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Homeobox A Cluster 7 (HOXA7) protein expression increased in the placentas of patients with preterm delivery

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Abstract

Objective: This study aimed to investigate Homeobox A Cluster 7 (HOXA7) protein expression in the placentas of patients with preterm labor by immunohistochemical method.

Methods: Placentas of 25 healthy women with term delivery and 25 women with preterm delivery were enrolled in this study. Placental samples were fixed in zinc-formalin and further processed for paraffin wax tissue embedding. Demographic and laboratory parameters of patients were recorded. HOXA7 immune activity was analyzed in the placental section with immunohistochemistry.

Results: In the placentas in the term delivery group, normal histology was observed with no placental lesions. In placentas in the preterm delivery group, degenerated villi, fibrin deposition, and cytotrophoblast delamination with vascular congestion were recorded. HOXA7 protein expression was very high in preterm placentas compared to term placentas. The expression was obvious, especially in cytotrophoblast cells and villous connective tissue.

Conclusion: HOXA gene family is required for proper placental formation. HOXA7 protein expression may be differently regulated in preterm placentas and may be involved in the pathogenesis of preterm delivery.

Keywords: HOX genes, placenta, preterm delivery

Introduction

There are 39 homeobox-containing (HOX) genes in mammals as four different linear clusters where each of which includes 9 to 11 genes. The homeobox A cluster (HOXA) gene family encodes proteins containing the DNA-binding homeobox motif and regulates embryonic developmental stages as well as embryonal segmentation. HOX genes are generally inactive in an adult human. Still, they can be activated in a controlled manner in different cellular events such as regeneration, hematopoiesis, wound healing, vascularization, and the female reproductive system.[1-4] The association of HOX genes with cancer is studied. Defective regulation of proteins belonging to the HOXA gene family has been reported in various cancers, including glioblastoma, hepatocellular carcinoma, prostate cancer, gastric cancer, epithelial ovarian cancer, breast cancer, and leukemia.[5-8] Although the role of HOXA genes in the development of various cancers has been well studied, the involvement of the genes during the placentation has not been fully understood.

Stress, infection, placental abruption, placenta previa, substance use, preterm birth or miscarriage history, smoking, advanced maternal age, fetal growth restriction, gestational diabetes, oligohydramnios, polyhydramnios, vaginal bleeding, premature preterm rupture of membranes, and environmental factors are supposed to play a role in the etiology of preterm birth.[9-14] Decidual activation and defects in the formation of membrane structures such as the mechanical integrity of the amnion and

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chorion membranes, collagen remodeling, and tissue failure could trigger preterm labor.[15-18] HOX transcription factors are activated especially in the embryonal period and are involved in many cellular events such as segmentation of the body, craniocaudal axis, and specification of tissues.[19,20] Amesse et al. found a lack of expression of some HOX gene products in abnormal trophoblastic cells and stated that HOX genes are required for trophoblastic cell homeostasis.[21] Since HOX genes have vital roles in embryogenesis, these genes may be involved in the pathogenesis of preterm delivery. Expression alterations of HOX genes may cause abnormal placentation and dysfunctional associated fetal membranes during placental development.

This study aimed to determine the expression level of HOXA7 gene family members in the placenta for the first time and to examine the expression density in the possible placental decidual layer.

Methods

Ethical approval was taken from Dicle University Medical Faculty, Ethics Committee for, Non-interventional studies (Date: 06/01/2023, No:10). A total of 25 healthy women with term delivery and 25 women with spontaneous preterm delivery without any systemic disorders were enrolled. Criteria for preterm labor were defined as labor between the 20th and 37th week of pregnancy.[22] Demographics, laboratory parameters, and pregnancy outcomes were recorded for each patient. Blood samples were collected at admission before labor and further analyzed for laboratory parameters. All patients were informed and accepted to participate in the study. A written consent form was signed by all participants.

