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The role of serum podocalyxin levels in recurrent pregnancy loss

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ABSTRACT

Objective: To measure serum levels of podocalyxin (PODXL) in recurrent miscarriages as a marker of vascular endothelial dysfunction.
Study design: In this case-control study, women who were hospitalized for singleton first-trimester pregnancy terminations due to missed abortion, anembryonic pregnancy, and inevitable abortion were included. There were 24 patients who were admitted for the first pregnancy termination, 39 patients who were admitted for recurrent pregnancy loss (RPL), and 25 fetal cardiac activity positive patients as the control group. Demographic features, medical and obstetric histories were recorded. The measurements of serum PODXL were done by a human enzyme-linked immunosorbent assay kit.
Results: Serum PODXL levels were found to be significantly higher in the RPL group than the control group and the first time miscarriage group (13.82 [10.09−113.54] vs. 11.78 [9.25−48.80], p = 0.016 and 13.82 [10.09–113.54] vs. 11.99 [8.20–20.47], p = 0.003; respectively). Serum PODXL levels were not statistically significantly different between the first miscarriage and the control group (p = 0.62). There were positive correlation between serum PODXL levels and the number of gravida and the number of miscarriages (r = 0.217, p = 0.042, and r = 0.291, p = 0.006; respectively).
Conclusion: Recurrent miscarriage patients had higher serum levels of PODXL than both normal pregnancies and first-time miscarriages. Our results suggest that maternal endothelial dysfunction might have a role in recurrent pregnancy losses.

Introduction

Miscarriage is defined as pregnancy loss of a fetus weighing less than 500 g or smaller than 20 gestational weeks. The incidence of spontaneous abortion is around 15–25 % after a clinical pregnancy [1]. The definition of recurrent pregnancy loss (RPL) is a more controversial issue as its definitions differ among societies as having two or more miscarriages [2,3] or consecutive three or more miscarriages [4]. In a recent study, the lifetime risk of one, two, three, and four or more miscarriages were found as 17.9 %, 3.9 %, 0.87 %, and 0.35 %, respectively [5]. Although genetic and endocrine disorders, uterine abnormalities, immunological factors, inherited and acquired thrombophilias, and as well as environmental factors are recommended to be investigated for RPL, an etiological factor cannot be demonstrated in 50–75 % of the cases [1,6,7]. Regardless of the reason, in 67–75 % of the cases, with a specific cause or unexplained RPL, a successful pregnancy result can be obtained in the next pregnancy with only supportive treatment [8–10].

Podocalyxin (PODXL), a sialoglycoprotein, is first identified as the major component of the transmembrane of the glomerular epithelial cells called podocytes [11]. However, PODXL has been also detected on vascular endothelial cells [12], mesothelial cells [13], platelets [14], hematopoietic progenitor cells [15], and neurons [16]. Due to the negative charge, PODXL acts as an anti-adhesive molecule, which is important in the formation and continuity of podocyte foot processes, and the urinary filtration barrier. Intriguingly, PODXL might show opposing proadhesive properties in the extrarenal locations functioning in adherence, migration, and intercellular communication of cells [17].

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Podocalyxin is expressed in the luminal surface of endothelial cells of blood vessels and is present in major and minor arterial and venous vessels of most tissues [12]. The role of PODXL in the vascular system is the maintenance of endothelial barrier integrity by regulation of vascular permeability, cell adhesion, and inflammation [17–19]. Urinary PODXL has been long recognized as a marker of podocyte injury, hence, serum PODXL measurements were proposed to reflect vascular endothelial injury [20,21]. Initially, Shoji et al. showed a positive correlation between serum PODXL levels and carotid intima-media thickness [20]. Likewise, serum PODOXL levels were found to be increased in peripheral arterial disease with type 2 diabetic patients [21]. In a detailed study, Chen et al. showed that maternal serum PODXL levels were increased in early-onset preeclampsia reflecting maternal endothelial injury or dysfunction [22].

