Original Research

Performance of an Interferon-Gamma Release Assay to Diagnose Latent Tuberculosis Infection During Pregnancy

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OBJECTIVE: To evaluate an interferon (IFN)-gamma release assay in diagnosing latent tuberculosis infection in pregnant adolescents and women at risk for exposure to Mycobacterium tuberculosis.

METHODS: This was a prospective study of women and adolescents receiving health care at Bellevue Hospital Outpatient Clinics in New York City. Each patient was assessed for M tuberculosis risk factors, had a tuberculin skin test placed, and an IFN-gamma release assay performed. The concordance between the tuberculin skin test and the IFN-gamma release assay was calculated and the results analyzed according to the likelihood of exposure to M tuberculosis. Mean mitogen IFN-γ levels were used across groups to compare reliability between trimesters and assay performance in pregnant compared with nonpregnant females of childbearing age.

RESULTS: A total of 140 pregnant and 140 nonpregnant females were enrolled in the study. The IFN-gamma release assay was highly specific, and IFN-gamma release assay positivity was associated with a greater likelihood of exposure to M tuberculosis. The overall agreement between the tuberculin skin test and IFN-gamma release assay results was 88% for all pregnant patients, corresponding to a κ of 0.452 (confidence interval 0.26–0.64). Interferon-γ release from the mitogen did not appear to have any temporal association with pregnancy trimester in cross-sectional or longitudinal studies. The IFN-gamma release assay performed equally well in pregnant and nonpregnant females.

CONCLUSION: The IFN-gamma release assay performed equally well in each trimester of pregnancy with comparable results to nonpregnant females. Interferon-gamma release assays are much more specific, at least as sensitive, and may be a better predictor of disease progression than the tuberculin skin test.

(Mycobacterium tuberculosis infects one third of the world’s population and causes death in approximately one million women yearly.1 In the United States, tuberculosis (TB) incidence is low as a result of great effort devoted to identifying and treating latently infected individuals with 6–9 months of isoniazid.2,3 In the United States, latent TB occurs in 4.2% of the population with higher rates among the foreign-born (18.7%) and individuals living in poverty (6.1%).4 Among individuals at increased risk for M tuberculosis infection, the prenatal setting provides an opportunity to identify females with latent TB and provides support for treatment completion. As of 2011, the Centers for Disease Control and Prevention (CDC) recommends interferon (IFN)-gamma release assays are the preferred diagnostic tests for pregnant women with risk factors for exposure to M tuberculosis.5 These recommendations were made before any data on IFN-gamma release assays performance during pregnancy, partially because the century old tuberculin skin test is neither very sensitive or specific.6 False-positive tuberculin skin test reactions occur secondary to cross-reactivity with environmental mycobacteria and bacille Calmette-
Guérin vaccination. Moreover, tuberculin skin test sensitivity is compromised during active TB, human immunodeficiency virus (HIV) coinfection, malnutrition, and states of immune suppression.13

Interferon-gamma release assays measure T cell responses to antigens transcribed from the region of difference-1, a region on the Mycobacterium genome specific for M tuberculosis and absent in bacille Calmette-Guérin and most other mycobacteria. In the Quantiferon-TB Gold In Tube assay (IFN-gamma release assay), M tuberculosis-specific antigens are incubated with whole blood, and IFN-γ, produced by the antigen specific T cells, is measured by an enzyme-linked immunosorbent assay. Similar to the skin test, IFN-gamma release assays detect the immune response to infection with M tuberculosis and do not distinguish clinical stage, ie, latent compared with active infection. Studies in adults and children using IFN-gamma release assays have shown this assay to have a specificity of 98% or greater and sensitivity relatively comparable to the tuberculin skin test, approximately 80%.7–10 Validating IFN-gamma release assays during pregnancy are important because an altered immune response occurs during pregnancy11–13 and IFN-gamma release assays are dependent on an individual’s Th1 response to M tuberculosis antigens. In this study, we examined the performance of the IFN-gamma release assay in pregnant adolescents and women receiving care at public hospital outpatient clinics in New York City.

