THE INFLUENCE OF TOBACCO SMOKING ON HUMORAL IMMUNE RESPONSE IN INSULIN DEPENDENT DIABETIC PREGNANCY

Medhat Haroun

Department of Bioscience & Technology, Institute of Graduate Studies & Research,
Alexandria University, Alexandria, Egypt

ABSTRACT

Tobacco smoking products have a heavy impact on the public health of developed as well as non-developed countries by being a main etiologic factor for the induction of cardiovascular diseases and tobacco-related cancer. The purpose of this study was to determine the influence of tobacco smoking on the measurement of the humoral immune response in Egyptian pregnant women with type 1 diabetes. Concentrations of serum immunoglobulin A, G and M in 35 smoking, 35 non-smoking pregnant women with type 1 diabetes and 35 matched normal women were measured by ELISA. Women were matched by age and working life with controls. Measurements suggested that diabetic smokers had decreased levels of IgG and IgM in their sera. It was found that normal individuals had mean IgA, IgG and IgM levels of 2.80 mg/ml, 9.33 mg/ml and 1.66 mg/ml, respectively while non-smoker women suffering from type 1 diabetes had mean levels of 3.47 mg/ml, 10.97 mg/ml and 2.05 mg/ml ($p < 0.0004$, $p < 0.0001$ and $p < 0.0002$). However, the mean level of IgA, IgG and IgM in diabetic smoker sera was determined to be 3.33 mg/ml, 8.07 mg/ml and 1.31 mg/ml, respectively ($p < 0.003$, $p < 0.0001$ and $p < 0.0001$). The obtained results suggest that toxic smoke components were immuno-suppressant and may well play a part in the complex immuno-pathogenesis interaction. The increased risk of smoking in insulin dependent diabetic pregnant women during pregnancy is a further reason to encourage pregnant women to quit tobacco smoking.

KEY WORDS

Pregnancy; Smoking; Type 1 diabetes; Immunoglobulins

INTRODUCTION

Type 1 diabetes mellitus is an organ-specific autoimmune disease in which the insulin-producing beta cells in the pancreatic islets are selectively eliminated resulting in decreased production of insulin and consequently increased levels of blood glucose (1, 2). Damage to the target organs in organ-specific autoimmunity can occur as the result of direct cellular damage by humoral or cell-mediated mechanisms or by stimulating autoantibodies or blocking autoantibodies (3).

Address for Correspondence:

Dr. Medhat Haroun
Associate professor of Biochemistry,
Department of Bioscience & Technology,
Institute of Graduate Studies & Research,
University of Alexandria,
163, Horreya Avenue, Chatby 21526,
P.O BOX: 832, Alexandria, Egypt.
Fax: +203 4285792
E-mail : mharounag@yahoo.com

There is overwhelming evidence that an association exists between smoking and a number of pathological conditions. Therefore, fetal growth not only impacts the outcome of the perinatal period, but also impacts adult well-being. Effects of nicotine are seen in every trimester of pregnancy, from increased spontaneous abortions in the first trimester, to increased premature delivery rates and low birth weight in the final trimester (4, 5). Smokers are exposed not only to nicotine, but also to a vast array of chemicals that are known to be harmful, including nicotine and carbon monoxide, the two main toxic substances. Smoking throughout pregnancy, caffeine and alcohol consumption may increase the risk of spontaneous abortion (6, 7).

Pregnancy in women with type 1 diabetes mellitus is associated with increased risk of maternal, perinatal, and neonatal complications (8, 9). Several previous studies link maternal smoking during pregnancy with intentional and mild cognitive difficulties in children (10, 11). Furthermore, mothers

who smoke cigarettes while pregnant have an additional risk of ~40% to 50% of having children with attentional and cognitive difficulties (12).

As there are no studies on the role of toxic tobacco components in the direct immune mechanisms or diabetic complications, the answer to the question whether the changes observed result from adaptative or cytotoxic processes remains an important and under investigated area of study. It was our objective to test the hypothesis that, in women with type 1 diabetes, smoking consumption during pregnancy are associated with an increased risk of immunopathological interaction outcomes.

MATERIALS AND METHODS

Anti-human IgA (IgG, IgM) antiserum (raised in rabbit), human IgA (IgG, IgM), rabbit anti-human IgA (IgG, IgM) conjugated to horseradish peroxidase (HRP), donkey anti-rabbit IgA conjugated to HRP, tetramethylbenzidine were purchased from Sigma (Sigma-Aldrich Company Ltd., UK) and all other chemicals were supplied from BDH (VWR International Ltd., UK).

Subjects

The study population consisted of 35 healthy women with normal deliveries (group 1) as a control group, 35 non-smoking pregnant women with type 1 diabetes (group 2) and 35 smoking pregnant women with type 1 diabetes (group 3) were used for analysis of IgA, IgG and IgM levels by ELISA. All groups were age matched (range 20-29 years). Patients, had no clinical or laboratory findings indicating infection or other diseases such as rheumatoid arthritis or viral infection. Group 3 were of women smoking 10-20 cigarettes a day for at least 5 years also during pregnancy. Cases in both group 2 and 3 were defined as women with a live fetus in gestational week 6-16. Glycaemic control early in pregnancy was good in most women and folic acid supplementation was adequate. We excluded other women because of first trimester spontaneous abortion, diagnosis of type 2 or secondary diabetes, or loss to follow up.

We recorded maternal characteristics (age, marital status, alcohol use, smoking and habits), duration of diabetes and treatment of diabetes (continuous subcutaneous insulin infusion or multiple insulin treatment, human insulin or analogue (lispro) insulin). The study had the approval of the local ethical committee and informed consent was obtained from all subjects. All sera were collected within four months

and stored in small aliquots at -20°C until tested under code.

Human immunoglobulin measurement by ELISA

Coating antibody [anti-human IgA (IgG, IgM) antiserum] was diluted 1 in 1000 in 1x coating buffer (0.02 M Tris-HCl, 1.5 M NaCl pH 9.0) and 100 μl was added to each of the wells of a microtiter plate (13,14). After overnight incubation at 4°C the plate was washed 4 times with PBST20 (0.1% (w/v) [Tween 20 in 1x PBS (phosphate buffered saline; 0.25 M NaCl, 0.0268 M KCl, 0.081 M Na_2HPO_4 and 0.0146 M KH_2PO_4]). Sites unoccupied by antibody were blocked by addition of 5% (w/v) Marvel (dried skimmed milk) in PBS for 1 h at room temperature followed by washing 6 times with PBST20. The human serum samples were initially diluted 1 in 2000 in 1x PBS, and 2 fold serial dilutions subsequently performed on the plate. Diluted samples were allowed to bind to the first antibody and the plate was then washed 6 times in PBST20.

Rabbit anti-human IgA (IgG, IgM) conjugated to HRP (second antibody) was diluted 1 in 1000 in 1x PBS, 100 μl was added to each well of the microtiter plate, incubated at room temperature for 1 h and then washed 6 times in PBST20. The amount of bound second antibody was determined by adding 200 μl of the substrate solution (tetramethylbenzidine 6 mg/ml in 0.1 M sodium acetate buffer pH 6.0) to each well. After incubation, in the dark at room temperature for 20 min, the reaction was stopped by adding 50 μl of 10% (w/v) H_2SO_4 to each well after that the absorbance was measured at 450 nm. A standard curve was constructed by plotting absorbance against concentration for the standard solutions and the concentration of immunoglobulin (mg/ml) in the samples was determined.

Statistical Analysis

After tabulating the data, the arithmetic mean for each group was calculated. The variation or variability in each group was represented by the standard deviation (SD). The means of the groups were compared to see if the differences were significant. Student's *t*-test was used to assess the significance of the difference between groups.