Recurrent pregnancy loss is another disease group in which vascular endothelial damage is proposed to play an active role in the pathophysiology, especially in idiopathic cases [23]. Thus, in this study, we aimed to investigate whether PODXL levels would be high as an indicator of vascular damage in recurrent miscarriages.

Materials and methods

This case-control study was conducted in Ankara Dr. Zekai Tahir Burak Women's Health Education and Research Hospital after getting approval from the ethics committee of the hospital (protocol no: 64/2019). There were a total of 88 patients in the study divided into three groups: the RPL group, the first miscarriage group, and the control group. The RPL group consisted of 39 patients who were hospitalized due to recurrent pregnancy loss with the diagnosis of missed abortion, anembryonic pregnancy, or inevitable abortion. Recurrent pregnancy loss was defined as two or more miscarriages before 20 weeks of gestation. The first miscarriage group consisted of 24 patients who were hospitalized for the first pregnancy termination due to similar diagnoses. For the control group, 25 pregnant women with low-risk pregnancies in the first trimester were included. Women with systemic illnesses including chronic hypertension, diabetes mellitus, and other cardiovascular, hematologic, autoimmune, and endocrine disorders were excluded. The control group of patients was followed until delivery and was excluded from the study if any obstetric problems have occurred during later stages of antenatal follow-ups. Demographic features, medical and obstetric histories, gestational week, and crown-rump length (CRL) measurements were recorded for all participants at the time of the blood sampling. The gestational age was calculated according to the first day of the last menstrual period (if known) or by ultrasound measurement.

Blood samples of the study group were drawn from the patients after hospitalization and before the operation. The blood samples of the control group were drawn when they were admitted to the antenatal outpatient clinic. They were centrifuged and stored at -80°C until analysis. The measurements of serum PODXL were done by human enzyme-linked immunosorbent assay (ELISA) kit for PODXL (product no: SEA768Hu, Cloud-Clone Corp., USA) by HEALES MB-530 device according to the manufacturer's instructions. The results were given as ng/mL. The detection range is reported to be between 0.156−10 ng/mL. The minimum detectable dose of this kit is typically less than 0.054 ng/mL. The intra-assay and inter-assay coefficient variations of the test were given as < 10 % and < 12 %, respectively.

For statistical analysis, data were transferred to a computer using the Statistical Package for the Social Sciences version 22.0 (SPSS, Chicago, IL). The distribution of the data was assessed using Shapiro Wilk's test. Continuous and the normally distributed variables were presented as mean ± standard deviation, and intergroup differences were investigated using Student's *t*-test. Continuous variables with non-normal distribution were expressed as median (minimum-maximum), and the differences between the variables were analyzed using the Kruskal-Wallis test. The relationship between serum PODXL levels and age, gravida, parity, BMI, number of miscarriages, gestational week, and CRL measurements was evaluated with Spearman's rank correlation (Rho). Data were analyzed at a 95 % confidence level and $p < 0.05$ was considered to be significant.

As our study was a pilot study, a power analysis was not done before initiation of the study. However, we performed a post hoc analysis, which showed a power of 0.81 when we accepted the effect size as 0.3 with an alpha = 0.05 significance level for 88 patients.

Results

Table 1 shows the demographic and clinical characteristics of the first miscarriage and RPL groups and the controls. In the first miscarriage group, the number of miscarriages was statistically significantly higher and the gestational week in which miscarriage occurred was statistically significantly lower than the control group ($p < 0.05$). In the RPL group, age, the number of gravida and miscarriages were higher than controls, whereas gestational week and CRL were lower than controls. When serum PODXL levels were examined between groups, there was no statistically significant difference between the first miscarriage and the control group (11.99 [8.20−20.47] vs. 11.78 [9.25−48.80], p = 0.62; respectively). However, serum PODXL levels were found to be significantly higher in the RPL group than the control group and the first time

Table 1
Demographic and clinic characteristics of the patients. Numbers are given as mean ± standard deviation or median (min.-max.) as appropriate.

