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Comparison of Glycosylation Patterns of Placental Proteins Between Normal Pregnancy and Missed Abortion

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A B S T R A C T

Contemporary understanding of missed abortion, as the case of spontaneous abortion where embryo is retained in uterus for four weeks or more after its death, is very poor. Aiming to improve the level of knowledge about this process, we have compared glycosylation patterns of placental proteins in normal pregnancy and missed abortion. Oligosaccharide branches were detected by Western-blot using SNA, DBA and PHA-E lectins. The comparison of samples of the same gestational age enabled identification of changes in protein glycosylation between normal and pathological placentas. Lectin DBA detects in normal placenta the glycoprotein GP 105 during the eleventh week, which is absent in missed abortion. PHA-E identifies GP 71 during fourteenth week only in normal placenta. However GP 25 recognized by SNA in missed abortion was not found in normal pregnancy at tenth week. These results indicate that abnormal placental development is associated with changes in glycoprotein structures, and that glycoconjugates might have an important role in placental development.

Key words: *glycoproteins, lectins, placenta, missed abortion*

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Introduction

Missed abortion describes a pregnancy in which the fetus had died but the uterus has made no attempt to expel the products of conception[1].

The reason why some abortions do not occur after death of fetus, while others do, is not clear, but Philipp and Kalousek found chromosomal abnormalities in 70% of cases[2]. Increasing maternal age is related to rising rates of both missed and spontaneous abortion[3].

The size of the yolk sac, as the first embryonic structure visualized sonographically within the gestational sac, was abnormally small or large in the majority of patients diagnosed as missed abortion[4]. Kurjak and others found no difference in the Doppler indices between a missed abortion and a normal pregnancy up to 11 weeks of gestation[5]. Trying to evaluate the usefulness of color Doppler for distinguishing between normal and abnormal pregnancies (missed abortion and anembryonic pregnancy), the other group of scientists conclude that it does not offer to the clinician an additional information in the diagnosis of early reproductive failure[6]. Some data show that luteal vascularization might be decreased in missed abortion but not in threatened abortion and anembryonic pregnancy[7,8].

Levels of human decidua-associated protein 200 (hDP200) were measured in amniotic fluid samples and a 3-fold decrease in the hPD200 level was observed between 9 and 20 weeks of normal pregnancy, the similar pattern was found in missed abortion[9]. The same group found no significant difference in the hPD200 levels in uterine fluid, comparing patients with ectopic pregnancy and patients with early missed abortion[10]. Zielberstein and Seibel suggest transvaginal amniotic puncture (TAP) for accurate cytogenetic assessment of missed abortion, which should lead to reevaluation of our current understanding and management of pregnancy loss[11]. There are findings that serum progesterone level can be used as a screening test with high sensitivity and specificity to predict a normal pregnancy[12,13]. Shahani and others have shown that early pregnancy factor (EPF) could be a useful marker of prognostic value in threatened abortions, because it is present in viable but absent from non-viable pregnancies[14,15]

For reason that the contemporary understanding of missed abortion is very poor, we have undertaken comparison of glycosylation patterns of placental proteins between several cases of missed abortion and normal pregnancy. Transmembrane glycoproteins belong to molecules, which participate in the two crucial processes for establishment and maintenance of normal pregnancy: implantation and placentation[16,17]. They facilitate two types of interactions: intercellular communications and communications of cells with extracellular matrix[18,19]. During implantation and placentation, cytotrophoblast cells secret proteases which are glycoproteins too[20–22]. The fact, that sugars are not just dull raw materials, but serve as sophisticated information-carriers with distinct biological specificities when bound to proteins, has attracted imposing attention from researches in recent years, and has promoted a universal re-evaluation of the importance of carbohydrates in biology and medicine. For reason of such an important role of glycoproteins we have undertaken in this study a systematic research of glycosylation of placental proteins in the period form the seventh till the seventeenth week of gestation trying to find differences between normal pregnancy and missed abortion.

Materials and Methods

The following materials were purchased: 5-bromo-4-chloro-3-indolyl phospha-

te (BCIP), nitro blue tetrazolium (NBT), ammonium persulfate (APS), glycine, magnesium chloride, sodium chloride, sodium dodecyl sulfate and Trizma base from Sigma (St. Louis, MO); bovine serum albumin (BSA), glycerol, p-mercaptoethanol, phenylmethylsulfonyl fluoride, Rotiophorese gel 30 and Tween 20 from Roth (Karlsruhe, D); ethanol, hydrochloric acid, methanol and acetic acid from Kemika (Zagreb, C); N,N,N',N'-Tetramethylethylenediamine from Merck (Darmstadt, D); Immobilion PVDF membrane from Millipore (Bedford, MA); digoxigenin-labeled SNA lectin and alkaline phosphatase-conjugated antidigoxigenin Fab fragments from Boehringer Mannheim (Mannheim; D); biotin-labeled PHA-E, UEA-I and DBA lectins, streptavidin alkaline phosphatase conjugate, bromphenol blue and Coomassie brilliant blue R-250 from Serva (Heidelberg, D).

The placental samples

Samples of 21 placental tissues were provided by healthy women undergoing elective termination of normal pregnancies at 10–14 weeks of gestation. The second group consisted of 11 pregnant women with missed abortion diagnosed by a routine transvaginal ultrasound. The operative methods were cervical dilatation followed by vacuum extraction or curettage. Gestational ages were assessed by the date of the last menses and checked by ultrasound and clinical examinations. Immediately after evacuation, placental tissues were frozen in liquid nitrogen and stored at –80 °C until used.

Placental tissues were mechanically homogenized in homogenizing buffer (50 mM Tris HCl, pH 7.5; 100 mM NaCl; 1 mM EDTA) containing 1mM phenylmethylsulphonyl fluoride. The homogenates were centrifuged at 5,000 g for 10 minutes and supernatants were stored at –80 °C.

Polyacrylamide gel electrophoresis

Gel electrophoresis of glycoproteins was performed in 10% polyacrylamide gels containing 0.1% sodium dodecylsulfate (SDS) (SDS-PAGE) according to Laemmli[23]. The protein samples were dissolved in a sample buffer (pH 6.8, Tris HCl 50 mM, Glycerol 10%, p-mercaptoethanol 5%, SDS 2%, Bromphenol-blue 0.1% in distillated H_2O) in ratio 1 : 1. They were denaturated for 5 minutes on the 95 °C before use. After migration of the samples (20 µg of total proteins in one slot) from the stacking gel into the separation gel at 80 V, proteins were separated at 120 V.

Western blotting

After electrophoresis, proteins were transferred to the PVDF immobilion membrane by the semidry blotting system (Pharmacia, Sweden) in the semidry buffer (Tris HCl 48 mM, Glycin 39 mM, SDS 1.4 mM, methanol 20%)[24]. Blotting was carried out at 0.8 mA/cm^2 over a period of 60 minutes. After blotting, the part of PVDF membrane with standard proteins was separated, and the rest of membrane was blocked overnight with 3% bovine serum albumin (pH 7.5).

Identification of proteins after blotting

After overnight blocking, membrane was incubated with lectins in lectin buffer ($MgCl_2$ 1 mM, $CaCl_2$ 1 mM in TBS). Lectin-glycoprotein complexes were detected with antidigoxigenin Fab fragments conjugated to alkaline phosphatase (SNA), or streptavidin-alkaline phosphatase conjugate (PHA-E, UEA-I and DBA), and visualized with BCIP (5-bromo-chloro-3-indolyl phosphate)/NBT (nitro blue tetrazolium)[25,26].

Results

Polyacrylamide gel electrophoresis in combination with the Western blotting

and analysis with the given set of lectins provided interesting results even within the given set of samples available so far. In the following photographs results obtained on placental glycoproteins from normal pregnancy vs. missed abortion in five stages of intrauterine development are shown. In all of them positions of protein standards (kD) are indicated on the left side of the gel, whereas positions of particularly interesting placental glycoproteins are marked on the right side of the picture.

