Insulin Resistance in Women with Recurrent Spontaneous Miscarriage of Unknown Aetiology

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Key Words

Insulin • Insulin, resistance • Miscarriage, recurrent • Miscarriage

Abstract

Objective: The objective of this study was to explore the role of insulin resistance in women with recurrent spontaneous miscarriage (RSM). Patients with ≥ 3 consecutive spontaneous miscarriages were classified as having RSM. **Subjects and Methods:** Thirty-five non-pregnant women presenting with RSM in our specialized RSM clinic constituted the study population, while 30 non-pregnant, parous, fertile women without RSM served as controls. The study and control patients were matched for age, ethnicity and body mass index (BMI). Blood was extracted from these patients for fasting blood glucose and fasting insulin simultaneously, and a battery of investigations including ultrasonography was performed on all the patients with RSM. **Results:** There was no significant difference in the mean fasting glucose for the study and control patients (5.5 ± 0.7 vs. 5.3 ± 0.6 mmol/dl) and in the mean fasting insulin (15.0 ± 5.1 and 12.9 ± 2.9 mU/l). There was a significant difference between the mean parity of the study and control patients (0.7 ± 0.7 vs. 1.7 ± 0.5 , $p < 0.0001$) and also the mean number of miscarriages (4.2 ± 1.2 vs. 0.1 ± 0.4 , $p <$

0.0001). There was, however, no significant difference in the insulin resistance of both groups as calculated using the Homeostasis Model Assessment and the fasting glucose insulin ratio of < 4.5 . **Conclusion:** Insulin resistance was not significantly associated with RSM in our study. Further studies may be needed.

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Introduction

Recurrent spontaneous miscarriage (RSM) remains a very disturbing event to the patients who are affected by this medical problem; they are always anxious to find out the underlying reasons for their miscarriages. RSM also remains a major challenge to the treating physicians because of their desire to satisfy the interests of their patients. Intensive research efforts including immunological and genetic studies are still in progress to unravel all the aetiological factors associated with this rather difficult problem so as to contribute positively towards the effective management. In spite of such research efforts, about 40–50% of RSM remain ‘unexplained’ [1, 2].

The aetiological factors associated with RSM in our clinic population and in many published reports include

chromosome anomaly (parental balanced translations), uterine malformations or anomalies, hypothyroidism, cervical incompetence, antiphospholipid syndrome (the most frequently identified aetiological factor in our clinic patients), bacterial infections (including bacterial vaginosis) and polycystic ovary syndrome (PCOS) [1, 3, 4–7]. PCOS has been associated with early RSM in many studies with an incidence of 21–56% being reported [5–7]; the exact role and mechanism of PCOS in RSM remain uncertain. PCOS is characterized by chronic anovulation, hyperandrogenism, menstrual abnormalities and obesity, with insulin resistance being a prominent feature of the presentation [8–10]; 40% of PCOS patients have insulin resistance. PCOS which does not respond readily to a variety of treatment options may progress to reproductive failure [1]. High levels of plasminogen activator inhibitor-1 (PAI-1), a significant inhibitor of fibrinolysis, have been reported in women with PCOS [11–15] and in women with early unexplained RSM [16, 17]. High fasting insulin levels and high PAI-1 levels have been associated with RSM [12, 15]. Treatment of some women with PCOS and early RSM using metformin has led to a decrease in fasting insulin level and PAI-1 levels and improved pregnancy outcome [15]. An increased incidence of insulin resistance has also been reported in unselected women with RSM [3]. The exact mechanism of how increased insulin resistance leads to RSM is, however, still unknown.

Insulin resistance can be diagnosed by the determination of the fasting glucose to fasting insulin ratio, a ratio of <4.5 being diagnostic of insulin resistance [18]. The Homeostasis Model Assessment (HOMA) has also been used to calculate the insulin resistance [19]; a result of >4.5 being diagnostic of insulin resistance.

In our search for further aetiological factors in our patients presenting with RSM, we embarked on a study of insulin resistance in our patients to ascertain if insulin resistance played any significant aetiological role in our patients with RSM.

Subjects and Methods

This was a cross-sectional study. The study was undertaken between October 2002 and June 2004 in the Department of Obstetrics and Gynaecology, Maternity Hospital, Kuwait. Thirty-five consecutive non-pregnant patients attending our special RSM clinic were selected as the study population. Patients who had ≥ 3 consecutive spontaneous miscarriages were classified as patients with RSM. Thirty non-pregnant, parous, fertile patients attending our general gynaecological clinic who did not have

RSM were selected as the control patients. The patients (study and control) were matched for age, ethnicity and body mass index (BMI).

The objectives and the plan for the study were discussed with all the patients, and they gave their consent to participate in the study. The study was approved by the Scientific/Institutional Review Board of the hospital.

