

HLA ANTIGENS IN ASYMPTOMATIC DIABETES. A 10-YEAR FOLLOW-UP STUDY OF POTENTIAL DIABETES IN PREGNANCY AND GESTATIONAL DIABETES

by

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INTRODUCTION

BOTH genetic and environmental factors have been implicated in the pathogenesis of diabetes mellitus. Juvenile-onset diabetes mellitus has been shown to be associated with increased frequencies of HLA-B8 and HLA-B15 (Nerup et al, 1974; Cudworth and Woodrow, 1975) and it is believed that a major genetic factor in this type of diabetes is the presence of a diabetogenic gene or genes linked to the major histocompatibility complex. While twin studies have suggested that genetic factors are also important in maturity-onset diabetes (Tattersall and Pyke, 1972) a definite association between antigens of the major histocompatibility complex and maturity-onset diabetes has not been demonstrated (Nerup et al, 1974; Cudworth and Woodrow, 1976).

Studies of genetic factors in diabetes mellitus have hitherto been confined mainly to symptomatic diabetes rather than asymptomatic diabetes, such as that which may occur in pregnancy. The relationship between this form of asymptomatic diabetes and symptomatic diabetes mellitus in later life is poorly understood. In particular, although some women with transiently abnormal glucose tolerance in pregnancy eventually develop symptomatic maturity-onset diabetes, it is uncertain whether they are at greater risk of doing so than the normal population (O'Sullivan, 1975; Hadden, 1979). Genetic factors may have a role in either the pathogenesis of asymptomatic diabetes in pregnancy or its subsequent progression to symptomatic maturity-onset diabetes.

As part of a long-term follow-up study (Hadden, 1979) we have examined the frequencies of HLA-B8 and HLA-B15 in women who had transiently abnormal glucose tolerance in an index pregnancy i.e. "gestational diabetics" and women regarded as having "potential diabetes in pregnancy" on the basis of selection criteria during the index pregnancy (previous big baby, family history of diabetes, etc) and have correlated our findings with the results of oral glucose tolerance tests 10 years later.

PATIENTS AND METHODS

Two groups of patients were studied. Group 1 consisted of 32 patients who had been classified during an index pregnancy as having "potential diabetes in pregnancy" and who were found to have an abnormal glucose tolerance test (GTT) at 10 year follow-up. This group was obtained from 625 patients attending the Antenatal Clinic of the Royal Maternity Hospital, Belfast between 1963 and 1965 who fulfilled one or more of the criteria for the diagnosis of potential diabetes in pregnancy in previous pregnancies or in the index pregnancy (Hadden and Harley, 1967). Of these, 234 attended for a 50g oral GTT 10 years later as part of a long term follow-up study and 32 were found to have abnormal glucose tolerance (2 hour plasma glucose greater than 7.2 mmol/l) (Hadden, 1979). Tissue typing was carried out in all of these 32 patients.

Group 2 consisted of 83 patients who had been classified during an index pregnancy as having "gestational diabetes". Between 1966 and 1968 over 1000 mothers attending the Antenatal Clinic of the Royal Maternity Hospital, Belfast fulfilled the selection criteria for the diagnosis of potential diabetes in pregnancy and had either an intravenous GTT or a cortisone-stressed oral GTT (Hadden et al, 1971). Of these 216 were shown to have an abnormal GTT in the index pregnancy. Frankly diabetic patients were excluded from the study. Ninety-three of these patients attended for a 50g oral GTT 10 years later. Tissue typing was carried out in 83 of these patients, 15 of whom had an abnormal GTT at follow-up.

At follow-up 10 years after the index pregnancy the patients were classified according to the results of a 50g oral GTT as "normal" (2 hour plasma glucose less than 7.2 mmol/l), "borderline" (2 hour plasma glucose 7.2—8.29 mmol/l) or "diabetic" (2 hour plasma glucose greater than 8.3 mmol/l). All patients with 2 hour plasma glucose greater than 7.2 mmol/l were considered to be "abnormal".