	Control N = 25	First miscarriage N = 24	RPL N = 39	P-value	
				1 vs. 2	1 vs. 3
Age	27 ± 5.75	27.58 ± 2.88	31.38 ± 6.31	0.77	**0.008**
Gravida	2 (1−6)	2 (1−6)	4 (2−7)	0.79	<**0.001**
Parity	1 (0−4)	1 (0−5)	1 (0−5)	0.996	
Miscarriage	0 (0−1)	1 (1−1)	2 (2−6)	**0.008**	<**0.001**
BMI	23.80 ± 2.40	23.43 ± 3.22	24.48 ± 3.05	0.81	0.61
Hb	12.37 ± 1.3	12.78 ± 0.98	12.7 ± 1.28	0.22	0.33
Htc	37.26 ± 3.06	37.88 ± 2.24	38.2 ± 3.07	0.42	0.23
WBC	9.13 ± 1.9	9.41 ± 2.8	9.16 ± 2.33	0.68	0.95
Plt ($x10^3$)	250 (150−369)	277.5 (165−605)	277 (173−458)	0.750	
Gestational week	10 (6−13)	7.5 (5−14)	7 (5−11)	**0.003**	<**0.001**
CRL	34.8 (6−69)	19 (2.5−87.5)	6.1 (2.5−57.0)	0.08	<**0.001**
Serum Podocalyxin (ng/mL)	11.78 (9.25−48.80)	11.99 (8.20−20.47)	13.82 (10.09−113.54)	0.62	**0.016**

BMI: Body mass index, Hb: Hemoglobin, Htc: Hematocrit, WBC: White blood cells, Plt: Platelets, CRL: Crown lump length.

miscarriage group (13.82 [10.09–113.54] vs. 11.78 [9.25–48.80], p = 0.016 and 13.82 [10.09–113.54] vs. 11.99 [8.20–20.47], p = 0.003; respectively). Fig. 1 demonstrates serum PODXL levels of the study groups shown as a boxplot.

We investigated the correlations between serum PODXL levels and age, gravida, parity, BMI, number of miscarriages, gestational week, and CRL measurement. We found positive correlation between serum PODXL levels and the number of gravidity and the number of miscarriages (r = 0.217, p = 0.042, and r = 0.291, p = 0.006; respectively).

Discussion

In this study, we aimed to investigate serum PODXL levels of women with recurrent miscarriage as a marker of maternal vascular dysfunction. Our results showed that recurrent miscarriage patients had higher serum levels of PODXL than both normal pregnancies and first-time miscarriages. These results might suggest that maternal endothelial dysfunction might have a role in RPL.

The role of PODXL during pregnancy begins with implantation and continues with embryogenesis and placental development. During the implantation period, the expression of PODXL increases in both blastocyst and receptive endometrium [24]. It has an effect as a progenitor in the formation of three germ leaves during embryogenesis, in the formation of megakaryocytes and platelets, and is also involved in the formation of neurons [13,16,25]. Lastly, PODXL is present in blood vessels in of various organs including the placenta [12,22]. In a case-control study performed in healthy pregnant women at 11–13, 23–27, 33–40 weeks of gestation, it was found that maternal serum podocalyxin concentration increased as the gestational week progressed and peaked at term [22]. Immunohistochemical staining showed that the expression of PODXL was localized to blood vessels of the placenta, not to trophoblastic cells. However, placental investigations of both preeclamptic patients and normotensive controls revealed similar levels of podocalyxin mRNA in PCR and immunohistochemical staining, thus the source of increased maternal serum PODXL was not due to placental development. The authors proposed that PODXL is released from maternal endothelial cells during pregnancy and increased levels of serum PODXL is detected in early-onset preeclampsia due to maternal endothelial dysfunction [22]. Subsequently, the same research group reported that first-trimester screening of serum PODXL levels showed higher levels in women who later develop preeclampsia [26] and also in women delivering small for gestational age babies after 34 gestational weeks without preeclampsia [27].