MATERIALS AND METHODS

This was a single-centered prospective study with all participants recruited from Bellevue Hospital, a large public hospital serving a diverse population in New York City. After obtaining informed consent, female adolescents and women in the outpatient clinic setting were enrolled in the study. Epidemiologic data were obtained on all pregnant patients, including information on risk factors for possible M tuberculosis exposure. For all pregnant patients, a tuberculin skin test was placed using the Mantoux technique and blood for the IFN-gamma release assay was drawn directly into the IFN-gamma release assay tubes. Tuberculin skin test results were checked 48–72 hours later and was considered positive when the area of induration was 10 mm or larger. All patients with a positive tuberculin skin test had a subsequent chest roentgenogram, usually performed in the second trimester of pregnancy. In nonpregnant females, only the IFN-gamma release assay was performed, because validation of the IFN-gamma release assay in this population has been performed many times in prior studies.7–10 HIV results were recorded when available but testing was not performed as part of this study. This study was approved by the institutional review boards of New York University and Bellevue Hospital.

The IFN-gamma release assay was performed according to the manufacturer’s instructions. Briefly, 1 mL of blood was drawn directly into three separate heparinized tubes: the nil control (containing only heparin), the mitogen control (containing phytohemagglutinin), and the M tuberculosis-specific antigens ESAT-6, CFP-10, and TB7.7 (Rv2654). Each tube was rotated several times to allow the blood to coat the entire wall. Within 2 hours of venipuncture, the tubes were placed in an incubator set at 37°C. After 24 hours of incubation, the tubes were centrifuged and the plasma was collected. The amount of IFN-γ in the plasma was measured by enzyme-linked immunosorbent assay with the reagents included in the test kit. The amount of IFN-γ in the nil tube was then subtracted from the values of IFN-γ (international units/mL) released in response to M tuberculosis-specific antigens or mitogen. The result was considered positive when the amount of IFN-γ was 0.35 international units/mL or greater and at least 25% greater than the nil value, as recommended by the manufacturer, and based on previous adult studies. The upper limit of positivity is an IFN-γ level of 10 international units/mL, and any value above 10 was beyond the range of reliability and therefore recorded in our data as “10 international units/mL.” Standards were conducted on each enzyme-linked immunosorbent assay in triplicate. Interferon-gamma release assay test results were calculated using the software provided by the manufacturer.

Sample size was calculated after enrolling 15 pregnant patients and 15 nonpregnant patients and obtaining the mean mitogen-nil for each group. Using an α error level 5% (95% confidence level) and a power of 80%, we calculated that 129 patients need to be enrolled into each group. To account for possible indeterminate IFN-gamma release assay results, we enrolled 140 into each group. Because the upper limit of IFN-gamma release assay accuracy is 10 international units/mL, all IFN-γ values greater than 10 international units/mL were recorded as 10 international units/mL for the analysis. Interferon-gamma release assay results were compared with tuberculin skin test results using χ² statistic. Because there is no gold standard to diagnose latent TB infection, IFN-gamma release assay results were further compared with a gradient of likely exposure to M tuberculosis based on risk factors such as contact with an index case or living in an endemic region. Univariable analysis of possible risk factors associated with a positive IFN-gamma release assay was evaluated by χ². The Mann-Whitney U test was used to compare IFN-γ levels. Analysis of variance and post hoc
None of the enrolled patients were on antimycobacterial medication before or at the time of enrollment. The demographic and clinical characteristics of the patients are listed in Table 1. Among the pregnant patients, the mean age was 18.5 years, ranging between 13.5–36.5 years. The majority (79%) of pregnant patients were Hispanic white. Historical data on patients revealed approximately 90% had at least one risk factor for exposure to *M tuberculosis*.

Among the 140 pregnant patients included in the analysis, nine (6.4%) had indeterminate IFN-gamma release assay results, eight as a result of a low mitogen response below the allowable cutoff (0.5 international units/mL), and one secondary to a mitogen minus nil result that was less than 25% of the nil (another criteria for IFN-gamma release assay validity). The IFN-gamma release assay and skin test results for the pregnant patients are presented in Figure 1.

Among the 131 valid IFN-gamma release assay results, 103 (79%) pregnant women and adolescents were tuberculin skin test-negative and 28 (21%) were tuberculin skin test-positive. All tuberculin skin test and IFN-gamma release assay-positive patients had chest roentgenograms performed, none of which had findings of active TB. Among the patients who had negative tuberculin skin test results, 100 (97%) of them also had a negative IFN-gamma release assay. The overall agreement between tuberculin skin test and IFN-gamma release assay results were 88% for all pregnant patients, corresponding to a $\kappa$ of 0.452 (confidence interval 0.26–0.64).