Two hundred normal blood donors resident in the same geographical area and tissue-typed concurrently with the patients were used as controls. Typing for HLA-B8 and HLA-B15 was carried out by a standard microlymphocytotoxicity test. In each instance not less than 3 different antisera of each specificity were used. Antisera against HLA-B15 were obtained from the National Tissue Typing Reference Laboratory, Bristol. Antisera against HLA-B8 were obtained locally and their specificity was checked in two other laboratories. Statistical analysis was carried out using the exact probability test.

RESULTS

Of the 32 patients (group 1) with potential diabetes in pregnancy who had abnormal GTT's 10 years later, 9 were classified as diabetic and 23 as borderline. The frequencies of HLA-B8 and HLA-B15 in this group compared with the 200 control subjects are shown in Table I. A significantly increased frequency of HLA-B15 but not HLA-B8 was found in this group compared with control subjects. This difference was due solely to the frequency of HLA-B15 in those patients classified as borderline.

TABLE I

Frequencies of HLA-B8 and HLA-B15 in 32 patients with potential diabetes in pregnancy who had abnormal glucose tolerance tests at follow-up compared with 200 control subjects.

	Number studied	HLA-B8			HLA-B15		
		Number positive	Per cent positive	Probability	Number positive	Per cent positive	Probability
Blood donors	200	69	34.5	—	9	4.5	—
Abnormal at follow-up	32	9	28.1	0.4277	6	18.8	0.0087
(i) Diabetic at follow-up	9	3	33.3	0.9999	0	0	0.9998
(ii) Borderline at follow-up	23	6	26.1	0.3666	6	26.1	0.0017

Of the 83 patients (group 2) with gestational diabetes, 7 were classified as diabetic, 8 as borderline and 68 as normal on the basis of their follow-up GTT's. The frequencies of HLA-B8 and HLA-B15 in this group compared with the 200 normal control subjects are shown in Table II. In the group as a whole and in those who were classified as normal at follow-up, but not in those who were abnormal at follow-up, the frequency of HLA-B8 was significantly decreased. The frequency of HLA-B15 in this group was nearly twice that of the control population, but this increase was not statistically significant.

TABLE II

Frequencies of HLA-B8 and HLA-B15 in 83 gestational diabetics compared with 200 control subjects.

	Number studied	HLA-B8			HLA-B15		
		Number positive	Per cent positive	Probability	Number positive	Per cent positive	Probability
Blood donors	200	69	34.5	—	9	4.5	—
Gestational diabetics	83	17	20.5	0.0106	7	8.4	0.2561
(a) Normal at follow-up	68	13	19.1	0.0097	6	8.8	0.2203
(b) Abnormal at follow-up	15	4	26.7	0.5806	1	6.7	0.5226
(i) Diabetic at follow-up	7	1	14.3	0.4249	0	0	0.9999
(ii) Borderline at follow-up	8	3	37.5	0.9998	1	12.5	0.3304

Of the 115 patients (groups 1 and 2) studied, 47 had an abnormal GTT at follow-up i.e. they had asymptomatic diabetes 10 years after an index pregnancy in which they had potential diabetes in pregnancy or gestational diabetes. The relative

frequencies of HLA-B8 and HLA-B15 in these asymptomatic diabetics are shown in Table III. Once again there was a significant increase in the frequency of HLA-B15 compared with control subjects and this increase was due solely to those patients classified as borderline at follow-up. There was however, no statistically significant difference between the frequency of HLA-B15 in the borderline group compared with the diabetic group ($p = 0.0782$) or with the group who were normal at follow-up ($p = 0.1044$).

TABLE III

Frequencies of HLA-B8 and HLA-B15 in 47 patients with either potential diabetes in pregnancy or gestational diabetes who had abnormal glucose tolerance tests at follow-up compared with 200 control subjects.

