Neonatal Apgar score and placental histological morphometry: Is there any relationship?

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Abstract: Background: Birth is a vital process resulted from the communicated employment of three biological factors; the uterus, placenta and newborn, hence, an attempt to find any correlation of some of the placental findings represented by (histological morphometric data) with the newborn's wellbeing was tried here, to found any pure placental based factors affecting level of Apgar score, which might be originated from neither maternal nor fetal causes, so that to pay attention for any possible similar problem in the future pregnancies. **Material and methods**: Placentas of selected one hundred newborns of low Apgar score at 1st minute of life (group I) and another one hundred newborns of high Apgar score also at 1st minute of lifewith their placentas (group II).Newborns of both groups were deliveredby normal second vaginal delivery. The research was carried out over the period from 2nd of January of 2013 till end of 30th of May 2014. Placentas of these newborn were studied histo-metrically via MOTIC IMAGE PLUS 2 program used in photomicrograph study of five randomly selected fields (at ×400 magnification), then the following data (as mean±SD) were compared statistically between the two groups: Number of chorionic terminal villi, number of syncytiotrophoblast cells (nuclei), number of syncytial knots and number of cross-sectional blood vessels. Also for three randomly selected terminal villi of different sizes (large, medium and small)the following parameters were obtained: surface area and perimeter of the villous sections. **Results:**Morphometric histological data in Table 1 showed that;placentas of Group I; had higher number of chorionic terminal villi setting asidesmall surrounding intervillous spaces. Also Group I had more crowded syncytiotrophoblast cells (nuclei) forming almost a continuous layer. The number of syncytiotrophoblast knots, and number of blood vessels within villous core were as well higher in placentas of Group I. What's more; cross-sectional surface area and perimeter of these terminal villi, in placentasof Group I were larger too. **Discussion:** This study was carried out to observe some morphometric microscopic changes in the placenta in case of low Apgar score without a known antenatal maternal or fetal contributing factors, hence, a pure placental cause was suspected here. According to the literature available, the placental grounds inducing low Apgar score had not been studied in human before, although Veronesi et al. (2005) had studied placental relation to Apgar score level in thoroughbred horses.Histological morphometric data might be explained as the followings: Larger number of villi in Group I could be present as a compensatory mechanism to placental insufficiency. Higher number of syncytiotrophoblast cells (nuclei) in placentas of Group I could be also as a compensatory means to balance the functional demand. Terminal villi were larger in Group I;might be due to additionalcontent of connective tissue fibers and cells in the villous core, which could be caused by reduction in villous perfusion stimulating the process of proliferation and activation of fibroblasts there. The increase in number of the syncytial knots in Group I might be as anindicator to the presence of oxidative stress due to poor perfusion. Increased number of blood vessels within villous core in placentas of Group I couldbe a compensatory reaction to chronic hypoxia to improve perfusion. Terminal villi had larger cross-sectional surface area and perimeter in Group I which could be a signof either a delay in maturation of placental tissue, or a compensatory mechanism to augment the function of placenta. **Conclusions:** Low Apgar score in newborns of apparently normal mothers might be caused by pure placental factor which could be documented by histological examination of placenta.

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1.Introduction:

By one process that is***" birth"*** the lifeindeed, is started. It is the critical outcome of interaction among three living entities; the mother, placenta and newborn; each of which is still an interesting concept for medical researchers to date[(1-3)].

Since Doctor Virginia Apgar in 1952 constructed a scoring way to review the clinical status of an infant at 1st minute of life till now, this way provides the best known practical means to assess newborn's wellbeingto locate any need for resuscitation [4].

Apgar score includes 5 issues: **A**ppearance, **P**ulse, **G**rimace, **A**ctivity, and **R**espiration, which could be assessed at 1st and/or 5th minutes from birth. Normal Apgar score at 1st minute has level of 7-10, and any level below 7 is regarded as a lowApgar score [5].

Despite its fundamental role in the health of the fetus and mother, the placenta is the least known human organ, since, the growing related facts are still underscored, and because placental development is vital in the lifelong health of both mother and offspring, this lack of knowledge regarding placental structure and physiology is largelyneeded nowadays [6].

Postnatal, it may be considered as "an experimental animal" for a large selection of researches [7]. It is the only organ known to be created in parenthood and is the purely one getting a distinct fate [8].