Endothelial dysfunction has been long proposed as an etiologic factor in a large spectrum of diseases including recurrent miscarriages, preeclampsia, and long-term cardiovascular diseases [28]. Similarly, it has been found that endothelial cellular microparticles, i.e. fragments of cell membranes released upon activation or apoptosis, are increased in recurrent miscarriage cases even in the non-pregnant state, thus suggesting endothelial dysfunction [29,30]. Moreover, another biological evidence of endothelial dysfunction in RPL has been obtained from the study of Banerjee et al. They demonstrated that while the expressions of pro-inflammatory cytokines were increased in the endometrium of RPL patients, the expression of the anti-inflammatory and angiogenesis-associated cytokines were decreased [31]. Our results are in accordance with the abovementioned studies as we found higher levels of serum PODXL levels in recurrent miscarriage patients than the controls and first-time miscarriage patients. Although the underlying mechanism of this increase is difficult to speculate, it might be from the inflammatory events involved in recurrent miscarriages as reported by Banerjee et al. Podocalyxin plays an active role in vascular integrity by controlling inflammation. Selective deletion of vascular PODXL in murine resulted in increased pulmonary vascular permeability of lungs in both normal and inflammatory conditions [32]. Similarly, it has been shown that endothelial PODXL deficient mice developed severe vasculitis in various blood vessels, in vivo, and showed delayed recovery of endothelial integrity after damage, in vitro. [18] Likewise, it was observed that the level of podocalyxin increased significantly in the human umbilical vein endothelium in response to IL-6 stimulation [22]. However, since we did not

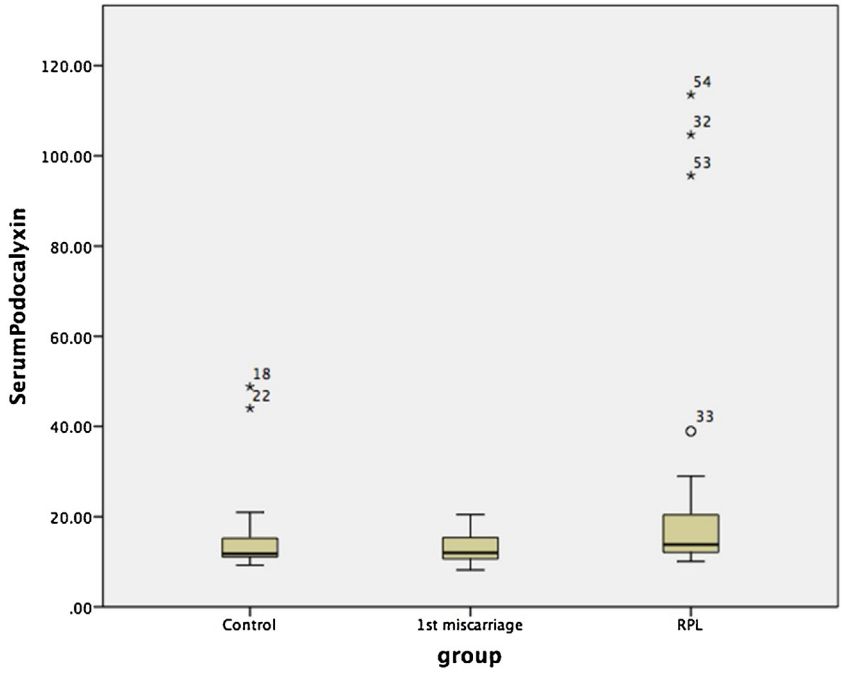

Fig. 1. Serum podocalyxin levels of the study groups shown as in Tukey style box and whiskers plot, and outliers are shown individually.

investigate any inflammatory markers neither in serum nor in the endometrium, this interpretation cannot go beyond speculation. Nevertheless, the correlation between serum PODXL levels and the number of miscarriages also supports our hypothesis.