	Number studied	HLA-B8			HLA-B15		
		Number positive	Per cent positive	Probability	Number positive	Per cent positive	Probability
Blood donors	200	69	34.5	—	9	4.5	—
Abnormal at follow-up	47	13	27.7	0.3062	7	14.9	0.0170
(i) Diabetic at follow-up	16	4	25.0	0.4269	0	0	1.0000
(ii) Borderline at follow-up	31	9	29.0	0.5443	7	22.6	0.0019

DISCUSSION

In this study HLA-B15 appears to be associated with abnormal glucose tolerance 10 years after an index pregnancy in which a woman was considered to have either gestational diabetes or potential diabetes in pregnancy. The frequency of HLA-B15 in these patients was comparable with that found in juvenile-onset diabetes in several series (Nerup et al, 1974; Cudworth and Woodrow, 1975) and in addition the frequency of HLA-B15 in our Northern Ireland control population was almost identical to that found in a large series in the Bristol area (Middleton and Martin, 1978). In contrast the frequency of HLA-B15 was only slightly increased in an unselected series of gestational diabetics. Although an unselected series of patients with potential diabetes in pregnancy was not examined in this study a similar absence of any significant HLA association might be expected especially since potential diabetes in pregnancy comprises a very heterogeneous group of patients. In this study therefore the increased frequency of HLA-B15 is associated with asymptomatic diabetes in middle life rather than with the original criteria for inclusion in the follow-up study. This study does not, however, suggest a correlation between HLA-B15 and progression from gestational diabetes or potential diabetes in pregnancy to symptomatic maturity-onset diabetes. In our series of patients who have now been followed from 10 to 15 years the incidence of symptomatic diabetes is very low and may be no greater than would be expected in a comparable group of the female population (Hadden et al, 1979). We cannot predict whether our patients who now have abnormal oral GTT's will eventually become symptomatic maturity-onset diabetics but prolonged follow-up is obviously required.

There have been several studies of the frequency of HLA antigens in maturity-onset diabetes but, in contrast to juvenile-onset diabetes no definite association with any HLA antigen has been found (Nerup et al, 1974; Cudworth and Woodrow, 1976). The association of asymptomatic diabetes with an increased frequency of HLA-B15 in this study therefore favours the suggestion that asymptomatic glucose intolerance does not necessarily progress to symptomatic maturity-onset diabetes. In this respect it is of interest that the increased frequency of HLA-B15 occurs predominantly in those women whose glucose tolerance tests were classified as "borderline" rather than "diabetic". It is thus possible that this study has revealed two subgroups of asymptomatic diabetes; (a) those with diabetic glucose tolerance tests in whom there is no HLA association and who may eventually become symptomatic maturity-onset diabetics and (b) those with mildly abnormal glucose tolerance tests associated with an increased frequency of HLA-B15 and in whom the outcome is uncertain.

A rather unexpected finding in this study was the decreased frequency of HLA-B8 in those patients with gestational diabetes compared with the control population, the difference being due mainly to the decreased frequency of HLA-B8 in those patients with gestational diabetes who had normal glucose tolerance at follow-up. This appears to be a true decrease in the frequency of HLA-B8 in the gestational diabetics rather than a falsely high frequency of HLA-B8 in the control population since the frequency of HLA-B8 has recently been shown to be higher in Northern Ireland, in common with other parts of Ireland, compared with other areas in the British Isles (Middleton and Martin, 1978). No obvious explanation exists for the decreased frequency of HLA-B8 in the gestational diabetics but it is perhaps relevant that patients who were already frankly diabetic during the index pregnancy and who might be expected to have an increased frequency of HLA-B8 were excluded from the study.