The exclusion criteria for both of the groups are as follows: previous history of preterm delivery, previous cervical surgery, preterm premature rupture of membranes (<37 weeks of gestation), multiple pregnancies, iatrogenic preterm delivery, intrauterine fetal demise, fetal major congenital malformations, gestational hypertensive disorders, placental abruption, anemia, systemic infections, and co-existing systemic diseases, including chronic hypertension, diabetes mellitus, hepatic disease, and known malignancy.

Histological tissue processing

Placentas were acquired from the Department of Obstetrics of Dicle University Faculty of Medicine. Placental samples for histologic analysis were excised and further analyzed for histological evaluation. Samples were fixed in zinc-formalin and dehydrated through grading alcohol series, immersed in xylene, and incubated in paraffin wax. 5 µm sections were cut from paraffin blocks and stained for immune staining of HOXA7.

Immunohistochemical examination

Placental sections were dewaxed, hydrated in grading alcohol series, and washed in distilled water. Slides were allowed to react with 3% hydrogen peroxide (H2O2) to block endogen peroxidase activity. After washing in PBS, sections were immersed in a blocking solution. Without washing, the previous solution was drained and sections were incubated with HOXA7 (catalog no: A14375, AFG Scientific, Northbrook, US) overnight at + 4°C. Sections were biotinylated and then react with streptavidin peroxidase for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used as a chromogen to observe color change. The reactions were stopped with PBS solution and sections were counter-stained with hematoxylin dye. Slides were imaged with Zeiss Imager A2 light microscope. All images were processed and quantified using ImageJ software.

ImageJ analysis

The staining intensity of HOXA7 expression was measured by Image J software (version 1.53, http://imagej.nih.gov/ij). Measurement was calculated by the method of Crowe et al.[23] Quantification was recorded by analyzing ten fields from each specimen per group. In specimens, the brown color stands for the positive expression of the antibody of interest while the blue color represents a negative expression of the antibody of interest. Signal intensity (expression) from a field was calculated by dividing the intensity of the antibody of interest by the whole area of the specimen. A value for staining area/whole area was calculated for each specimen from ten fields. An average value was measured for groups and analyzed for semi-quantitative immunohistochemistry scoring.

Statistical analysis

Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US). The data were recorded as mean±standard deviation. Statistical distribution was evaluated with the Shapiro-Wilk test. Binary group comparisons were done with the Mann-Whitney U test and Student t-test. Significance was considered for p-values <0.05. The categorical variables were analyzed by Chi-square test. The number of patients for each group was calculated by G Power analysis (version 3.1). Cohen's criteria were defined according to the study of Alviggi et al.[24]

Results

Demographic and laboratory parameters and pregnancy outcomes of patients with term and preterm labor were shown in Table 1. As expected, the gestational week at

delivery, and birth weight were significantly lower, and the mean leukocyte count and C-reactive protein (CRP) levels, and the rates of cesarean delivery were significantly higher in women with preterm labor than in women with term labor.

Table 1. Demographic and laboratory parameters, and pregnancy outcomes of patients

Parameters	Term delivery group (n=25)	Preterm delivery group (n=25)	Significance (p-value)
Maternal age, years	28.45 ± 3.45	29.92 ± 4.72	0.703
Body mass index, kg/m²	28.62 ± 5.83	28.31 ± 6.39	0.183
Gravida, n	2.12 ± 1.03	3.53 ± 1.32	0.228
Parity, n	0.93 ± 0.83	1.11 ± 1.02	0.176
CRP (mg/dL)	2.17 ± 45	5.03 ± 1.06	0.003
Leukocyte count (10⁹/L)	12.28 ± 2.39	15.16 ± 1.12	<0.001
Cesarean delivery, n (%)	7 (%28)	21 (%84)	<0.001
Gestational week at delivery	38.34 ± 1.28	33.76 ± 1.48	<0.001
1-min APGAR score	8.15 ± 1.25	7.30 ± 1.43	0.011
5-min APGAR score	9.62 ± 1.05	9.21 ± 1.52	0.180
Birth weight, g	3252.81 ± 323.28	2160.45 ± 218.61	0.001

Histological analysis

Hematoxylin eosin staining of placental sections was presented in Figure 1. The placental sections of patients in the term delivery showed normal histological appearance. There were no placental lesions (Figures 1a and 1b). In placentas in the preterm delivery group, increased fibrin deposition, cytotrophoblast delamination, vascular congestion, and degenerated villi were observed (Figures 1c and 1d).