Lectin SNA provides results on placental glycoproteins from the 10th week of normal pregnancy presented in Figure 1. Glycoprotein patterns of placentas from missed abortion obtained by electrophoresis and detection with SNA lectin in 10th week of pregnancy (lane 2) in comparison to control placenta from normal pregnancy (lane 1) of the same gestational age. Glycoproteins with molecular weights of 80 kD and 43 kD are more abundant in patterns of pathological sample than in normal placenta. Gp 25 is absent in normal and present only in placenta from missed abortion. Gp 74 is equally abundant in both samples.

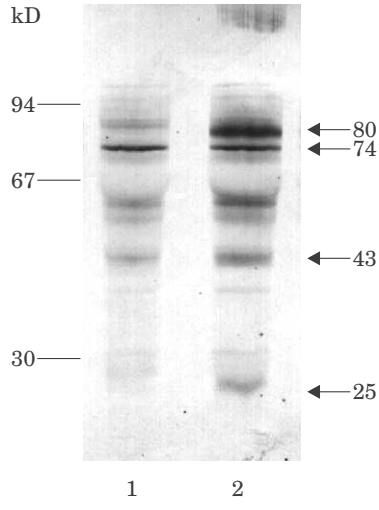

Fig. 1. Glycoprotein pattern of placentas from missed abortion obtained by electrophoresis and detection with SNA lectin in tenth week of pregnancy (2) in comparison to control placenta from normal pregnancy (1) of the same gestational age.

Glycoprotein pattern of placentas from missed abortion in 11th week of pregnancy (lane 2) obtained by electrophoresis and detection with PHA-E lectin in comparison to control placenta from normal pregnancy (lane 1) of the same gestational age are given in Figure 2. Glycoprotein with molecular weight of 61 kD is more abundant in pathological placenta than in placenta from normal pregnancy, while gp 71 and 74 are equally abundant in both samples.

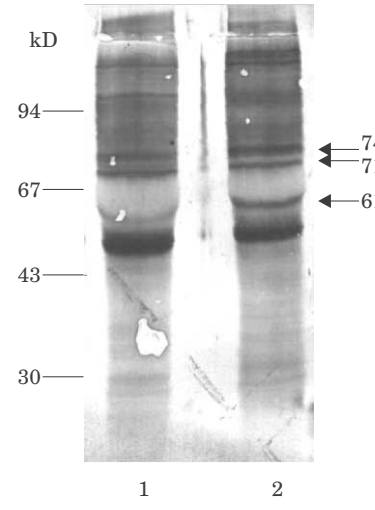

Fig. 2. Glycoprotein pattern of placentas from missed abortion obtained by electrophoresis and detection with PHA-E lectin in eleventh week of pregnancy (2) in comparison to control placenta from normal pregnancy (1) of the same gestational age.

Glycoprotein pattern of placentas from missed abortion also from the 11th week of pregnancy (band 2) but obtained by detection with DBA lectin in compari-

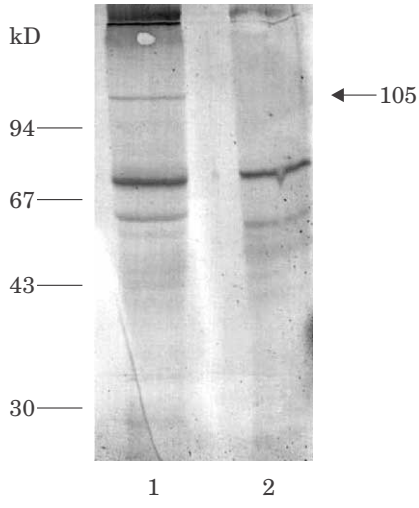

Fig. 3. Glycoprotein pattern of placentas from missed abortion obtained by electrophoresis and detection with DBA lectin in eleventh week of pregnancy (2) in comparison to control placenta from normal pregnancy (1) of the same gestational age.

son to control placenta from normal pregnancy (band 1) of the same gestational age are given in the Figure 3. Glycoprotein with molecular weight of 105 kD is absent from the sample of placenta from missed abortion, while it is clearly present in the normal sample.