Structurally, placenta contains tissues from both mother and embryo. The embryonic side is the chorion, which is formed by trophoblast, and the maternal side is the decidua basalis[9]. The trophoblast includes cytotrophoblast and syncytiotrophoblast constructing the chorionic villi together, to provide a wider surface for exchange. There are thousands of chorionic villi, each branching many times to be bathed in lakes of maternal blood where diffusion of gasses and nutrients occurs there [10].

2.Material and Methods

This study was conducted by Department of Anatomy, Histology and Embryology, College of Medicine, Al-Mustansiryia University, in cooperation with the Department of Gynecology and Obstetrics at Al- Yarmook Teaching Hospital in Baghdad, Iraq. The study was approved by the local scientific committee of both institutes. It was carried out on 200 mothers with their placentas and newborns, who were admitted to delivery room, for normal 2nd vaginal deliveries, at Al-Yarmook Teaching Hospital. They were chosen as pregnant at term of 38-40 wk of gestation, having single fetus, non-smoker and apparently normal women; according to their history, clinical examinations, laboratory investigations and ultrasound examination. The fetal condition also was checked by Doppler ultrasonic study, and any prenatal tired fetus had beenruled out from this study. Any mother had complicated or prolonged labor and even postpartum mother loss also had been excluded from this research. A verbal consent was obtained from each patient to be a part of thisstudy. Newborns of these mothers were scored by pediatrician at 1st minute after delivery at the level of Apgar score. Any low Apgar score was proposed by this study to be caused by placental grounds, since there were no obvious maternal or fetal causes.

Newborns and placentas were divided into two variable groups, according to the Apgar score of newborns. Each group consisted of 100 newborns with each own placenta. The 1st group contained placentas and newborns having Apgar score <7 (Group I) and the 2nd group contained placentas and newborns having Apgar score of ≥7 (Group I) which is the normal level of Apgar score, hence it was considered as the control group.

After being expelled, placentas were immediately examined grossly and tissues for histological examination, were taken from the central part of cotyledon which identified by the typical central subchorionic blood lake, half way between the maternal (decidua basalis) and fetal surfaces (chorion). The block was excised at mid-pointof the largest diameter extending between the insertion of umbilical cord andperiphery of placenta [11 &12]. Each block was approximately 1 × 1 × 0.5 cm and was prepared for routine paraffin section by fixing it immediately in 10% formal solution then stained with haematoxylin and eosin. From each tissue block; five serial sections of 5 µmthickness were examined for histological morphometric study using light microscopy [13 &14].

Morphometric information were obtained via the computerized program namely; MOTIC IMAGE PLUS 2, at 400× magnification; the variables were taken from randomly selected 5 fields obtained from five regions in each slide (4 corners and the center of the specimen), then the image was captured in high definition using the same device's built-in camera that displays the image on screen: and the following data were obtained from these 5 fields: (a) Mean number of chorionic terminal villi ,(b) Mean number of syncytiotrophoblast cells (nuclei), (c) Mean number of syncytial knots and (d) Mean number of cross-sectional blood vessels. Then, three randomterminal villi of different sizes (large, medium and small) were studied, in each section measured 1 μm^2; the following parameters were obtained: (a) Mean surface area of the villous section and (b) Mean villous perimeter.

Data were put as mean ± SD, next; by using Paired Samples T- test via the computerized program that is PASW Statistics 18, used on personal computer, the variables were compared and statistical significance was considered at P value less than or equal to 0.05 [15].

3.Results:

All of morphometric results were presented on table1. At 400× magnification: placentas of Group I (Apgar score < 7) had higher number of chorionic terminal villi which had more villous connective tissue

fibers and cells, letting aside smallerintervillous spaces.Also, in placentas of Group I the lining syncytiotrophoblast cells (nuclei) were more crowded forming almost a continuous layer surrounding the core of villi. The number of syncytiotrophoblast knots at periphery of villi and number of blood vessels at villous core werehigher in Group I placentasas well. In addition; cross-sectional surface area and perimeter of the terminal villi, in placentas of Group I was higher, too. All differences of compared data were significant (P<0.05).