In most instances in which an increased frequency of HLA-B15 has been associated with symptomatic diabetes there has been a concomitant increase in the incidence of HLA-B8 (Nerup et al, 1974; Cudworth and Woodrow, 1975). Cudworth and Woodrow (1976) found that in juvenile-onset diabetics the relative risk was additive when both HLA-B8 and HLA-B15 were present. They suggested that this finding was in favour of there being more than one HLA-linked gene, possibly operating by different mechanisms, involved in the pathogenesis of juvenile-onset diabetes. It may therefore be that our patients in whom HLA-B15 is associated with asymptomatic impaired glucose tolerance have lacked an additional genetic or environmental factor which would have favoured the development of juvenile-onset diabetes. In favour of this suggestion is the finding of Nelson et al (1975) that there is an increased frequency of HLA-B15 but not HLA-B8 in identical twins discordant for juvenile-onset diabetes. This suggests that an HLA-B15 linked diabetogenic gene may not necessarily be phenotypically expressed.

The mechanisms by which HLA-linked disease susceptibility genes result in expression of the disease concerned are uncertain but one possibility is that such genes determine susceptibility to virus infection. There is good evidence that virus infection can result in diabetes in experimental animals (Craighead, 1975) but in human diabetes the evidence in favour of initiating virus infection e.g. by Coxsackie

B4 virus, is much weaker (Gamble, Taylor and Cumming, 1973; Hadden et al, 1972). Cudworth et al (1977) have produced evidence that HLA-B15 positive juvenile-onset diabetics have higher neutralising antibody titres to Coxsackie virus types B1—B4 at the time of diagnosis. Antibody titres to Coxsackie virus were not determined in this study. Further work is required to determine the mechanism by which the presence of HLA-B15 or an HLA-B15 linked gene may lead to the development of asymptomatic diabetes mellitus.

SUMMARY

In a 10-year follow-up study the frequencies of HLA-B8 and HLA-B15 were examined in women classified in an index pregnancy as having potential diabetes in pregnancy or gestational diabetes. In 32 patients who had been considered as having potential diabetes in pregnancy and who had an abnormal GTT ten years later, the frequency of HLA-B15 (18.8 per cent) was significantly increased compared with 200 control subjects (4.5 per cent; $p=0.0087$). In 83 patients originally classified as having gestational diabetes, 15 of whom had an abnormal GTT ten years later, the frequency of HLA-B15 (8.4 per cent) showed a small increase compared with controls (4.5 per cent; $p=0.2561$) while the frequency of HLA-B8 (20.5 per cent) was significantly lower than controls (34.5 per cent; $p=0.0106$).

Of a total of 115 patients studied 47 had an abnormal GTT at follow-up and in these 47 patients the frequency of HLA-B15 was also significantly increased compared with controls (14.9 per cent; $p=0.017$). This increase was most marked in those women with borderline GTT (22.6 per cent; $p=0.0019$).

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BOOK REVIEW

MANUAL OF GYNAECOLOGIC AND OBSTETRIC EMERGENCIES. By B. Taber, M.D. (Pp 929. Illustrated. £17.25). Philadelphia, London, Toronto: W. B. Saunders and Eastbourne; Holt-Saunders. 1979.

THERE is little doubt that one can find in this book hints on the management of almost every emergency possible in obstetrics and gynaecology. There is information on topics as far ranging as breech presentation to burns and syncope to sexual assault.

The book is well laid out with a good index and each subject is discussed in a similar and logical manner. The information is sound and supported by illustrations and recent references although some procedures and drugs have a strong American flavour.

It is not unreasonable to ask what place there is for a manual devoted to emergency procedures and one must agree with the sentiments of the emergency physician writing the foreword that "in the various fields of medicine the chances are that a knowledge of obstetrics and gynaecology will be necessary at some time or another". Although he suggests that the manual may fill the need of the psychiatrist, dermatologist, orthopaedic or general surgeon faced with a problem, I find it difficult to agree with him that it will be read by any physician who might ask himself "Do I know enough of the most wondrous elements of the physiology of the female of our species?"

However, this book would be of use to specialists in other fields and indeed to general practitioners who wish to remind themselves what is current practice in a wide variety of situations in obstetrics and gynaecology.

J.W.K.R.

ERRATUM

It is regretted that the portrait of Mr. T. S. Holmes (Figure 1 page 9 of the previous number) was incorrectly named Mr. H. P. Hall.