

**Inflammation among women with histories of gestational diabetes and diagnosed diabetes in the National Health and Nutrition Examination Survey
III**

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Running Title: Inflammation in women with histories of GDM

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Objective: We compared inflammatory markers among women with histories of gestational diabetes (hGDM), women with diagnosed diabetes (DM), and unaffected women in a population-based sample.

Research Design and Methods: We conducted cross-sectional analyses of 6346 non-pregnant women in the Third National Health and Nutrition Examination Survey (1988-1994). Women were classified as having hGDM (n=87), diagnosed DM (n=244), or neither condition (n=6015). Inflammatory markers included ferritin, leukocyte counts, and C-reactive protein (CRP) levels.

Results After adjustment, women with diagnosed DM had the most marked differences in inflammatory markers compared with unaffected women. Differences between unaffected women and women with hGDM were minimal.

Conclusions: Women with diagnosed DM have less favorable inflammation profiles than unaffected women and greater ferritin levels than women with hGDM. After adjustment, women with hGDM who have not developed diagnosed DM have similar inflammation profiles to unaffected women.

Women with histories of GDM (hGDM) are at increased risk for future glucose intolerance, and this risk may be associated with inflammation.(1-4) The association has not been examined in population-based studies and may not be robust after adjustment for body mass index (BMI).(1-4) Using the National Health and Nutrition Examination Survey III (NHANES III), a population-based cross-sectional study, we compared inflammatory markers among unaffected women vs. women with hGDM vs. women with diagnosed diabetes (DM).

METHODS

Study Population - The sampling strategy and data collection methods for NHANES III have been previously described.(5) We excluded women who were currently pregnant or had missing data regarding pregnancy status or previous DM or hGDM diagnoses, for a sample of 6346 women. Women were classified as having diagnosed DM if they reported a diagnosis of DM outside of pregnancy; as having hGDM if they reported having a diagnosis of DM made only during pregnancy; and as unaffected if they did not have hGDM or diagnosed DM. Therefore, the categories of unaffected women (n=6015), women with hGDM (n=87) and women with diagnosed DM (n=244) were mutually exclusionary. In NHANES III, undiagnosed DM was assessed in a subsample of nondiabetic persons who were randomly assigned to a morning fasting examination.

Information on demographic and behavioral factors were collected by interview.(5) The poverty index was calculated as the poverty-income-ratio (PIR).(6) Measurements of height, weight, and waist circumference were performed in a standardized manner. Leg length was calculated by subtracting sitting height from standing height.(7) Measurement procedures for inflammatory markers for ferritin,(8) leukocytes,(9) and CRP(6) in the NHANES III have been previously described.

Statistical analysis - All analyses accounted for the multistage, stratified, cluster-sampling design of NHANES III by using survey sample weights. We conducted tests for trend across unaffected women, women with hGDM, and women with DM across exposure variables. We calculated predicted marginal probabilities and 95% confidence intervals in multivariate models. In a sensitivity analysis, we excluded women who had fasting glucose ≥ 126 mg/dl and were also classified as having hGDM or as unaffected. Statistical analyses were conducted using SUDAAN 9.0 (Research Triangle Institute, North Carolina).

RESULTS

Women with DM were significantly older than women with hGDM or unaffected women (46 years vs. 32 years vs. 36 years, $p<0.0001$) and were more likely to report that they were Non-Hispanic Black ($p<0.0001$), a family history of DM ($p<0.0001$), the least favorable PIR ($p=0.0036$), the fewest number of alcohol beverages per day ($p<0.0001$), and the greatest waist circumference ($p<0.0001$). Waist circumference increased across unaffected women, women with hGDM, and women with DM from 85.9 cm to 92.4 cm to 103.2 cm ($p<0.0001$). Few women were multiparous; all of the women in the hGDM and DM groups and $>91\%$ of unaffected women had at least one delivery. Women with DM had the highest ferritin compared to women with hGDM or unaffected women (135.1 ng/ml vs. 59.4 ng/ml vs. 62.7 ng/ml, $p<0.0001$), the highest leukocyte counts (8.4 cells/ml vs. 8.0 cells/ml vs. 7.2 cells/ml, $p<0.0001$), and the highest CRP (0.88 mg/l vs. 0.51 mg/l vs. 0.40 mg/l, $p=0.0012$).