Figure 4 shows placental glycoprotein patterns of placentas from missed abortion obtained by detection with SNA lectin during 13th week of pregnancy (lane 2) in comparison to control placenta from normal pregnancy (lane1) of the same gestational age. Glycoprotein with molecular weight of 71 kD is present only in control while it is absent from pathological placenta. Gp 74 is equally abundant in both samples.

Figure 5 shows glycoprotein patterns of placentas from 14th week of pregnancy obtained from missed abortion (lane 2) and detected with PHA-E lectin, in comparison to control placenta from the nor-

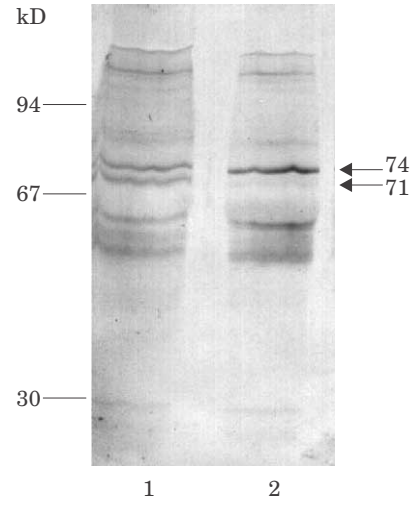

Fig. 4. Glycoprotein pattern of placentas from missed abortion obtained by electrophoresis and detection with SNA lectin in thirteenth week of pregnancy (2) in comparison to control placenta from normal pregnancy (1) of the same gestational age.

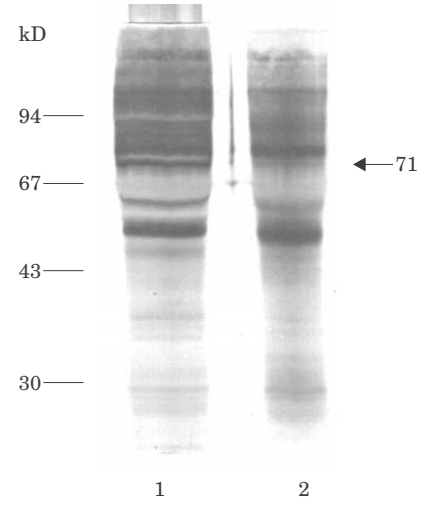

Fig. 5. Glycoprotein pattern of placentas from missed abortion obtained by electrophoresis and detection with PHA-E lectin in fourteenth week of pregnancy (2) in comparison to control placenta from normal pregnancy (1) of the same gestational age.

mal pregnancy of the same gestational age (lane 1). Glycoprotein with molecular weight of 71 kD is generally barely visible in pathological placenta during this week.

Discussion

The reason why some abortions do not terminate after death of the fetus, while others do, is not clear. Yamamoto and others investigated the proportion of CD56 (+)3(+)T cells in maternal peripheral and decidual lymphocytes in normal pregnancy and spontaneous abortion and found decreased proportion of those cells in decidual lymphocytes in missed abortion, which may be due to an immunologic event leading to this disorder of pregnancy[27].

Significantly lower levels of ceruloplasmin and copper values compared to normal pregnancy of the same gestational age were demonstrated in cases of missed abortion, so the ceruloplasmin activity and copper measurements are valuable in predicting the prognosis of threatened abortion[28].

Vuorela and others used immunohistochemistry to analyze the expression of vascular endothelial growth factor (VEGF) family of proteins, together with their receptors and the Tie (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains) receptors in placental and decidual tissue of women with missed abortion and normal early terminated pregnancies. Compared with controls, the missed abortion group showed: diminished placental trophoblastic VEGF immunoreactivity; weaker VEGFR-1 and -2 immunoreactivity in decidual vascular endothelium; reduced placental trophoblastic Tie-1 receptor immunoreactivity; and reduced decidual vascular endothelial Tie-1 and -2 receptor immunoreactivity[29].