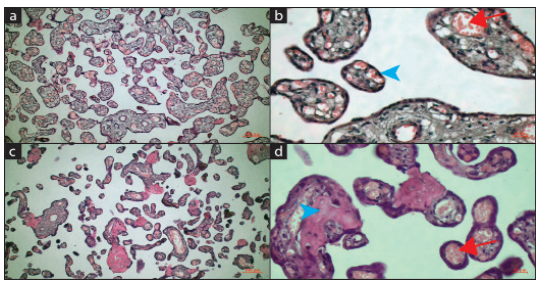

Fig 1. Hematoxylin eosin staining of the placental sections. a-b) Histologically normal villi (blue arrow) and capillaries (red arrow) in term placentas; c-d) Fibrin deposition with edema (blue arrow), vascular congestion (red arrow) in preterm placentas.

Figure 2 shows the immune reaction of HOXA7 protein in the placentas of women with preterm and term delivery. HOXA7 expression was redundantly negative in cytotrophoblast cells in term placentas. Slight expression was observed in villi connective tissue (Figures 2a and 2b). The HOXA7 immune reaction was increased in cytotrophoblast cells and villous connective tissue (Figures 2c and 2d).

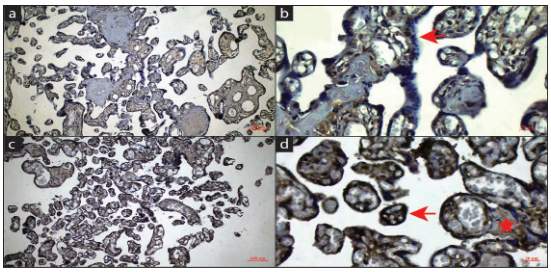

Fig 2. The immune activity of HOXA7 protein in placental sections. a-b) Negative expression in villi (red arrow) in term placentas; c-d) Dense HOXA7 expression in cytotrophoblast cells (red arrow) and in villous connective tissue (red star) in term placentas.

Semi-quantitative measurement of HOXA7 expression by immunohistochemistry staining was shown in Table 2 and illustrated in Figure 3. A similar trend of immunostaining findings was also observed in the semi-quantitative analysis of HOXA7 expression. Signal intensity (expression) HOXA7 was 29.20 ± 4.61% in the term delivery group while it was 36.17 ± 5.89% in the preterm delivery group which showed a statistical increase compared to term placentas.

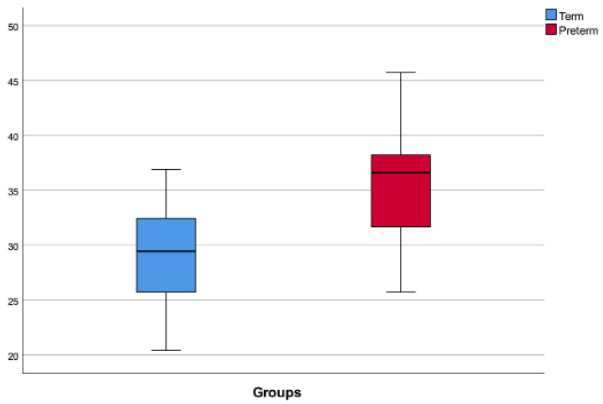

Fig 3. Mean intensity of HOXA7 signal (expression).