To the best of our knowledge, our study is the first preliminary study investigating this topic, however, it has some limitations to consider. The major limitation of our study is that the etiologic factors were not sought due to the design of the study. Thus, whether there is a causal relationship between serum PODXL levels and RPL could not be determined. However, the correlation between the number of miscarriages and serum PODXL levels suggests a casual relationship rather than an outcome. Besides, our study was conducted with a relatively small number of patients. Should we have more cases, it would be ideal to adjust serum PODXL concentrations to multiple of median (MoM) values for the respective gestational age. On the other hand, when interpreting our results, it should be emphasized that serum PODXYL values were slightly higher in the recurrent miscarriage group with a much wider range and a few outliers. Thus, our results need to be validated in prospective larger studies in which etiologic factors are also taken into account. After that, we could develop new clinical approaches to RPL cases.

Conclusion

In this pilot study, our results showed that serum PODXL levels were higher in RPL patients suggesting a vascular endothelial dysfunction. However, our data are not fully sufficient to explain whether there is a causal or consequential relationship. Therefore, the exact role of PODXL in RPL could be determined in larger studies investigating serum PODXL levels, particularly in unexplained RPL. We hope our study will highlight future prospective studies, which would be conducted with blood sampling in early pregnancy of women with a history of unexplained RPL and those without and follow-up regarding the occurrence of a miscarriage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Practice Committee of the American Society for Reproductive M. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril 2012;98:1103–11.
[2] Eshre Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open 2018;2018: hoy004.
[3] Practice Committee of the American Society for Reproductive Medicine. Electronic address aao. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2020;113:533–5.
[4] Gynaecologists RCoOa. The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. Green-top guideline No 17. . p. 1–17.
[5] Lidegaard O, Mikkelsen AP, Egerup P, Kolte AM, Rasmussen SC, Nielsen HS. Pregnancy loss: a 40-year nationwide assessment. Acta Obstet Gynecol Scand 2020.
[6] Kumar A, Dey M, Arora D. Relevance of invasive testing in era of non-invasive testing for prenatal chromosomal abnormalities. Gynecol Obstet Reprod Med 2020;1–6.
[7] Önalan Etem E, Yüce H, Erol D, Derya Deveci Ş, Çelik H, Elyas H. Cytogenetic investigation in couples with recurrent abortion and poor obstetric history. Int J Gynecol Obstet Reprod Med Res 2010;16:4–8.
[8] Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Hum Reprod 1999;14:2868–71.
[9] Lund M, Kamper-Jorgensen M, Nielsen HS, Lidegaard O, Andersen AM, Christiansen OB. Prognosis for live birth in women with recurrent miscarriage: what is the best measure of success? Obstet Gynecol 2012;119:37–43.
[10] Sakar MN, Balsak D, Demir S, Budak MS, Tahaoglu AE, Gungor SE, et al. The ability of a single serum progesterone measurement to predict the prognosis of first trimester pregnancy. Gynecol Obstet Reprod Med 2020;26:1–5.
[11] Kerjaschki D, Sharkey DJ, Farquhar MG. Identification and characterization of podocalyxin–the major sialoprotein of the renal glomerular epithelial cell. J Cell Biol 1984;98:1591–6.
[12] Horvat R, Hovorka A, Dekan G, Poczewski H, Kerjaschki D. Endothelial cell membranes contain podocalyxin–the major sialoprotein of visceral glomerular epithelial cells. J Cell Biol 1986;102:484–91.