Although glycoconjugates have been proven to play a vital role in cellular metabolism and intercellular interactions, they have been almost completely overlooked in studies evaluating differences between normal human pregnancy and missed abortion. In this study we have for the first time demonstrated changes of glycosylation patterns between placental proteins in normal pregnancy and missed abortion.

By comparing glycosylation patterns from normal placenta and placenta from missed abortions, we observed differences in all analyzed stages of gestation, as well as with each of the used lectins (SNA, DBA, and PHA-E). These differences included both appearance of new glycoproteins in pathological samples, and the disappearance of »normal« glycoproteins that were present in control patterns from normal pregnancies.

Glycoproteins detected by lectin SNA demonstrated differences between tenth and fourteenth week of embryonic development, so that e.g. during tenth week we observed the appearance of new glycoproteins of 25 and 80 kD in samples from pathological placentas.

Among glycoproteins that have been detected by PHA-E lectin, the differences were apparent during all studied weeks, being most prominent however during the eleventh and fourteenth week. So during the eleventh week of gestation glycoprotein 61 kD is evidently most abundant in the sample from pathological placenta, while during fourteenth week the gp 71 kD can only be detected in control samples from normal placentas.

The presence of numerous differences in glycoprotein patterns indicated the existence of a link between developmental errors associated with fetal death and protein glycosylation. Glycoconjugates have vital roles in implantation, placentation and maintenance of pregnancy, and changes in the content and composition of glycoproteins could lead towards inade-

quate recognition mechanisms between human embryo and mother. The crucial questions of weather the observed changes in glycosylation patterns are causal factors leading to fetal death, and further to its mysterious retention *in utero,* and consequently how important these changes are in etiopathogenesis of missed abortion, remain the stimulus for further investigation.