There were three women and adolescents who had a negative skin test and a positive IFN-gamma release assay.

<table>
<thead>
<tr>
<th>Table 1. Pregnant Patients’ Characteristics (n=140)</th>
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<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>White ethnicity</td>
</tr>
<tr>
<td>Non-Hispanic</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Any risk factor for exposure to <em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>Vaccinated with BCG</td>
</tr>
<tr>
<td>Born in an endemic region</td>
</tr>
<tr>
<td>Infected with HIV</td>
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</tbody>
</table>

Data are mean (range) or n (%).

BCG, bacille Calmette-Guérin; HIV, human immunodeficiency virus.
Each of those patients had risk factors for exposure to Mycobacterium tuberculosis. Among these patients, one had a skin test of 6.5 mm and the other two skin test results were 0 mm. Two of the IFN-gamma release assay results were considered low positive with IFN-γ/H9253 values of 0.65 for two patients, and the third woman had an IFN-γ/H9253 result of 1.51 international units/mL.

The proportion of positive IFN-gamma release assays in pregnant patients with increasing gradients of Mycobacterium tuberculosis exposure is shown in Table 2. As the likelihood of exposure to Mycobacterium tuberculosis increased, the proportion of pregnant patients with a positive IFN-gamma release assay also increased. Among the 12 women and adolescents with no known risk factors for Mycobacterium tuberculosis, none tested positive on either the IFN-gamma release assay or tuberculin skin test diagnostic test. In this “control group,” the estimated IFN-gamma release assay specificity was 100%. Among the 26 patients with a low-to-moderate exposure risk to Mycobacterium tuberculosis and a positive skin test, 10 (38%) were both tuberculin skin test- and IFN-gamma release assay-positive.

Univariable analysis of potential risk factors revealed that contact with a known index case was the only risk factor significantly (P<.001) associated with a positive IFN-gamma release assay result, yet there were only two patients with this history. No other identifiable risk factors were significantly associated with having IFN-gamma release assay positivity.

Many studies have described a direct correlation between tuberculin skin test induration size and likelihood of Mycobacterium tuberculosis exposure.14–17 In this regard, we would expect larger skin test induration reactions to indicate true-positive Mycobacterium tuberculosis infections. When we compared skin test size with IFN-gamma release assay positivity, we observed a significant association (P=.02) between a larger induration size and percentage of positive IFN-gamma release assay results (Fig. 2).

Pregnant patients were enrolled during each trimester of pregnancy. We enrolled 21 women and adolescents during the first trimester (gestational age 1–13 weeks), 44 in the second (14–26 weeks) and 75 in the third (27 weeks or greater). The mean IFN-γ response after phytohemagglutinin stimulation (mitogen minus nil) from 140 patients enrolled in their first, second, and third trimesters of pregnancy was 8.6 international units/mL (standard deviation [SD] 2.73), 6.75 (SD 3.76), and 7.08 (SD 3.58), respectively. There was no significant difference (P=.126) between IFN-γ levels detected in each trimester of pregnancy. Additionally, the percentage of indeterminate results was relatively consistent throughout each trimester (Table 3).

In a subset of 25 pregnant patients, we performed a longitudinal study and repeated the IFN-gamma release assay a second time during a subsequent
Table 3. Mean Interferon-Gamma (International Units/mL) From Mitogen Control Analyzed by Trimester Initially Enrolled

<table>
<thead>
<tr>
<th>Trimester</th>
<th>First Trimester (n=21)</th>
<th>Second Trimester (n=44)</th>
<th>Third Trimester (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>8.6±2.73</td>
<td>6.75±3.76</td>
<td>7.08±3.58</td>
</tr>
<tr>
<td>Indeterminate [n (%)]</td>
<td>1 (4.7)</td>
<td>3 (6.8)</td>
<td>5 (6.7)</td>
</tr>
</tbody>
</table>

SD, standard deviation.  
Overall P value=.13 using analysis of variance test. Indeterminate values are included in analysis.

trimester of pregnancy or postpartum. None of the patients converted from a negative to positive (or vice versa) result with repeated testing. Possible variation in individual T cellular immunity was measured from a patient’s response to phytohemagglutinin assay stimulation. Although there was a mean decrease in response from the first to the third trimester, this difference was not statistically significant (P=.105) and no other difference in mitogen response was detected between trimesters. One of the patients had an initial indeterminate IFN-gamma release assay as a result of a mitogen response just below the cutoff value with a repeated valid result in the subsequent trimester.