Table 2. Image J analysis of HOXA7 signal

	Term delivery group	Preterm delivery group	Significance (p-value)
HOXA7 signal	29.20 ± 4.61	36.17 ± 5.89	<0.001

Discussion

Placental lesions including villi and vessels are common findings in preterm delivery.[25] Obimbo histologically studied the placentas of women with placentas and found increased fibrinoid accumulation, degenerated villi, vascular congestion, and trophoblastic delamination. [26] Other studies reported histological evidence of chorioamnionitis and villitis in preterm placentas.[27-29] This study reported increased capillaries, fibrin deposition, and vascular congestion in preterm placentas compared to term placentas (Figure 1). Our findings are consistent with previous studies.

HOX genes are members of transcription factor-encoding genes with a crucial role in embryogenesis. [30] Their role in cancer is also well-studied. Li et al. studied the prognostic value of HOXA genes (1-13) in laryngeal squamous cell cancer and stated that HOXA genes involves in many cellular events during tumorigenesis and suggested investigating their role further.[31] Ekanayake et al. studied the role of HOXA10 in mammalian female fertility as a prospective marker and found that gonadal steroids directly modulate the expression of the HOXA10 gene and stated that alterations in HOX genes can cause reproductive competence of the female.[32]

Considering their function, it is not surprising they may have a role in the development of the human placenta of women with preterm labor. In a study of mouse models with mutant HOX genes, mice showed placental defects and fetal anomalies.[33] A study showed that the Psx homeobox gene was specifically expressed in extraembryonic tissues and predominantly in the placenta in the early stages of development and its expression continued at the end of pregnancy.[34] This gene also was shown to involve in the differentiation of trophoblastic cells in the murine placenta.[35] Topaloğlu et al. studied HOXA10, HOXB6, and HOXC6 expression in the bovine placenta during gestation. They found that these genes were expressed in trophoblastic cells of the placenta, suggesting the genes may have vital roles in the proliferation and differentiation of placental cells.[36] The present study reveals that HOXA7 protein was slightly expressed in the placentas in the term delivery group. In placentas in the preterm delivery group, HOXA7 immune activity was increased especially in villi structures such as cytotrophoblast and connective tissue. (Table 2, Figures 2 and 3). These findings were consistent with previous studies. Our findings suggest that the HOXA7 gene is required for placental development. HOXA7 expression may modulate the integrity and enforcement of fetal membranes. Transcriptional control of the HOXA7 gene may give a clue for normal and abnormal placentation associated with preterm delivery.

Although this study is the only study investigating the HOX genes in the human placenta in preterm deliveries, it has some limitations. First, histopathology in placentas of patients with preterm delivery is known however the number of patients was low for immunohistochemical scoring to make a powerful conclusion. A large number of patients would give better results. To show the possible role of the HOXA7 gene in placentation with immunostaining is not enough. The hypothesis should be supported with more molecular techniques such as western blot, immune fluorescent, and flow cytometry.

Conclusion

Formation of the placenta is a complex process that requires a family of numerous gene activation. HOXA genes are expressed in the early stages of placentation. This study reported that preterm placentas had higher expression of HOXA7 protein. HOXA7 protein may be involved in the pathogenesis of preterm delivery and regulated during placental formation.

References

1. Rezsohazy R., Saurin A.J., Maurel-Zaffran C., Graba Y. Cellular and molecular insights into HOX protein action. Development. 2015;142:1212–1227. [PubMed] [CrossRef]
2. Dunwell T.L., Holland P.W. Diversity of human and mouse homeobox gene expression in development and adult tissues. BMC Dev. Biol. 2016;16:40. [PubMed] [CrossRef]
3. Rux D.R., Wellik D.M. HOX genes in the adult skeleton: Novel functions beyond embryonic development. Dev. Dyn. 2017;246:310–317. [PubMed] [CrossRef]
4. Alharbi R.A., Pandha H.S., Simpson G.R., Pettengell R., Poterlowicz K., Thompson A., Harrington K., El-Tanani M., Morgan R. Inhibition of HOX/PBX dimer formation leads to necroptosis in acute myeloid leukemia cells. Oncotarget. 2017;8:89566–89579. [PubMed] [CrossRef]
5. Quagliata L, Matter MS, Piscuoglio S, Arabi L, Ruiz C, Procino A, et al. Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. Hepatology. 2014;59:911–923. [PubMed] [CrossRef]
6. Luo Z., Rhie S.K., Lay F.D., Farnham P.J. A Prostate Cancer Risk Element Functions as a Repressive Loop that Regulates HOXA13. Cell Rep. 2017;21:1411–1417. [PubMed] [CrossRef]
7. Wu DC, Wang SSW, Liu CJ, Wuputra K, Kato K, Lee YL, et al. Reprogramming Antagonizes the Oncogenicity of HOXA13-Long Noncoding RNA HOTTIP Axis in Gastric Cancer Cells. Stem Cells. 2017;35:2115–2128. [PubMed] [CrossRef]
8. Richards EJ, Permuth-Wey J, Li Y, Chen YA, Coppola D, Reid BM, et al. A functional variant in HOXA11-AS, a novel