The patients presenting with RSM were screened as outlined in our previous study [4] to establish the aetiology of the RSMs. The study patients were screened for chromosome anomaly (parental karyotype studies), uterine anomaly including cervical incompetence, antiphospholipid syndrome, PCOS, infections including bacterial vaginosis, and endocrinological disorders (including hypothyroidism, diabetes mellitus, hyperprolactinaemia, hypothalamic/pituitary disorders). The control patients presented with a variety of gynaecological complaints including vaginal discharge, slight menstrual disturbances (inter-menstrual spotting, pre-menstrual spotting and staining, dysmenorrhoea), mild/moderate pelvic inflammatory disease, urinary tract infections and small dermoid cysts, and they were all investigated and treated appropriately.

Blood was withdrawn from the study and control patients specifically for the determination of the fasting blood glucose and fasting insulin levels. The patients had a 12-hour overnight fast before the blood was extracted from the patients between 8.00 and 10.00 a.m. on the day of the test. The fasting glucose was determined soon after the collection of the blood samples. The blood samples for fasting insulin were spun down immediately after collection and frozen and analysis performed later.

Fasting serum insulin was determined by an ELISA (DSL-10-1600 ACTIVE, Diagnostics Systems Laboratories, Webster, Tex., USA). The intra- and inter-assay coefficients of variation were 3.1 and 5.4%, respectively. Glucose concentration was determined by the oxygen rate method employing a Beckman oxygen electrode on the Beckman LX20 automated analyzer (Beckman Corp., Brea, Calif., USA). The intra- and inter-assay coefficients of variation were 2.1 and 3.7%.

The fasting glucose to fasting insulin ratio was calculated for each patient. This ratio was equal to the insulin resistance for each patient.

The HOMA for insulin resistance was also calculated as follows: $IF \times GF/22.5$, where IF = fasting insulin level (mU/l) and GF = fasting glucose in SI units (mmol/l), as described by Kauffman and Castracane [20].

Statistical Analysis

The data from the two groups were subjected to statistical analysis using Fisher's exact two-tailed test, an alternate Welch t test when the standard deviations between the groups were not equal and also the χ^2 test, using the SPSS 13 package of Windows 2000.

Results

The Kuwaitis constituted 60 and 56% of the study population and the control group, respectively; there was no significant difference in this distribution of Kuwaitis in

the study and control groups, $p = 0.985$ (table 1). There was also no significant difference in the distribution of non-Kuwaitis in the study and control groups. The percentage of Kuwaitis and non-Kuwaitis using the services of Maternity Hospital, Kuwait, is usually 66.7% Kuwaitis to 33.3% non-Kuwaitis, and this is essentially the ratio reported in this study. Ethnicity did not play any significant role in the study.

The mean number of spontaneous previous miscarriages was significantly higher in the study group compared with the control group: 4.2 ± 1.2 versus 0.1 ± 0.4 ($p < 0.0001$). Conversely the control group had a significantly higher parity than the study group: 1.7 ± 0.5 versus 0.7 ± 0.7 , ($p < 0.0001$). As expected there was no significant difference in the age and BMI of the study and control groups, since the patients were matched for these events. The patients were well matched for the BMI, although the BMI in the study and control groups were elevated.

Seven patients in the study group also presented with PCOS; only 2 (28.6%) of these patients with PCOS had

confirmed insulin resistance, and since these numbers were rather small, a test for statistical significance was considered unnecessary. PCOS was not diagnosed in any of the control patients. The aetiological factors associated with RSMs in the study population followed the trend previously reported in our clinic population. There were no cases of type I (insulin-dependent) and type II (non-insulin-dependent) diabetes mellitus in the study population. Three patients in the study group had hyperprolactinaemia, while no such case was reported in the control group. Testosterone levels were estimated in the study population but not in the control group.

The mean fasting blood glucose and fasting insulin levels were higher in the study group compared with the control group (table 2). Although there was no significant difference in the fasting blood glucose in both groups, 5.5 ± 0.7 versus 5.3 ± 0.6 mmol/l ($p = 0.264$), the fasting insulin levels in the study group were significantly higher than those reported for the control group, 15.0 ± 5.1 versus 12.9 ± 2.9 mU/l ($p = 0.042$). The mean fasting insulin reported in the study group was, however, lower than the level diagnostic of insulin resistance: ≥ 20 mU/l.

Insulin resistance was detected in more patients in our study population (6 patients, 17.1%) than controls (2 patients, 6.7%), using the fasting glucose to insulin ratio method for assessing insulin resistance, but was not significantly different ($p = 0.211$). The HOMA insulin resistance scores for the study and control patients were 3.7 ± 1.6 (range 2.6–9.5) and 3.1 ± 0.9 (range 2.5–4.9), and the HOMA insulin resistance index of >4.5 identified insulin resistance in 8 (22.9%) and 2 (6.7%) in the study and control patients, respectively; these data were not significantly different ($p = 0.093$, odds ratio = 4.00, 95% confidence interval = 0.709–22.566; table 2).

Table 1. The clinical characteristics of the population studied

Characteristics	Study group n = 35	Control group n = 30	p value
Age, years	28.6 ± 4.3	27.5 ± 4.6	0.323*
Parity	0.7 ± 0.7	1.7 ± 0.5	$<0.0001^{**}$
Miscarriages	4.2 ± 1.2	0.1 ± 0.4	$<0.0001^{**}$
BMI	29.4 ± 6.5	29.1 ± 6.3	0.851*
Kuwaiti, %	60.0	56.7	0.985*
Non-Kuwaiti, %	40.0	43.3	0.985*

* Not significant; ** statistically significant.

Table 2. Biochemical data of the population studied

Biochemical data	Study group n = 35	Control group n = 30	p value
Fasting glucose, mmol/l	5.5 ± 0.7	5.3 ± 0.6	0.264*
Fasting insulin, mU/l	15.0 ± 5.0	12.9 ± 2.9	$<0.0428^{**}$
Fasting glucose, mg/dl	99.3 ± 12.1	96.8 ± 10.6	$<0.400^{**}$
Glucose:insulin ratio	7.2 ± 2.0	7.8 ± 1.6	0.211*
HOMA-IR (>4.5)	8 (22.9%)	2 (6.7%)	0.093*
HOMA-IR score	3.7 ± 1.6 (range 2.5–9.5)	3.1 ± 0.9 (range 2.5–4.9)	
Glucose:insulin ratio (<4.5)	6 (17.1%)	2 (6.7%)	0.112*

* Not significant; ** significant. IR = Insulin resistance.

Insulin resistance was detected in 2 of our 7 study patients classified as having PCOS. The results of insulin resistance determined by both methods were virtually the same.

Discussion

The aetiological factors involved in RSM in the patients studied has followed the pattern previously reported from our centre [4]. The incidence of unexplained RSM, 40–50%, in most studies, however remains high, serving as a strong incentive to continue the search for other aetiological factors in this clinical problem; this was a motivating factor in the initiation of this study on insulin resistance in RSM.

Since the difference in fasting glucose to insulin ratio and HOMA insulin resistance index between the study and control groups was not statistically significant (table 2), it follows that insulin resistance was not significantly associated with RSM in our study population. The incidence of insulin resistance in our study population (17.1%) is lower than the rate of 27.0% reported in another study [3], in which there was an increased prevalence of insulin resistance in patients with RSM.

The gold standard for assessing insulin resistance is the clamp technique [21]. This technique is rather elaborate, time-consuming and complex for the average clinician. The fasting glucose to fasting insulin ratio [18] has been identified as quite a sensitive method for diagnosing insulin resistance, while the HOMA method [19] has been reported to correlate well with the clamp technique [22, 23] and thus provides quite a good assessment of insulin resistance in clinical practice. The HOMA method has been found quite reliable for insulin resistance studies [24]. The fasting glucose to fasting insulin ratio and the HOMA method were used for assessing insulin resistance in the current study, and so one could safely state that insulin resistance was adequately quantified in the current study.

HOMA has also been used to assess insulin resistance in some studies on PCOS [25, 26].

Insulin resistance has been reported in about 40% of patients with PCOS, as previously stated in this paper. Insulin resistance has also been reported to correlate positively with plasma levels of PAI-1 [27]. High levels of PAI-1 have been reported in women with PCOS [11–15] and in women with early unexplained RSM [16, 17]. In the current study, only 7 cases had PCOS, and only 2 of them had insulin resistance. This observation is keeping

with the previous reports which indicate that not all patients with PCOS present with insulin resistance [3, 9, 24]. However, insulin resistance was also reported in other patients in the study population; some other cases in both the study and control groups who were not cases diagnosed as having PCOS were also diagnosed as having insulin resistance, as already reflected under the results. PAI-1 levels were not estimated in the current study; however, their association with insulin resistance, PCOS and unexplained RSM as stated above from other studies, areas that were also covered in the current research, makes the linkage relevant, and appropriate deductions can thus be extrapolated. Altered levels of PAI-1 have been associated with disorders of follicular rupture, failed implantation/implantation difficulties and miscarriages [28]. This is a probable pathway for the explanation of insulin resistance (as an individual entity independent of its linkage with PCOS stated above) being associated with RSM. This may appear a speculative approach for now, but it is an area that could be pursued in further studies to establish definitive answers if possible.

The exact mechanisms linking insulin resistance with RSM, however, are still unknown and certainly call for further studies.

There are some obvious limitations in our study. The population studied was rather small. The fasting glucose and fasting insulin determination could also be affected if the patients did not abide by some of the pre-conditions for such estimations: adequate diet before the tests and appropriate fasting period before the study. The determination of fasting insulin levels could also have been affected by the natural fluctuations of insulin levels in patients.

The above points notwithstanding, our study has indicated that insulin resistance is not significantly associated with RSM. The higher levels of fasting blood glucose and fasting insulin and higher percentages of insulin resistance in the study population (in which PCOS was not a major contributor) strongly indicate that insulin resistance should be further evaluated in patients with RSM. There is quite a probability that insulin resistance might play some role in RSM. Larger studies are therefore called for to help in unravelling any real connection between insulin resistance and RSM.

Conclusions

Higher levels of fasting blood glucose and fasting insulin and higher percentages of insulin resistance were reported in the study patients with RSM, although the

differences were not statistically significant. Insulin resistance has not been identified as a significant factor in our patients with RSM. A larger study is required to investigate further the relationship between insulin resistance and RSM.

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