RESULTS

Results showed that smoking diabetic women of group 3 have a lower level of serum IgG and IgM compared to normal women in group 1. In addition, non-smoking diabetic women of group 2 have a higher level of serum IgA, IgG and IgM compared to group 1. The quantitative analysis of serum IgA, IgG and IgM

level (mean $\pm$ SD) found that group 2 had a mean level of IgA, IgG and IgM (3.47 ± 0.85 , 10.97 ± 0.90 and 2.05 ± 0.27 mg/ml, respectively) higher than group 1 (2.80 ± 0.41 , 9.33 ± 0.84 and 1.66 ± 0.34 mg/ml, respectively). These represented significant increases in IgA, IgG and IgM level in non-smoking diabetic sera of group 2 compared to group 1 ($p < 0.0004$, $p < 0.0001$ and $p < 0.0002$). On the contrary, the mean levels of IgG and IgM in smoking diabetic sera of group 3 were (8.07 ± 0.85 and 1.31 ± 0.19 mg/ml, respectively) lower than group 1. These represented significant decreases in IgG and IgM level in diabetic sera of group 3 compared to group 1 ($p < 0.0001$ and $p < 0.0001$). Analysis of the obtained results for smoking diabetic women showed significant increases in IgA level (3.33 ± 0.77 mg/ml, $p < 0.003$) compared to normal individuals of group 1. Average values of the parameters examined together with standard deviation are shown in Fig. 1, 2 and 3.

DISCUSSION

A high number of immune abnormalities, both humoral and cellular, occur either transiently or permanently in type 1 diabetes (15). Despite this abundance of reports, most researchers consider that whether the role of immunological factors is primarily pathogenetic, co-causative, secondary or simply chronologically associated to pathogenetic events has not been definitively answered (1, 16). Our finding of low levels in both IgG and IgM in smoking pregnant diabetic sera, leading to humoral immune abnormalities in immunoglobulin estimation by ELISA, represents a move in this direction.

Concentrations of immunoglobulins in serum are affected by tobacco smoking. A series of large studies have shown lower serum levels of IgG in smokers than in non-smokers. The precise mechanism(s) by which smoking affects serum immunoglobulin levels and the component(s) of cigarette smoke responsible for the effect have not been clarified (17). However, evidence of a smoke-response relationship, with lower levels of IgG with increased smoking, has been presented (18-21). In this study, we found another smoke-response relationship with lower levels of IgM in diabetic pregnant women.

A major problem when discussing immunoglobulin class and subclass deficiency is the lack of a generally recognized definition of what is meant by "deficient", partly due to the wide range of subclass levels in normal subjects. Several large studies have shown that the frequency distributions of IgG subclasses in healthy adults are skewed, with the possible exception of IgG1, (22-24) which was also true in our study (data not shown).

Figure (1) : Serum IgA levels in groups of diabetic and unaffected control. Comparison of average serum IgA (mean $\pm$ SD).

Figure (2) : Serum IgG levels in groups of diabetic and unaffected control. Comparison of average serum IgG (mean $\pm$ SD).

Figure (3) : Serum IgM levels in groups of diabetic and unaffected control. Comparison of average serum IgM (mean $\pm$ SD).

Smoking diabetic women in group 3 had a lower level of serum IgG and IgM compared to normal subjects in group 1. Immunodeficiency and autoimmune phenomena may occur concomitantly in the same individual. Many immune deficiency syndromes, mainly humoral defects, are associated with autoimmune disorders (25). Some immunodeficiency disorders involve a deficiency in a single immunoglobulin class or subclass. IgM is always the first class of antibody made by a developing B cell, although many B cells eventually switch to making other classes of antibody. Furthermore, IgG molecules are the only antibodies that can pass from mother to fetus via the placenta. Cells of the placenta that are in contact with maternal blood have Fc receptors that bind blood-borne IgG molecules and direct their passage to the fetus. Hence, the failure to switch to IgG- producing B-lymphocytes, or an impaired survival of such cells, may be an important molecular mechanism in IgG deficiency. Then, IgG deficiency may result from impaired switching from class IgM to IgG.

