A study on placental expression of neuronal markers in intrauterine growth restriction

Upendhar Reddy Pulluru¹, Sudhakara Babu Chelli ², Venkateshwar Reddy Muchinthala¹, Steevaan V.³, Sai Charitha¹, Sai Priya Reddy⁴, Govindarajan Sumathy⁴

¹Department of Anatomy, SVS Medical College, Mahbubnagar, Telangana, India
²Department of Anatomy, Government Medical College, Nizamabad, Telangana, India
³Department of Pathology, Toxicology, Palamur Biosciences Private Limited, Mahabubnagar, Telangana, India
⁴Department of Anatomy, Sree Balaji Dental College & Hospital, Chennai, Tamil Nadu, India

(Received: April 2023          Revised: October 2023          Accepted: October 2023)

Corresponding author: Govindarajan Sumathy. Email: sumathyravi93@gmail.com

ABSTRACT

Introduction and Aim: The restriction of intrauterine growth (IUGR) has a 20% recurrence rate and is one of the leading causes of postnatal illness and death. The diagnosis of intrauterine retardation refers to the infant's increased risk of neurological issues over an extended period, neonatal morbidity, and mortality, and in utero death.

Materials and Methods: One hundred placenta samples were collected and divided into cases and controls. Clearance from the ethics committee was taken from the institute prior to the commencement of this study. Exclusion criteria include the patients with multiple pregnancies, unknown gestational age, gestational diabetes, and HIV. The inclusion criteria are the singleton pregnancy, normal and cesarean section, maternal age between 18-35 years and GA between 34 – 41 weeks. Standard immunohistochemistry protocols were followed for the study and glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE) markers were used as neuronal markers.

Results: Strong immunoreactivity of glial fibrillary acidic protein and neuron specific enolase was observed in fetal growth restriction placenta indicating perinatal brain damage of neonate.

Conclusion: In our study we observed strong positive immunoreactivity of GFAP and NSE in IUGR only. This study suggests that these markers are used to predict brain damage in IUGR neonates.

Keywords: IUGR; GFAP; NSE; immune expression; brain damage; neonates.

INTRODUCTION

The term "intrauterine growth limitation" is also used for intrauterine growth restriction (IUGR). One of the key causes of the processes underlying FGR is placental illness (1). The placenta can be examined both in gestation and after delivery to learn important details about the health of the fetus (2). The placenta is frequently removed soon after delivery without adequate evaluation, and researchers have long stressed the advantages of a thorough anatomical study. The possibility of long-term neurological problems, ailments in neonatal or in utero death has increased in infants who are born with intrauterine retardation (3,4).

MATERIALS AND METHODS

The study was carried out at SVS Medical College & Hospital in Mahbubnagar, Telangana, in the OBG & Anatomy departments. Prior to the start of the study, the institute granted ethical clearance (SVSMC/IEC Approval No. 03 / 2020-456). After obtaining informed consent, placental samples from both normal and fetal growth restriction patients were collected. With a 100 percent sample size, the controls and cases were split equally. Sections of 2 microns were selected on positively coated slides and all the standard immunohistochemistry procedures were followed. Slices of tissue were covered by glial fibrillary acidic protein for a period of half to one hour and neuron specific enolase for 30 minutes on a separate slide and kept it warm in a closed chamber at room temperature using Pathnsitu poly excel detection system. The slides were counterstained with hematoxylin for 120 seconds and viewed under LEICA / 1000 DM microscope. Readings were noted by a pathologist who was blind at study. For the statistical analysis, SSPS 25 was employed.

RESULTS

An independent t-test was performed after taking the mean of the quantitative data and assigning an intensity score (0-no reaction; 1-mild; 2-moderate; 3-strong; 4-very strong) based on staining. In graph 1, there was a significant increase in GFAP observed in cases compared to controls (p=0.001). In IUGR placenta, the expression of NSE was more in cases when compared to normal with p= 0.001 which is significant.
Fig. 1: Comparison of the neuronal markers between two groups

Arrow mark is showing the expression of (GFAP) glial fibrillary acidic protein marker in control group without any intensity. Placental (villi) tissue is very light brown stained in (cases) IUGR group.

Fig. 2: The expression of GFAP marker in cases (IUGR) and control (normal)
DISCUSSION

GFAP is a highly specific intermediate filament protein found only in brain astrocytes, belonging to class III. It is employed in the distinction between non-glial cell tumors and astrocytes.

When it comes to identifying tumors with neuroendocrine origins, like pheochromocytomas, and peripheral nerves, NSE-gamma is a helpful marker. This antibody can be used in conjunction with other markers like neurofilament, synaptophysin, and chromogranin A.

Since NSE has primarily been studied in cerebrospinal fluid, adult cerebral infarction patients' serum levels have been found to correlate with lesion volume, long-term development, and acute neurological symptoms (5). NSE can be used to gauge the degree of brain damage in cases of congenital cytomegalovirus infection in neonates, and it has been demonstrated to predict neurodevelopment in children experiencing perinatal asphyxia (6). Furthermore, higher fetal amniotic NSE levels are linked to neonatal mortality, necrotizing enterocolitis (NEC), and the requirement for intubation in addition to neonatal intraventricular hemorrhage (7-9). In our study, fetal growth restriction (FGR) placenta expressed NSE while in normal placenta we didn’t observe any staining and we found a significant association between cases and control.

Despite being a useful indicator of brain damage, NSE in IUGR has not been the subject of many investigations. Neuron-specific enolase (NSE) and brain-derived neurotrophic factor (BDNF) ranges in umbilical twine blood in full-time period newborns with asymmetrical intrauterine boom retardation resulted from persistent placental insufficiency were studied (10). When allopregnanolone synthesis was reduced, GFAP expression rose, confirming the important regulatory function of this steroid. Astrocytes express GFAP during development and when pathological processes activate them (11). In a study by Kelleher et al., (12), there are no noteworthy correlations between GFAP expression and IUGR.

This is in line with another study that showed there was no discernible variation in GFAP-positive cells in the brains of growth-restricted fetal guinea pigs. Our study was in contrast to the above author where we found a significant association between GFAP and FGR.

Both stressors, regardless of gender, decreased plasma allopregnanolone, but Liu et al., found that neither stressor had an impact on GFAP or MAP2 expression (13). Allopregnanolone treatment has also shortened the length of GFAP-fantastic astrocytes at the location of the experimental lesions in adult rat models of stressful brain injury (14). Given that neither GFAP nor B-50 were detected in the placenta or umbilical cord, these proteins appear to be promising indicators of neuronal injury in neonates as per observations by Wijnberger et al., (8).

CONCLUSION

GFAP and NSE were expressed in IUGR placenta, and no reaction was observed in normal placenta. An increased number of these markers suggest neurodegeneration or generation. In our study we observed positive reactions of GFAP and NSE in IUGR only. This study suggests that these markers are used to predict brain damage in IUGR neonates.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of the pathologists of Palamur Biosciences Private Limited, Telangana.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES