CHORIONIC STRUCTURES IN MATERNAL BLOOD

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Summary

Chorionic villi are the exchange structures of the placenta where human fetuses receive oxygen and nutrients from maternal blood, This article reports an improvement of a published method to recover them from the blood of pregnant, women, quotes their number and illustrates their size and trypsin impregnation. The reasons to explain the presence of chorionic structures and villi in the circulating maternal blood, the possible significance of their existence in maternal blood and the mechanisms to remove them are discussed. Their size and trypsin impregnation are illustrated. This paper discusses the significance of the presence of such allogeneic structures in the circulating blood of pregnant women and presents a brief discussion on the role of trypsin and its inhibitor in pregnancy homeostasis.

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Introduction

The embolization of pulmonary vessels by chorionic cells in women dying with eclampsia, has been reported by Schmorl[1] in 1904 and Veit[2] has described the deportation of chorionic villi to maternal blood. Even when these reports received only scant confirmations, the deportation of chorionic villi to maternal blood was accepted as an actual fact by obstetric knowledge and treatises[3]. Most of the published evidences on the subject reports only the migration of fetal cells or free fetal DNA to maternal

blood, a feature observed in all pregnancies[4]. Chorionic villi have been observed at uterine or pelvic veins but not at peripheral blood simultaneously sampled[5] Luz [6] has published a technique for their retrieval from maternal blood. Few studies have quantified the number of chorionic cells or structures migrated maternal blood[7, 8, 9]. Fetal DNA presence and fate in maternal plasma has been properly studied[10, 11]. A phase contrast microscope was used by Luz[12] to study the number of chorionic villi found in fresh aprotinine (trasylol®Bayer) treated blood samples, obtained from a group of 30 normal pregnant women at the end of pregnancy.

In a study of this subject, Luz has counted and measured the chorionic villi recovered from peripheral maternal blood, in a group of 10 normal pregnant women[13].

. A mean of 5.76 ± 0.88 villus and 15.4 ± 3.07 chorionic sheets > 100 µm per ml of maternal blood have been found. Villus mean longitudinal size was 289.3 ± 13.05 mµ and mean transverse diameter was 116.4 ± 5.75 µm. (Figure 1). The presence of trypsin at the syncytial envelopment of

villi and in a sheet of trophoblast being detached is illustrated. (Figure 2). This article is intended to report an improvement of the published method to recover chorionic structures from maternal blood, to present the large dimensions of some of them and to display their impregnation with trypsin.

These observations present new insights on the characteristics and size of chorionic structures at the circulating maternal blood. The recovery technique described in this article may help the persons interested on the subject to confirm our reported observations. New facts here reported could enhance the understanding of some unclear aspects on the immunology and the physiology of a pregnant women

Method

3 ml of maternal blood are obtained at a cubital vein, with a syringe soaked in heparin, immediately verted to a centrifuge tube containing methylic alcohol, gently agitated and kept in vertical position for 30 to 60 minutes, then centrifuged at 2,000 rpm by 30 minutes. The plasma over the sediment is removed with a small pipette leaving intact a linear portion of 3 to 5 mm

directly over the sediment. The removed plasma is substituted by an equal amount of Bouin's fixative, centrifuged again for 15 minutes at 2,000 rpm and kept at a vertical position for 6 hours. The centrifuge tube is next sectioned just above the sediment and split into two halfs by a vertical incision. The 2 complete, hardened pieces of the sediment are embedded in paraffin, sectioned at 3 µm and stained with HE or Masson's trichrome. Microscope glass slides are scrutinized at 250x magnification.

Results

Large pieces of trophoblastic villi and sheets of detached syncytiotrophoblast (Figure 1) have been recovered from each blood sample studied[13]**.** They present several degrees of degenerative alterations.. Their nuclei are pale or not stained by hematoxylin. Our recent studies have detected trypsin at the syncytial envelopment of villi and in the detached chorionic sheets (Figure 2). Monoclonal antigens specific to chorionic tissues recognized them only 33 % of cases, in a study performed by Bill Kalionis. Morphology has offered, up to now, the best criteria for their identification.