ELISA measurements showed that non-smoking diabetic women in group 2 had a higher level of serum IgA, IgG and IgM compared to normal women in group 1. The dramatic increase determined by ELISA was expected due to diabetic complications in support to previous studies (26-28). It has been estimated that exposure to environmental tobacco smoke in pregnancy results in birth weight reduction by approximately 20–30g (29, 30). Maternal cigarette use during pregnancy was associated with a significantly elevated risk of having a newborn with a congenital digital abnormality (31, 32). In addition, cigarette smoking is established as the major environmental risk factor for low lung function (33). We hypothesized that exposure to cigarette smoke (either personal smoking or environmental tobacco smoke) could potentially interact with spurious immuno-precipitation, as well as a circulating immunoglobulin which is capable of binding other autologous immunoglobulins may well interact with other immune factors, thus participating in the complex immunopathological events which occur in type 1 diabetes mellitus.

In conclusion, this study indicates the risk factor of tobacco smoking on humoral immune response in pregnant women with insulin dependent diabetes that in turn has a terrible consequence on fetus and may increase the risk of spontaneous abortion. However, further studies are needed to clarify the most vulnerable periods of pregnancy and the role of individual pollutants, and to examine whether the impaired reproductive outcomes have any long-term consequences on child health.

REFERENCES

1. Boitard, C., Timsit, J., Larger, E. and Dubois, D. (1997) Immune mechanisms leading to type 1 insulin-dependent diabetes mellitus. *Horm. Res.* 48,58-63.
2. Atkinson, M.A. and Maclaren, N.K. (1994) The pathogenesis of insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 331,1428–1436.
3. Kallan, A.A., de Vries, R.R. and Roep, B.O. (1996) T-cell recognition of beta-cell autoantigens in insulin-dependent diabetes mellitus. *APMIS.* 104,3-11.
4. Rush, D., Kristal, A., Blanc, W., Navarro, C., Chauhan, P., Campbell Brown, M., Rosso, P., Winick, M., Brasel, J. and Naeye, R. (1986) The effects of maternal cigarette smoking on placental morphology, histomorphometry, and biochemistry. *Am. J. Perinatol.* 3,263-272.
5. Economides, D. and Braithwaite, J. (1994) Smoking, pregnancy and the fetus. *J. R. Soc. Health* 114,198-201.
6. Rasch, V. (2003) Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. *Acta. Obstet. Gynecol. Scand.* 82,182-188.
7. Khoury, J.C., Miodovnik, M., Buncher, C.R., Kalkwarf, H., McElvy, S., Khoury, P.R. and Sibai, B. (2004) Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. *J. Matern Fetal Neonatal Med.* 15,44-50.
8. Hawthorne, G., Robson, S., Ryall, E.A., Sen, D., Roberts, S.H. and Ward Platt, M.P. (1997) Prospective population based survey of outcome of pregnancy in diabetic women: results of the Northern Diabetic Pregnancy Audit, 1994. *BMJ.* 315,279-281.
9. Evers, I.M., de Valk, H.W. and Visser, G.H. (2004) Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ.* 328,915.
10. Naeye, R.L. and Peters, E.C. (1984) Mental development of children whose mothers smoked during pregnancy. *Obstet. Gynecol.* 64,601-607.
11. Keeping, J.D., Najman, J.M., Morrison, J., Western, J.S., Andersen, M.J. and Williams, G.M. (1989) A prospective longitudinal study of social, psychological and obstetric factors in pregnancy: response rates and demographic characteristics of the 8556 respondents. *Br. J. Obstet. Gynaecol.* 96,289-297.

12. Fergusson, D.M., Horwood, L.J. and Lynskey, M.T. (1993) Maternal smoking before and after pregnancy: Effects on behavioral outcomes in middle childhood. *Pediatrics* 92,815-822
13. Engvall, E. and Perlmann, P. (1971) Enzyme-linked immunosorbent assay (ELISA): quantitative assay of immunoglobulin G. *Immunochem.* 8,871-874.
14. Engvall, E. (1977) Quantitative enzyme immunoassay (ELISA) in microbiology. *Med. Biol.* 55,193-200.
15. Eisenbarth, G.S. (1986) Type 1 diabetes mellitus: A chronic autoimmune disease. *N. Engl. Med. J.* 314,1360-1368.
16. Stene, L.C., Barriga, K., Norris, J.M., Hoffman, M., Erlich, H.A., Eisenbarth, G.S., McDuffie, R.S. Jr. and Rewers, M. (2004) Perinatal factors and development of islet autoimmunity in early childhood: the diabetes autoimmunity study in the young. *Am. J. Epidemiol.* 160,3-10.
17. Higgins, S. (2002) Smoking in pregnancy. *Curr Opin Obstet Gynecol.* 14,145-151.
18. Mili, F., Flanders, W.D., Boring, J.R., Annest, J.L. and Destefano, F. (1991) The associations of race, cigarette smoking, and smoking cessation to measures of the immune system in middle-aged men. *Clin. Immunol. Immunopathol.* 59,187-200.
19. McMillan, S.A., Douglas, J.P., Archbold, G.P., McCrum, E.E. and Evans, A.E. (1997) Effect of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentrations. *J. Clin. Pathol.* 50,819-822.
20. Williams, G.M., O'Callaghan, M., Najman, J.M., Bor, W., Andersen, M.J., Richards, D. U. C. (1998) Maternal cigarette smoking and child psychiatric morbidity: a longitudinal study. *Pediatrics* 102,111.
21. Marshall, A.L., Chetwynd, A., Morris, J.A., Placzek, M., Smith, C., Olabi, A. and Thistlethwaite, D. (2004) Type 1 diabetes mellitus in childhood: a matched case control study in Lancashire and Cumbria, UK. *Diabet. Med.* 21,1035-1040.
22. French, M.A. and Harrison, G. (1984) Serum IgG subclass concentrations in healthy adults: a study using monoclonal antisera. *Clin. Exp. Immunol.* 56,473-475.
23. Plebani, A., Ugazio, A.G., Avanzini, M.A., Massimi, P., Zonta, L., Monafo, V. and Burgio, G.R. (1989) Serum IgG subclass concentrations in healthy subjects at different age: age normal percentile charts. *Eur. J. Pediatr.* 149,164-167.
24. Hill, S.L., Mitchell, J.L., Burnett, D. and Stockley, R.A. (1998) IgG subclasses in the serum and sputum from patients with bronchiectasis. *Thorax* 53,463-468.
25. Simonte, S.J. and Cunningham-Rundles, C. (2003) Update on primary immunodeficiency: defects of lymphocytes. *Clin. Immunol.* 109,109-118.
26. Pietropaolo, M. and Eisenbarth, G.S. (2001) Autoantibodies in human diabetes. *Curr. Dir. Autoimmun.* 4,252-282.
27. Peng, S.L., Szabo, S.J. and Glimcher, L.H. (2002) T-bet regulates IgG class switching and pathogenic autoantibody production. *Proc. Natl. Acad. Sci.* 99,5545-5550.
28. Haroun, M. (2002) Effect of anti-immunoglobulin antibodies on serum IgA in type 1 diabetes mellitus. *Bull Alex. Fac. Med.* 38,169-174.
29. Windham, G.C., Eaton, A. and Hopkins, B. (1999) Evidence for an association between environmental tobacco smoke exposure and birthweight: *Paediatr. Perinat. Epidemiol.* 13,35-57.
30. Sram, R.J., Binkova, B., Dejmek, J. and Bobak, M. (2005) Ambient air pollution and pregnancy outcomes: a review of the literature. *Environ. Health Perspect* 113,375-382.
31. Czeizel, A.E., Kodaj, I. and Lenz, W. (1994) Smoking during pregnancy and congenital limb deficiency. *B.M.J.* 308,1473-1476.
32. Man, L.X. and Chang, B. (2006) Maternal cigarette smoking during pregnancy increases the risk of having a child with a congenital digital anomaly. *Plast. Reconstr. Surg.* 117,301-308.
33. Sherrill, D.L., Lebowitz, M.D. and Burrows, B. (1990) Epidemiology of chronic obstructive pulmonary disease. *Clin. Chest Med.* 11,375-387.