[13] Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, Graf T, et al. Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. J Exp Med 2001;194:13–27.
[14] Miettinen A, Solin ML, Reivinen J, Juvonen E, Vaisanen R, Holthofer H. Podocalyxin in rat platelets and megakaryocytes. Am J Pathol 1999;154:813–22.
[15] Kerosuo L, Juvonen E, Alitalo R, Gylling M, Kerjaschki D, Miettinen A. Podocalyxin in human haematopoietic cells. Br J Haematol 2004;124:809–18.
[16] Vitureira N, Andres R, Perez-Martinez E, Martinez A, Bribian A, Blasi J, et al. Podocalyxin is a novel polysialylated neural adhesion protein with multiple roles in neural development and synapse formation. PLoS One 2010;5:e12003.
[17] Larrucea S, Butta N, Arias-Salgado EG, Alonso-Martin S, Ayuso MS, Parrilla R. Expression of podocalyxin enhances the adherence, migration, and intercellular communication of cells. Exp Cell Res 2008;314:2004–15.
[18] Horrillo A, Porras G, Ayuso MS, Gonzalez-Manchon C. Loss of endothelial barrier integrity in mice with conditional ablation of podocalyxin (Podxl) in endothelial cells. Eur J Cell Biol 2016;95:265–76.
[19] Cait J, Hughes MR, Zeglinski MR, Chan AW, Osterhof S, Scott RW, et al. Podocalyxin is required for maintaining blood-brain barrier function during acute inflammation. Proc Natl Acad Sci U S A 2019;116:4518–27.
[20] Shoji M, Takemoto M, Kobayashi K, Shoji T, Mori S, Sagara JI, et al. Serum podocalyxin levels correlate with carotid intima media thickness, implicating its role as a novel biomarker for atherosclerosis. Sci Rep 2018;8:245.
[21] El-Ashmawy HM, Selim FO, Hosny TAM, Almassry HN. Association of serum podocalyxin levels with peripheral arterial disease in patients with type 2 diabetes. J Diabetes Complications 2019;33:495–9.
[22] Chen Q, Wang Y, Li Y, Zhao M, Nie G. Serum podocalyxin is significantly increased in early-onset preeclampsia and may represent a novel marker of maternal endothelial cell dysfunction. J Hypertens 2017;35:2287–94.
[23] Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv 2007;62:335–47 quiz 53-54.
[24] Imakawa K, Bai R, Kusama K. Integration of molecules to construct the processes of conceptus implantation to the maternal endometrium. J Anim Sci 2018;96:3009–21.
[25] Doyonnas R, Nielsen JS, Chelliah S, Drew E, Hara T, Miyajima A, et al. Podocalyxin is a CD34-related marker of murine hematopoietic stem cells and embryonic erythroid cells. Blood 2005;105:4170–8.
[26] Mansilla M, Wang Y, Hyett J, da Silva Costa F, Nie G. Serum podocalyxin for early detection of preeclampsia at 11-13 weeks of gestation. Placenta 2018;71:13–5.
[27] Rolnik DL, Wang Y, Hyett J, Silva Costa FD, Nie G. Serum podocalyxin at 11-13 weeks of gestation in the prediction of small for gestational age neonates. J Perinatol 2019;39:784–90.
[28] Germain AM, Romanik MC, Guerra I, Solari S, Reyes MS, Johnson RJ, et al. Endothelial dysfunction: a link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? Hypertension 2007;49:90–5.
[29] Carp H, Dardik R, Lubetsky A, Salomon O, Eskaraev R, Rosenthal E, et al. Prevalence of circulating procoagulant microparticles in women with recurrent miscarriage: a case-controlled study. Hum Reprod 2004;19:191–5.
[30] Pasquier E, De Saint Martin L, Bohec C, Collet M, Dignat George F, Mottier D. Unexplained pregnancy loss: a marker of basal endothelial dysfunction? Fertil Steril 2013;100:1013–7.
[31] Banerjee P, Ghosh S, Dutta M, Subramani E, Khalpada J, Roychoudhury S, et al. Identification of key contributory factors responsible for vascular dysfunction in idiopathic recurrent spontaneous miscarriage. PLoS One 2013;8:e80940.
[32] Debruin EJ, Hughes MR, Sina C, Lu A, Cait J, Jian Z, et al. Podocalyxin regulates murine lung vascular permeability by altering endothelial cell adhesion. PLoS One 2014;9:e108881.