Discussion

The first point to be stressed from this article is the fact these chorionic structures have been found and recovered from maternal blood in all studied cases. This was obtained sampling maternal blood with a protease inhibitor, or by the use of an immediate fixation of the blood sample. Douglas[5] has recovered chorionic cells from uterine or pelvic vessels but none at the peripheral blood, even when simultaneously sampled,. He believed this was caused by the trapping of the chorionic structures by the capillary network of the lungs. The presence of such large structures in peripheral blood of pregnant women, as reported in this article can be explained by the existence of arterio-venous shunts that are enlarged during pregnancy[14, 15. 16, 17], allowing the bypass of large structures from arteries directly to veins (figure 1). Their absence as observed in most previous studies can be ascribed to their removal by maternal mechanism and not to their capture by lung's capillaries. Their constant presence in blood samples obtained by us[6, 12, 13] can be explained by the use of an inhibitor of proteases or by the use of an immediate fixation, any one procedure

interfering with the maternal removal mechanisms. Other points be considered from our reported observations are the number and the size of these structures found in maternal blood[12, 13]. A total of 15.4 ± 3.1 syncytial sheets larger than 100 µm (range 22-114) and 5.8 ± 0.9 villi per ml of maternal blood have been found in a group of 10 normal pregnant women at the end of pregnancy. As the total volume of blood in a normal human pregnancy can be estimated as of 5,500 ml, our findings suggest the actual presence of 116,600 allogeneic structures in the maternal blood, 84,700 of them being chorionic sheets and 31,900 being chorionic villi. They are large, in182 measured villi their mean longitudinal diameter was 289.3µm ± 68.6 (range 68.6 to 1,372µm) and their mean transverse diameter was 116.4 ± 5.23µm (range 9,2 to 372.4 µm)).

These data could be important to evaluate the actual amount of chorionic structures present in maternal blood and help to the understanding its possible significance.

Other point to be considered from our data, is the immunologic significance of the presence of so many structures the in circulating blood

of pregnant women. Jerzak[18] has considered this as a currently neglected factor with influence on the development of the maternal tolerance to her fetus Some studies have already associated the number of chorionic structures in maternal blood with pregnancy diseases as pre-eclampsia [19, 20, 21. 22, 23. 24]. The use of the blood sampling method here reported may provide additional informations concerning the amount of chorionic tissues circulating in maternal blood in normal and abnormal pregnancies. The maternal mechanisms to remove them from circulating blood are aspects not currently studied. Thomas[7] has studied in vitro the effects of trypsin upon chorionic villi. He has verified this enzyme, in vitro, at a concentration of 100 micrograms per ml will destroy fresh human chorionic villi in 3 to 4 minutes and promotes the detachment of the syncytial envelopment from the core of the villus. Few evidences have been reported on the existence of these removal mechanisms. Lo[11] has described the short mean half-life of free fetal DNA in maternal plasma. Dhallan et al[25, 26] have significantly increased the percentage of free fetal DNA from 6.3 to 34.7 % of total maternal plasma DNA, by the use of

formaline in the treatment of the blood samples. This effect could be caused by a direct action of formaline, decreasing or stopping the maternal mechanism for the elimination of these allogeneic fetal/placental tissues from her blood.

Impregnation with trypsin has been found by us in the syncytiotrophoblast envelopment of the villi and in other chorionic structures recovered from maternal blood **(**Figure 2).

An important point, quoted in this article, is the biological effect of an urinary protease inhibitor in an experimental model of premature labor in mice. This substance has significantly increased the duration of pregnancy in induced premature labors in mice and has decreased the intensity of their placental apoptosis[27, 28, 29, 30, 31, 32, 33], suggesting both, trypsin and its inhibitor are significant parameters for the maintenance of mice pregnancies. They are possible participants on the homeostasis of human pregnancy, as it is suggested by the in vitro effect of trypsin[7], by the trypsin presence in the chorionic tissues recovered from maternal blood,

here reported, and the evidences of their rapid removal from maternal blood[25, 26].

Conclusions

1) a great number of large chorionic structure is daily deported to maternal blood
2) they seem to be participants in the development of pregnant women immune tolerance to her fetus
3) the possible action of trypsin and its inhibitor in trophoblast could be relevant to the physiology of human pregnancy

LEGENDS

Figure 1: A chorionic villus recovered from peripheral maternal blood.with a thickened syncytiotrophoblast and two detaching syncytial knots. The 63x inserted at the left upper part of the figure demonstrates this villus is part of a much larger structure. Actual size can be estimated by the 50µm scale

Figure 2: Trypsin impregnates on the syncytiotrophoblast from a degenerated villus and on a detaching sheet of trophoblast

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