We enrolled and obtained the IFN-gamma release assay on 140 nonpregnant adolescent girls and women from similar age and socioeconomic backgrounds as the pregnant cohort. There was no statistical difference (P=.482) in the mitogen control between each group of adolescents and women. The mean IFN-γ (mitogen-nil) response in the nonpregnant and pregnant cohort was 8.5 (SD 2.69) and 7.2 (SD 3.57), respectively (Table 4). Although not statistically significant (P=.255), there were twice as many indeterminate responses, as a result of low mitogen, in the pregnant (n=9) compared with the nonpregnant cohort (n=4).

**DISCUSSION**

Although the IFN-gamma release assay has been extensively studied in adults and children, there exists little data on IFN-gamma release assay performance during pregnancy. Evidence from this investigation and other studies in nonpregnant adults demonstrate IFN-gamma release assays to be much more specific than the tuberculin skin test for *M tuberculosis* detection. Although sensitivity cannot be directly measured, we observed the percentage of positive IFN-gamma release assay results to increase as the gradient of exposure increased in tuberculin skin test-positive patients. Interferon-gamma release assay positivity also correlated with larger tuberculin skin test induration size, which is often used as an indicator of true infections with *M tuberculosis*.

We observed an excellent agreement between the tuberculin skin test and IFN-gamma release assay. However, almost two thirds of pregnant patients with positive skin tests had negative IFN-gamma release assays, a finding that was similarly observed in another at-risk pregnant population. Although this may indicate increased IFN-gamma release assay specificity, it is also possible that the IFN-gamma release assay may be less sensitive than the tuberculin skin test in certain circumstances. For example, studies have shown that as time from *M tuberculosis* exposure increases, IFN-gamma release assay sensitivity may decrease. Our study involved a large immigrant population, and exposure to *M tuberculosis* may have occurred remotely during childhood. New York City is a low TB endemic region and contacts of TB disease cases are actively investigated, allowing documentation of recent infection. It is, however, most important to consider the actual risk of TB progression in the setting of a remote exposure and negative IFN-gamma release assay. This appears unlikely because with progression to disease, there is bacilli replication and subsequent antigen release triggering effector memory T cell responses that are detected on the IFN-gamma release assay.

Discordant IFN-gamma release assay-positive and tuberculin skin test-negative results were much less common and occurred in three pregnant patients. Similar discordant positive IFN-gamma release assay

Table 4. Interferon-Gamma Level From the Mitogen (PHA) Control Minus the Nil*

<table>
<thead>
<tr>
<th>Cohort</th>
<th>n</th>
<th>Mean Mitogen IFN-γ (International Units/mL)</th>
<th>SD (International Units/mL)</th>
<th>Indeterminate [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not pregnant</td>
<td>140</td>
<td>8.5</td>
<td>2.69</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>140</td>
<td>7.2</td>
<td>3.57</td>
<td>9 (6.4)</td>
</tr>
</tbody>
</table>

IFN, interferon; SD, standard deviation.  
P=.482  
† Cellestis reports values greater than 10 international units/mL of IFN-γ may fall beyond the linear range of the IFN-gamma release assay software. Maximum IFN-γ levels were therefore recorded as 10 international units/mL.
and negative tuberculin skin test have been observed in other studies and a meta-analysis identified IFN-gamma release assay-positive and tuberculosis skin test-negative in 6% of all persons, accounting for 23% of all positive IFN-gamma release assay results.7

Interestingly, IFN-gamma release did not appear to have any temporal association with pregnancy trimester. In both cross-sectional and longitudinal analysis, there was no significant difference in mitogen-stimulated IFN-γ release. This observation has important diagnostic value, because it is not uncommon to have at-risk populations (urban poor or immigrants) present to prenatal care in the second or third trimesters. The ability to perform the IFN-gamma release assay without losing sensitivity to any given trimester increases its diagnostic value. This is in contrast to the skin test, which may have a higher anergic rate during pregnancy,28 making it unclear if a lack of induration indicates a negative tuberculin skin test or anergy.