Table (1): Number of Villi, Number of Syncytiocytotrophoblast cells, Number of blood vessels, Sectional surface area of the villi in μm^2, Perimeter of the villi in µm and Number of Syncytiocytotrophoblast Knots:

Histological Data	Apgar score of the newborn	N	Mean± SD	*P* value
Number of Villi	low Apgar score <7	100	10.762±4.468	
	high Apgar score ≥7	100	8.458±3.171	0.0001
Number of Syncytiocytotrophoblast cells (nuclei)	low Apgar score <7	100	14.676±5.578	
	high Apgar score ≥7	100	8.795±3.227	0.0001
Number of Syncytiocytotrophoblast Knots	low Apgar score <7	100	1.527±.798	
	high Apgar score ≥7	100	0.829±.621	0.0001
Number of blood vessels	low Apgar score <7	100	5.677±2.747	
	high Apgar score ≥7	100	4.121±1.779	0.0001
Sectional surface area of the villi in μm^2	low Apgar score <7	100	9307.903±2331.894	
	high Apgar score ≥7	100	6909.057±2604.512	0.0001
Perimeter of the villi in µm	low Apgar score <7	100	135.571±35.048	
	high Apgar score ≥7	100	101.131±28.326	0.0001

-N is the sample number, SD is the sample mean standard deviation.
-Data wererepresented as Mean ± SD.
-Statistical comparison of data was considered significant at *P* value equal or less 0.05.

4.Discussion:

The placenta was chosen in the current study because it is regarded as a mirror to the intrauterine fetal condition [16]. The fetus, placenta and mother form a triangle of dynamic equilibrium and any disturbance in one of them would affect the others[17]. Literature review showed that previous studies, dating back to 7th decade of previous century, had considered pure placental factor in some intrauterine growth retardation [18-22].

Apgar score was used here because it is still regarded as the best tool to assess the fetal-to-neonatal transition [23]. Apgar score of 7-10 at the 1st minute is considered to be normal [4&5]. In this study cases of low Apgar score due to fetal or maternal factors were excluded, hence the low Apgar score was considered to be merely due to placental causes. Morphometric examination of human placentas wasregarded by many scientists,as an indirect way to study the histophysiology of pregnancy. The ongoing study involved three types of villi, just like previous similar work[24]. The Motic Image plus II program was very useful in this study. It was implicated to measure the irregular and complex structures of the terminal chorionic villi images, and compare the obtained data in a mathematically unbiased manner. Tissues were taken only from the intermediate part of the cotyledon half way between the maternal and fetal surfaces and midway between the insertion and periphery at its largest diameter,because this technique was used by others, to avoid the structural difference in tissues between parabasal and subchorionic areas [11 & 12].

On examination of haematoxylin and eosin stained sections of both groups:At Table 1villi number was found to be increased in Group 1 just like that of placenta delivered beyond 41 weeks of gestation. This could be taken place as a compensatory mechanism to equalize placental insufficiency[25]. It had been reported that there is a higher incidence of stillbirth, low birth weight babies and poor fetal outcome in cases with high syncytiotrophoblast cell count [26-29]. In the present study, the mean number of syncytiotrophoblast cells (nuclei) in low Apgar score group placentas was significantly higher than the other group (Table 1), seen as more continuous adjacent nuclei, to go with functional demand and to repair the damaged syncytium, just like what was found in a previous study of placentas in stillbirths and low birth weight [26-28]. Table 1proved increased number of syncytial knotsin Group 1 same as findings noticed in idiopathic intrauterine growth retardation, toxaemia of pregnancy, and in pre-eclampsia[27-29]. Abundance of syncytial knots are indicators of oxidative stress owing to poor fetal circulation[29]. Table 1pointed up increased number of blood vessels in the villous core in placentas of Group 1, which considered as a placental

compensatory reaction to improve chronic low grade perfusion and fetal hypoxia [30& 31]. Table 1showed a large number of terminal villiof small cross-sectional surface area, in placentas of Group 1. This surface area represents the interface for exchange between maternal and fetal circulation, hence, it is a context of medical research till now [30]. The instant findings in Group 1 were similar to that of delay in maturation of placental tissue, or a compensatory mechanism to improve function of the placenta [30-32]. Table 1 showed larger terminal villi having more connective tissue fibers and cells in Group 1 which could be caused by reduced fetal villus perfusion leading to proliferation and activation of the fibroblastic cells of villous stroma[29 &31]. The perimeter of villous section which corresponds to the feto-maternal exchange surface increased in Group 1 imitating that in placentas of diabetic mothers [33].

This study was undertaken to observe some morphometric microscopic changes in the placenta in cases of pure placental cause of low Apgar score and according to the literature available, this had not been studiedin human placentas before, although Veronesi*et al.* (2005) had worked on placental relation to Apgar score of thoroughbred horses[34].

Currently, Apgar score is the only definite method for evaluation of fetal status [3], so, the current research tried to correlate this score with the underlying placental histopathology which is an interest context of researches till now [35].

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