Wallis test was used to assess the difference in fasting or postprandial TSBA between seasons, according to Shapiro-Wilk test for normality.

The statistical analyses were conducted using statistical software package SAS 9.3 and the significance level was set at 5%.

Ethical approval

This study was performed according to the principles of the Declaration of Helsinki and was approved by the ethics committee of Careggi University Hospital (reference number 18008_bio).

Results

A total of 612 women participated in the study. The ethnic distribution was 525 Caucasian, 50 South-Asian, 27 Hispanic, and 10 Sub-Saharan African women. We collected 528 fasting samples and 377 postprandial samples. In 293 patients both samples were taken, exceeding the minimum suggested 120 samples for determining reference intervals and confidence intervals according to the Clinical Laboratory and Standards Institute[31].

In our analysis, we found that TSBA concentrations for both groups of samples were not normally distributed, but rather had a positive skewness distribution, in accordance with other studies[26,28] (figure 1-2). We found a median of 7.6 μmol/L for the fasting TSBA with an upper reference limit (97.5^{th} percentile) of 14.1 μmol/L (95% CI 12.7-15.5 μmol/L). We found a median of 9.1 μmol/L in the postprandial samples with an upper reference limit of 20.2 μmol/L (CI 95% 17.3-32.3 μmol/L). We established reference intervals of 4.4-14.1 μmol/L for fasting TSBA, and 4.7-20.2 μmol/L for postprandial TSBA levels (table 1).

When applying the currently used thresholds to our normal asymptomatic pregnant population, we found that TSBA levels exceeded 10 μmol/L, the most commonly used threshold, in 15.7% of the fasting and in 38.5 % of postprandial measurements. The fasting threshold [?]6 μmol/L was present in 83.3% of the pregnant population, and postprandial [?]15 μmol/L was present in 6.9% of the measurements.

When both measurements were performed (293 patients), the postprandial values were significantly higher than the fasting measurement, with a mean increase of 1.77 μmol/L (22%). On correlation analysis, fasting TSBA levels were moderately correlated with postprandial TSBA levels (Pearson's coefficient 0.44, P value < 0.0001) (table 2-3).

We observed higher fasting TSBA levels in pregnancies obtained with the use of assisted reproductive technology (ART) compared to spontaneous conception (8.59 μmol/L and 7.98 μmol/L, respectively, P value 0.02). The reasons for the association between ART and higher TSBA levels are unclear, but they could be related to some metabolic disturbances regarding infertility itself[33], or due to hormonal maintenance therapy used for ART. We also noticed higher fasting TSBA levels in nulliparous compared to multiparous (mean 8.6 μmol/L and 7.75 μmol/L, respectively, P value 0.04), although the reason for this association is unclear.

Interestingly, we also found a correlation between fasting TSBA and fetal gender, with the male gender being associated with higher levels of TSBA (8.47 μmol/L versus 7.6 μmol/L, P value <0.001). To our knowledge, this is the first time a correlation with fetal gender has been made and can be explained by differences in hormonal metabolism by the fetal liver. The magnitude of the observed differences, however, may not be clinically relevant.

Fasting TSBA levels were not correlated with maternal age, BMI, weight gain and neonatal birth weight, and no association was found with gestational diabetes mellitus, hyperthyroidism, hypertensive disorders, Intrauterine growth restriction (IUGR) or progesterone therapy (table 2-3).

We found that postprandial TSBA values were significantly lower in pregnancies complicated by IUGR (median 8.59 μmol/L versus 9.98 μmol/L in non-IUGR fetuses, P value 0.03), although this difference is difficult to explain and may not be clinically relevant. Postprandial values were not associated with any of the other variables examined (table 2-3).

We noticed a seasonal pattern for both fasting and postprandial TSBA levels, with highest values in the winter season, a decline during spring and summer, and minimum values in the autumn (p values <0.01 and 0.02, respectively) (figure 3-4).

ICP is known to be more common in South America and in Northern Europe[5]. Nevertheless, we did not find a strong correlation with ethnicity, probably due to the fact that the majority of patients in our study was of Caucasian origin.

Discussion

Main findings

In our study we aimed to establish pregnancy-specific reference ranges for fasting and postprandial TSBA levels. Despite using restrictive exclusion criteria, we demonstrated that a normal reference range and upper reference limit in pregnancy are different from those established for the general non-pregnant population. Compared to the reference range of normal adults (0.28-6.5 μmol/L)[21,36,37], we found significantly higher fasting TSBA (4.4-14.1 μmol/L) and postprandial TSBA (4.7-20.2 μmol/L) reference intervals in the pregnant population.

Our study confirms that TSBA levels increase in response to food intake and that differences between fasting and postprandial measurements are clinically and statistically significant[11,29].

Strengths and limitations

The main strength is the large cohort of pregnancies analysed (612 patients), which to our knowledge is the largest sample of healthy pregnant women that has been examined for TSBA so far.

We believe the strengths also include the prospective design, the accurate selection of eligible patients, the generalizability, and the clinical relevance of the results. Since no correlation has been reported between

TSBA levels and gestational age within the third trimester[26], our results could be applicable to the third trimester of pregnancy. The duration of the study over one year allowed us to evaluate the seasonality of TSBA levels.

Finally, all blood samples were analysed in the same laboratory and were taken ad hoc for the study purposes.

The low variability of ethnicity was due both to the majority of Caucasian patients in our hospital and to the difficulty of collecting informed consent from non-Italian speakers. This limited our ability to evaluate variations in normal TSBA levels between different ethnic groups.

Even though widely used in clinical laboratories, the most important limitation of the enzymatic method is low sensitivity, as the lowest concentration of TSBA measurable is around 1.5 μmol/L[18, 29]. Nevertheless, 100% of the measured values resulted above the evaluation limit.