long non-coding RNA, inhibits the oncogenic phenotype of epithelial ovarian cancer. Oncotarget. 2015;6:34745-34757. [PubMed] [CrossRef]

9. Oğlak SC, Bademkıran MH, Obut M. Predictor variables in the success of slow-release dinoprostone used for cervical ripening in intrauterine growth restriction pregnancies. J Gynecol Obstet Hum Reprod. 2020;49(6): 101739. [PubMed] [CrossRef]
10. Cobo T, Kacerovsky M, Jacobsson B. Risk factors for spontaneous preterm delivery. Int J Gynaecol Obstet. 2020;150(1):17-23. [PubMed] [CrossRef]
11. Gedik Özköse Z, Oğlak SC, Ölmez F. The comparison of maternal and neonatal outcomes between planned and emergency cesarean deliveries in placenta previa patients without placenta accreata spectrum. Ginekol Pol. 2022;93(3):217-223. [PubMed] [CrossRef]
12. Ölmez F, Oğlak SC, Can E. The Implication of Aquaporin-9 in the Pathogenesis of Preterm Premature Rupture of Membranes. Z Geburtshilfe Neonatol. 2022;226(4):233-239. [PubMed] [CrossRef]
13. Oğlak SC, Olmez F, Tunç Ş. Evaluation of antepartum factors for predicting the risk of emergency cesarean delivery in pregnancies complicated with placenta previa. Ochsner J. 2022;22(2):146-153. [PubMed] [CrossRef]
14. Oğlak SC, Yavuz A, Olmez F, Gedik Özköse Z, Süzen Çaypınar S. The reduced serum concentrations of β-arrestin-1 and β-arrestin-2 in pregnancies complicated with gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2022;35(25):10017-10024. [PubMed] [CrossRef]
15. Oyen ML, Cook RF, Calvin SE. Mechanical failure of human fetal membrane tissues. J Mater Sci Mater Med. 2004;15(6):651-8. [PubMed] [CrossRef]
16. Joyce EM, Moore JJ, Sacks MS. Biomechanics of the fetal membrane prior to mechanical failure: review and implications. Eur J Obstet Gynecol Reprod Biol. 2009;144 Suppl 1(Suppl 1):S121-7. [PubMed] [CrossRef]
17. Can E, Oğlak SC, Ölmez F. Maternal and neonatal outcomes of expectantly managed pregnancies with previable preterm premature rupture of membranes. J Obstet Gynaecol Res. 2022;48(7):1740-1749. [PubMed] [CrossRef]
18. Oğlak SC. An Introduction to the Special Issue on Premature Rupture of Membranes in Pregnancy Including Its Etiology, Management, and Maternal and Neonatal Outcomes. Clin. Exp. Obstet. Gynecol. 2023;50(5):95. [CrossRef]
19. Acampora D, D'Esposito M, Faiella A, Pannese M, Migliaccio E, Morelli F, et al. The human HOX gene family. Nucleic Acids Res. 1989;17(24):10385-402. [PubMed] [CrossRef]
20. Holland PW, Booth HAF, Bruford EA. Classification and nomenclature of all human homeobox genes. BMC Biol. 2007;5:47. [PubMed] [CrossRef]
21. Amesse LS, Moulton R, Zhang YM, Pfaff-Amesse T. Expression of HOX gene products in normal and abnormal trophoblastic tissue. Gynecol Oncol. 2003;90(3):512-8. [PubMed] [CrossRef]
22. American College of Obstetricians and Gynecologists; Committee on Practice Bulletins—Obstetrics. ACOG practice bulletin no. 127: Management of preterm labor. Obstet Gynecol. 2012;119(6):1308-17. [PubMed] [CrossRef]