The IFN-gamma release assay performed equally well in pregnant and nonpregnant females of the same age and socioeconomic cohort and there was no significant difference in mitogen-stimulated IFN-γ levels between the two groups. Although not significant, approximately twice the number of indeterminate results was observed in the pregnant patients as a result of low mitogen responses and larger randomized studies may be able to assess the reproducibility and significance of these findings. Meta-analysis studies on IFN-gamma release assay report an average indeterminate rate of approximately 2–6%,29,30 which is within the range observed in this study. Possible causes of a low mitogen response include malnutrition, immune suppressive medication, HIV infection, concurrent viral or bacterial infection, genotype HLA-DRB1*0701, age younger than 5 years, active TB, and technical error.31–33 Most indeterminate results are transient. In clinical practice, indeterminate IFN-gamma release assay results in apparently healthy and immune competent adults or children are repeated in 1–2 months. In most instances, repeating the IFN-gamma release assay with a new blood sample results in a valid IFN-gamma release assay.

If the IFN-gamma release assay were to be used as the diagnostic test for latent TB infection in pregnant patients, less than half of women and adolescents would have been identified as positive and a chest roentgenogram during pregnancy to rule out active TB would have been avoided. Moreover, if treatment decisions were based on IFN-gamma release assay results, as is the case now in nonpregnant adults and children, far fewer people would have avoided a 9-month regimen of anti-TB preventive medication. The cost of the IFN-gamma release assay is between $30 and $60, considerably more than a tuberculin skin test. However, this additional cost is offset by a decrease in the number of positive test results and the associated costs of evaluating and treating tuberculin skin test-positive patients.34

Several studies, including one performed in HIV-infected women, provide evidence that a positive IFN-gamma release assay is at least as good as the tuberculin skin test in predicting a progression to TB disease.9,35–37 A more reliable indicator of progression may be a high or rising IFN-γ level.9,38–40 Therefore, positive IFN-gamma release assay results in at-risk populations allow the selection of individuals most likely to benefit from prophylaxis and, at the very least, identify women who may need close monitoring and follow-up.

The very women at risk for exposure to M tuberculosis, ie, foreign-born and urban poor, often seek medical care only during pregnancy.41 We are therefore currently missing an opportunity to identify and treat latent TB infection in pregnancy when compliance and treatment can be easily monitored.42 A recent study performed in New York City observed that less than 10% of women diagnosed with latent TB infection during pregnancy completed 6 months or more of isoniazid postpartum.43 The most important reasons for noncompliance include nonreferral for evaluation of a positive tuberculin skin test result, missed appointments, and nonadherence with prescribed treatment.43 Initiating latent TB infection treatment during prenatal care visits would eliminate some of the reasons for failure of treatment. Using decision analysis, antepartum isoniazid is found to be cost-effective and reduces mortality and morbidity compared with postpartum treatment if noncompliance with postpartum treatment is only 10%,44

Currently in the United States, the CDC and the American College of Obstetricians and Gynecologists recommend withholding treatment of latent TB infection in pregnant women not at risk for hematogenous disease45,46 based on studies that were not well-controlled47,48 and failed to show a significant increase in hepatitis during pregnancy.49 Protocols for monitoring patients on isoniazid have been revised over the past 20 years and now include monthly monitoring of clinical symptoms for each patient and liver enzymes for pregnant women.45 These protocol changes have resulted in an approximate sixfold decrease in hepatotoxicity in the nonpregnant population.45,50 Isoniazid is safe during pregnancy and is not a teratogen.45,52 Likewise, women can breast feed while taking isonia-
azid because only small concentrations (less than 20% of therapeutic levels) are excreted in breast milk.42,53

In conclusion, replacing the tuberculin skin test with a diagnostic test that is more specific and possibly better at predicting disease progression could improve screening and appropriate identification of individuals at risk of M tuberculosis exposure. If larger randomized studies in pregnant women confirm the IFN-gamma release assay to be a more appropriate diagnostic than the tuberculin skin test, the American College of Obstetricians and Gynecologists and the CDC should reconsider new TB diagnostic and even management guidelines in the pregnant population.

REFERENCES