Another limitation is that common hospital food was provided, not standardized for macronutrients and potentially different from each other.

Interpretation

Evidence that normal pregnancy may be associated with a mild sub-cholestatic state has been described in early studies. Although based on a limited number of patients, an increase has been demonstrated in the mean of single and total BA concentration in uncomplicated pregnancies with no other evidence of ICP[13-16].

Our results are consistent with early observations that pregnancy is a sub-cholestatic state[13-16], and this can be explained by the cholestatic effect of reproductive hormones. Normal pregnancy is an hyperestrogenic state and therefore is associated with a physiological elevation of TSBA. Thus, obstetricians need to be aware that healthy pregnant women have increased levels of TSBA when assessing women who may have ICP.

Early studies described that ICP have a seasonal pattern with increased incidence in some countries during winter months, suggesting a possible association with an environmental trigger. This pattern, however, was not yet demonstrated in clinical studies. Two possible explanations are low levels of natural selenium and of vitamin D during the winter, as both deficiencies have been reported in women with ICP[1,5,19,20].

It has recently been suggested that higher thresholds should be used for the diagnosis of ICP, also given the low risk of stillbirth demonstrated recently for TSBA levels < 100 μmol/L[26, 29].

The assessment of diagnostic criteria for ICP is complex, as the classic methodologies used to establish diagnostic thresholds for other pathologies are difficult to apply here. Currently, in clinical practice, ICP is diagnosed by elevated TSBA levels that are measured after the insurgence of pruritus, a non-specific symptom that is not necessarily associated with ICP. Thus, the diagnosis is based on TSBA alterations (as pruritus without TSBA elevation will not be diagnosed as ICP) by using non-pregnant reference ranges. In non-pregnant patients TSBA levels are used as a biomarker for hepatic injury, while in pregnant patients, the outcome of interest is not liver dysfunction but rather the absolute elevation in circulating TSBA levels[4]. Beside pruritus, TSBA elevation is not associated with adverse outcome unless reaching a severe ICP (> 100 μmol/L for stillbirth and > 40 μmol/L for other adverse outcomes), thereby the diagnostic threshold cannot be tested by maternal or neonatal outcomes either.

For these reasons, an important limitation when studying the normal distribution of TSBA levels in pregnancy is that the accuracy (sensibility and specificity) of the upper limit value of normality among the ICP population cannot be calculated, as ICP was originally diagnosed by the same criteria that needs to be tested.

Our results demonstrate that the non-pregnant reference range cannot be used in the pregnant population. In accordance with the RCOG clinical guidelines for ICP[25], we suggest using in clinical practice the upper limit

value of our normal pregnancy-specific refence range. Specifically, 14 μmol/L for fasting TSBA values and 20 μmol/L for postprandial TSBA values. A similar threshold (19 μmol/L) was suggested by a recent study that re-evaluated the diagnostic thresholds for ICP. Although using different methodologies, our postprandial threshold was similar to the random threshold described by Mitchell et al[26]. In accordance, we also suggest that patients with otherwise unexplained pruritus with TSBA levels below the proposed thresholds should repeat the TSBA measurements since pruritus often precedes an elevation in TSBA levels[2,34].

There is ongoing debate regarding which value should be used in clinical practice to diagnose ICP. Postprandial TSBA assessment may be a more sensitive test, whereas elevated fasting TSBA levels are a more specific indicator of severe liver disease[11,25]. Some authors suggest measuring TSBA levels in the fasting state due to the large overlap between normal non-fasting values and commonly used thresholds for diagnosis[29]. On the other hand, considering that the adverse perinatal outcomes of ICP are associated with peak TSBA concentration, a non-fasting measurement has greater clinical relevance[26].

We suggest using both values of TSBA. We recommend distinguishing the fasting and postprandial status and avoiding random samplings due to different reference ranges and upper limit values that should be applied. Fasting TSBA levels may be the most specific for the diagnosis of ICP and therefore better for confirming ICP. Fasting measurements are also more predictable, have less variability, and correlate better with certain risk factors. The postprandial measurement, on the other hand, is essential for risk stratification. We suggest using postprandial TSBA levels to assess the severity of the disease and to follow-up patients with ICP for subsequent management of the pregnancy and eventual active management.

Conclusions

In our study we demonstrated that a normal pregnancy is a sub-cholestatic state and is associated with physiological elevation of TSBA levels compared to non-pregnant adults. By defining pregnancy-specific reference ranges, we can avoid unnecessary diagnosis of ICP that is strongly correlated with maternal anxiety and active management.

We recommend that higher threshold should be used for the diagnosis of ICP. We suggest using the upper limit in the normal pregnant population: fasting TSBA values [?]14 μmol/L and postprandial TSBA values [?]20 μmol/L. We also suggest both values should be measured, as each provide different information: the fasting measurement is more specific for the diagnosis and the postprandial is essential for risk stratification and severity assessment. Since TSBA values usually increase after food intake, the measurement should not be random, as two different thresholds should be used for the fasting and postprandial measurements.

Disclosure of Interests

The authors have no conflicts of interest to disclose.

Contribution to Authorship

MH and CL performed data curation, investigation and wrote the manuscript together with VS. VS also provided a critical review and text editing. LT performed the data analysis.

MDT was the project administrator, conceived the idea and designed the study. MDT, FP and AG provided validation and visualization of the final manuscript.

Details of Ethics Approval

This study was performed according to the principles of the Declaration of Helsinki (2013) and was approved by the ethics committee of Careggi University Hospital (approval date 18/12/2020, reference number 18008_-

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