REFERENCES

1. HOWIE, P. W., Abortion and ectopic pregnancy. In: WHITFIELD, C. R. (Ed.): Dewhurst's textbook of obstetrics and gynecology for postgraduates. (Blackwell Science, Oxford, 1995). — 2. PHILLIP, T., D. K. KALOUSEK, Am. J. Med. Genet., 111 (2002) 43. — 3. MC FADYEN, I. R., Eur. J. Obstet. Gynecol. Reprod. Biol., 20 (1985) 381. — 4. CEPNI, I., T. BESE, P. OCAL, E. BUDAK, M. IDIL, M. F. AKSU, Acta. Obstet. Gynecol. Scand., 76 (1997) 969. — 5. KURJAK, A., S. KUPEŠIĆ, T. HAFNER, V. LATIN, M. KOS, R. D. HARRIS, Croat. Med. J., 39 (1998) 4. MC FADYEN, I. R., Eur. J. Obstet. Gynecol. Reprod. Biol., 20 (1985) 381. — 6. DILMEN, G., N. O. TURHAN, N. C. SECKIN, Int. J. Gynecol. Obstet., 52 (1996) 285. — 7. ALCAZAR, J. L., C. LAPARTE, G. LOPEZGARCIA, J. Ultrasound Med., 15 (1996) 645. — 8. ALCAZAR, J. L., C. A. ORTIZ, Eur. J. Obstet. Gynecol. Reprod. Biol., 102 (2002) 83. — 9. HALPERIN, R., E. HADAS, I. BUKOVSKY, D. SCHNEIDER, Gynecol. Obstet. Invest., 45 (1998) 225. — 10. HALPERIN, R., D. HALPERIN, E. HADAS, I. BUKOVSKY, D. SCHNEIDER, Gynecol. Obstet. Invest., 46 (1998) 150. — 11. ZILBERSTEIN, M., M. M. SEIBEL, Gynecol. Obstet. Invest., 44 (1997) 217. — 12. AL – SEBAI, M. A. H., C. R. KINGSLAND, M. DIVER, L. HIPKIN, I. R. MC FADEYN, Br. J. Obstet. Gynaecol., 102 (1995) 364. — 13. AKSOY, S., H. CELIKKANAT, S. SENOZ, O. GOKMEN, Eur. J. Obstet. Gynecol. Reprod. Biol., 67 (1996) 5. — 14. SHAHANI, S. K., C. L. MONIZ, A. D. BORDEKAR, S. M. GUPTA, K. NAIK, Gynecol. Obstet. Invest., 37 (1994) 73. — 15. HAQ, A., B. A. MOTHI, K. AL-HUSSEIN, M. AL-TUFAIL, J. HOLLANDERS, K. JAROUDI, N. AL-WAILI, M. SHABANI, Eur. J. Med. Res., 6 (2001) 209. — 16. GETSIOS, S., C. D. MAC CALMAN, Dev. Biol., 257 (2003) 41. — 17. YOSHIMURA, Y., J. Reprod. Dev., 48 (2002) 215. — 18. BLANKENSHIP, T. N., A. C. ANDERS, Microsc. Res. Tech., 60 (2003) 390. — 19. CHEN, C. P., J. D. APLIN, Placenta, 24 (2003) 316. — 20. STRICKLAND, S., W. G. RICHARDS, Cell, 71 (1992) 355. — 21. KEMP, B., B. HUPPERTH, W. RATH, Geburtshilfe und Frauenheilkunde, 62 (2002) 550. — 22. ALEXANDER, C. M., E. J. HANSELL, O. BEHRENDTSEN, M. L. FLANNERY, S. N. KISHNANI, S. P. HAWKES, Z. WERB, Development,122 (1996) 1723. — 23. LAEMMLI, U. K., Nature, 227 (1970) 680. — 24. TOWBIN, H., T. STAEHELIN, J. GORDON, Proc. Natl. Adad. Sci. USA, 76 (1979) 4350. — 25. DUNN, M. J., Detection of proteins on blots using avidin – biotin system. In: WALKER, J. M. (Ed.): Basic protein and peptide protocols. (Humana Press, New Jersey, 1994). — 26. HASELBECK, A., W. HOSEL, Immunological detection of glycoproteins on blots based on labeling with dioxigenin. In: HOUNSELL, E. F. (Ed.): Glycoprotein analysis in biomedicine. (Humana Press, New Jersey, 1994). — 27. YAMAMOTO T., Y. TAKAHASHI, N. KASE, H. MORI, Am. J. Reprod. Immunol., 42 (1999) 355. — 28. OZGUNES, H., M. S. BEKSAD, F. B. ATAC, S. INMEZ, O. SAKA, K. KAYAKIRILMAZ, S. DURU, Trace. Elem. Electrolytes, 11 (1994) 139. — 29. VUORELA, P., O. CARPEN, M. TULPPALA, E. HALMESMAKI, Mol. Hum. Reprod., 6 (2000) 276.

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USPOREDBA GLIKOZILACIJE PROTEINA POSTELJICE U NORMALNOJ TRUDNOĆI I ZADRŽANOM POBAČAJU

S A Ž E T A K

Dosadašnja saznanja o zadržanom pobačaju, obliku spontanog pobačaja kod kojeg se zametak nakon odumiranja zadržao četiri ili više tjedana u maternici vrlo su oskudna. Stoga smo proveli usporedbu u glikozilaciji proteina posteljice u urednoj trudnoći i zadržanom pobačaju. Oligosaharidni ogranci detektirani su Western blot metodom uz uporabu lektina SNA, DBA i PHA-E nakon prethodnog razdvajanja proteina diskontinuiranom SDS-PAG elektroforezom. Usporedbom prepoznatih šećera u uzorcima iste gestacijske starosti uočene su razlike u glikozilaciji proteina normalne i patološke posteljice. Lektin DBA u jedanaestom tjednu detektira GP 105, a nema ga u uzorku zadržanog pobačaja. PHA-E prepoznaje prisustvo GP 71 u četrnaestom tjednu samo u uzroku normalne posteljice. GP 25 prepoznat je lektinom SNA samo u uzorku zadržanog pobačaja dok u kontrolnom uzorku uredne posteljice desetog tjedna, nije detektiran. Dobiveni rezultati podupiru pretpostavku da oligosaharidne strukture glikoproteina imaju ključnu ulogu tijekom razvoja posteljice.