23. Crowe AR, Yue W. Semi-quantitative Determination of Protein Expression using Immunohistochemistry Staining and Analysis: An Integrated Protocol. Bio Protoc. 2019;9(24): e3465. [CrossRef]
24. Alviggi C, Conforti A, De Rosa P, Strina I, Palomba S, Vallone R, et al. The Distribution of Stroma and Antral Follicles Differs between Insulin-Resistance and Hyperandrogenism-Related Polycystic Ovarian Syndrome. Front Endocrinol (Lausanne). 2017;8:117. [PubMed] [CrossRef]
25. Min AM, Saito M, Simpson JA, Kennedy SH, Nosten FH, McGready R. Placental histopathology in preterm birth with confirmed maternal infection: A systematic literature review. PLoS One. 2021;16(8):e0255902. [PubMed] [CrossRef]
26. Obimbo MM, Zhou Y, McMaster MT, Cohen CR, Qureshi Z, Ong'ech J, et al. Placental Structure in Preterm Birth Among HIV-Positive Versus HIV-Negative Women in Kenya. J Acquir Immune Defic Syndr. 2019;80(1):94–102. [PubMed] [CrossRef]
27. Ategeka J, Wasswa R, Olwoch P, Kakuru A, Natureeba P, Muehlenbachs A, et al. The prevalence of histologic acute chorioamnionitis among HIV infected pregnant women in Uganda and its association with adverse birth outcomes. PLoS One. 2019;14(4):e0215058. [PubMed] [CrossRef]
28. Gichangi PB, Nyongo AO, Temmerman M. Pregnancy outcome and placental weights: their relationship to HIV-1 infection. East Afr Med J. 1993;70(2):85–9
29. Feist H, Hussein K, Stucki-Koch A, Wohlschlaeger J, Hager T, Blocker T, et al. Villitis of unknown etiology and chronic deciduitis are not associated with human papilloma virus and enterovirus infection. Virchows Arch. 2020;477(1):73–81. [PubMed] [CrossRef]
30. Chen SL, Qin ZY, Hu F, Wang Y, Dai YJ, Liang Y. The Role of the HOXA Gene Family in Acute Myeloid Leukemia. Genes (Basel). 2019;10(8):621. [PubMed] [CrossRef]
31. Li J, Ye M, Zhou C. Expression Profile and Prognostic Values of HOXA Family Members in Laryngeal Squamous Cell Cancer. Front Oncol. 2020;10:368. [PubMed] [CrossRef]
32. Ekanayake DL, Małopolska MM, Schwarz T, Tuz R, Bartlewski PM. The roles and expression of HOXA/HOXa10 gene: A prospective marker of mammalian female fertility? Reprod Biol. 2022;22(2):100647. [PubMed] [CrossRef]
33. Li Y, Behringer RR. Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. Nat Genet. 1998;20(3):309-11. [PubMed] [CrossRef]

34. Han YJ, Park AR, Sung DY, Chun JY. Psx, a novel murine homeobox gene expressed in placenta. Gene. 1998;207(2):159-66. [PubMed] [CrossRef]

35. Chun JY, Han YJ, Ahn KY. Psx homeobox gene is X-linked and specifically expressed in trophoblast cells of mouse placenta. Dev Dyn. 1999;216(3):257-66. [CrossRef]

36. Topaloğlu U, Aydın Ketani M. The distribution of some homeobox proteins in the bovine placenta during gestation. Theriogenology. 2021;166:71-82. . [PubMed] [CrossRef]