When we adjusted for patient covariates, the most striking differences were seen between women with DM and unaffected women. After adjustment for demographic and behavioral factors (Table, Model 1), women with DM had greater ferritin, leukocyte, and CRP than unaffected women. After further adjustment for waist

circumference (Table, Model 2), women with DM had greater inflammatory marker levels than unaffected women, but the differences in CRP were no longer significant. As in unadjusted analyses, we observed no significant differences in inflammatory markers between unaffected women and women with hGDM. Women with hGDM had lower inflammatory markers than women with DM, but the differences were statistically significant only for ferritin, and not for CRP and leukocyte counts.

CONCLUSIONS

This population-based cross-sectional study suggests that differences in inflammatory markers are greatest between women with diagnosed DM and unaffected women. Ferritin levels distinguished women with DM from unaffected women and hGDM before and after adjustment for other risk factors, but ferritin did not differ significantly between unaffected women and women with hGDM.

Our findings are consistent with results from previous studies of women with hGDM vs. unaffected women, which have not shown robust associations after adjustment of BMI or other markers of adiposity.(1-4) Our findings are also consistent with studies that have compared unaffected women vs. women with DM and have found more robust associations.(10)

The study has several limitations. NHANES III was cross-sectional and may not reflect disease progression in individuals. It is possible that the relationship between inflammation and hGDM would have been stronger had we been able to distinguish which women were to develop DM and if we were also able to adjust for time since delivery. It is also possible that examination of other markers with greater discrimination would have shown an association. There may have been unmeasured confounding with other inflammatory conditions not included in our analysis. Finally, the number of women with hGDM was greater than in most other

reports but still small, leading us to report group differences only when the 95% confidence intervals were mutually exclusive.

In conclusion, the differences in inflammatory markers between women with hGDM and unaffected women were much smaller than differences seen in women with DM. Prospective longitudinal studies using inflammatory markers with greater discrimination may elucidate how inflammation progresses in women who develop GDM, then DM after delivery.

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Table. Predicted probabilities and 95% confidence intervals for inflammatory markers, by categories of history of gestational diabetes (hGDM) or DM (diagnosed diabetes).

	no GDM or DM	history of GDM	diagnosed DM	R2
<u>Ferritin (ng/ml)</u>				
Model 1-adjusted for age, race, DM family history, parity, smoking, alcohol	66.3 (62.2, 70.4)*	72.7 (55.0, 90.3)*	120.3 (96.4, 144.2)	0.08
Model 2-further adjusted for waist circumference	66.4 (62.1, 70.7)*	68.0 (50.2, 85.9)*	112.8 (88.5, 137.1)	0.10
Model 3-further adjusted for leg length	63.4 (59.8, 67.1)*	61.3 (44.7, 77.9)*	108.4 (86.9, 129.9)	0.09
Model 4-further adjusted for poverty index ratio	63.5 (60.0, 67.1)*	61.3 (44.1, 78.6)*	106.5 (85.6, 127.4)	0.09
<u>Leukocyte count (cells/ml)</u>				
Model 1-adjusted for age, race, DM family history, parity, smoking, alcohol	7.2 (7.1, 7.4)*	7.5 (6.7, 8.4)	8.5 (8.1, 8.9)	0.11
Model 2-further adjusted for waist circumference	7.2 (7.1, 7.4)*	7.4 (6.5, 8.3)	8.1 (7.8, 8.4)	0.16
Model 3-further adjusted for leg length	7.2 (7.1, 7.3)*	7.3 (6.5, 8.1)	8.0 (7.7, 8.3)	0.16
Model 4-further adjusted for poverty index ratio	7.2 (7.1, 7.3)*	7.3 (6.4, 8.1)	8.0 (7.7, 8.4)	0.16
<u>C-reactive protein (mg/l)</u>				
Model 1-adjusted for age, race, DM family history, parity, smoking, alcohol	0.4 (0.4, 0.5)*	0.5 (0.2, 0.7)	0.8 (0.5, 1.1)	0.03
Model 2-further adjusted for waist circumference	0.4 (0.4, 0.5)	0.4 (0.2, 0.6)	0.7 (0.4, 1.0)	0.10
Model 3-further adjusted for leg length	0.4 (0.4, 0.4)*	0.4 (0.2, 0.6)	0.7 (0.4, 0.9)	0.12
Model 4-further adjusted for poverty index ratio	0.4 (0.4, 0.4)*	0.4 (0.2, 0.6)	0.7 (0.4, 1.0)	0.14

* indicate significant differences vs. women with diagnosed DM at p<0.05.