Original article:

A comparative quantitative study of calcium content of human placenta

DR. MAITRAYEE MONDAL[1], DR. PALLAB KUMAR SAHA[2], DR. MALLIKA SENGUPTA[2], DR. GURUPRASAD MONDAL[1]

[1]Associate Professor, [2]Assistant Professor
North Bengal Medical College and Hospital, Sushrutanagar, Darjeeling
Corresponding author – Dr Pallab Kumar Saha

Abstract:
Calcium is a vital element for the mineralization of the growing foetus. Quantitative estimation of calcium content of palacenta of normal and hypertensive group with history of antepartum haemorrhage showed no significant difference. The study was carried out to see whether calcified placenta was the cause of poor foetal outcome.

Keywords: APH Placentae, Aliquote, Muffle furnace, AAS (Atomic Absorption Spectophotometer)

INTRODUCTION

Calcium is important in regulation of normal cellular function. It is vital for proper mineralization of the foetal skeleton. It's deficiency may lead to poor foetal outcome. Foetus receives calcium though transport from mother by syncytiotrophblast, by an active process[1]. Placenta is the vital organ for the survival of the growing foetus[2]

In this study quantitative study of calcium content of placenta of two groups that is normal gestation and patients with history of antepartum haemorrhage was carried out. Since qualitative studies are in plenty a quantitative study was desirable to estimate the calcium content of the placenta.

MATERIAL AND METHODS

A total of 50 placentae were studied for chemical analysis out of which 25 were from term pregnant mother without any obstetric complication and 25 placentae from mother with pregnancy with complication like pregnancy induced hypertension with history of Antepartum haemorrhage. Placentae of different groups were collected after delivery from labour room and emergency OT of R. G. Kar Medical College, Kokata, from June 2016 to December 2017. Pacental weight was recorded after removal of membranes and after cutting the umbilical cord and cleaned, total placentae was cut into small pieces and grinded into a mixer grinder to get a homogenized mass. A small amount of this placental mass was taken with a spatula in a crucible and weighed and charred in an electric heater for about 1 hour. Weight of the mass taken was recorded for every placentae. Then the mass of the placentae in the crucible was placed in a Muffle furnace at 500^0 C for 2-3 hours to turn into an ash in the West Bengal University of Animal & Fishery Science. 20 ml of 1:1 HCL was then added to the ash in crucible and heated to boiling temperature for 5-10 minutes then it was cooled to room temperature and transferred to volumetric flask by filtering in Whatman filter paper (No. 1) with repeated hot water washing. Distilled water was then added to the solution to make it 100 ml.

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Calcium content was then estimated in Atomic Absorption spectrophotometer. Total calcium of the sample mass (in gm.) taken from each placentae = AAS reading (PPM mlg/kg.) X Dilution factor.

Dilution factor

$$= \frac{\text{Aliquote made (100ml)}}{Sample\ weight\ (gm.)}$$

From this formula calcium % i.e. mg. % of each placentae can be calculated.

This procedure was followed according to Association of official analytical chemist (USA AOAC,1995)[3].

Then data were tabulated and appropriate analysis was done in North Bengal Medical College.

RESULT

Control = Term pregnant mother without any obstetric complication

Table 1 (calcium content of control group)

Serial no.	Wt. of placenta (in gm)	Dilution factor (100/ sample of mass in gm)	AAS reading in PPM (mg/kg)	Total calcium content of sample (in gm) {AAS x DF}/ 1000	Total calcium content of sample (mg/kg)
1	24.80	4.032	32.223	0.129	129
2	16.63	6.013	9.795	0.059	59
3	22.48	4.448	16.949	0.075	75
4	22.60	4.425	17.401	0.077	77
5	15.25	6.557	16.470	0.108	108
6	14.90	6.711	10.729	0.072	72
7	19.24	5.197	16.548	0.086	86
8	26.20	3.816	16.247	0.062	62
9	18.55	5.391	14.283	0.077	77
10	20.15	4.963	16.723	0.083	83
11	19.15	5.222	17.426	0.091	91
12	23.64	4.230	23.168	0.098	98
13	22.10	4.525	14.586	0.066	66
14	19.70	5.076	15.366	0.078	78
15	18.20	5.495	12.739	0.070	70
16	17.25	5.797	15.698	0.091	91
17	23.80	4.202	20.228	0.085	85
18	18.10	5.525	14.660	0.081	81
19	16.90	5.917	12.337	0.073	73

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20	22.55	4.434	15.787	0.070	70
21	25.95	3.854	14.271	0.055	55
22	20.85	4.796	12.927	0.062	62
23	18.45	5.420	14.576	0.079	79
24	24.80	4.032	14.137	0.057	57
25	18.90	5.291	13.419	0.071	71

For control group

Mean- 78.2

SD- 16.6107, S.error- 3.322.

Case = Pregnant mother with complication like pregnancy induced hypertension with history of Antepartum haemorrhage

Table 2 (calcium content of cases)

Serial no.	Wt. of placenta (in gm)	Dilution factor (100/ sample of mass in gm)	AAS reading in PPM (mg/kg)	Total calcium content of sample (in gm) {AAS x DF}/ 1000	Total calcium content of sample (mg/kg)
1	11.31	8.841	18.965	0.167	167
2	22.60	4.425	14.463	0.064	64
3	25.50	3.922	18.103	0.071	71
4	19.74	5.066	17.165	0.087	87
5	14.57	6.863	12.169	0.083	83
6	17.35	5.764	15.961	0.092	92
7	22.38	4.468	14.247	0.063	63
8	18.87	5.299	16.565	0.087	87
9	20.36	4.912	17.548	0.086	86
10	16.40	6.097	16.729	0.102	102
11	20.12	4.970	18.639	0.092	92
12	25.65	3.898	16.357	0.064	64
13	15.75	6.349	17.483	0.111	111
14	23.60	4.237	17.170	0.073	73
15	21.20	4.717	18.019	0.085	85
16	18.85	5.305	15.457	0.082	82
17	27.60	3.623	15.611	0.056	56
18	30.21	3.310	8.865	0.029	29

19	19.65	5.089	17.881	0.091	91
20	20.85	4.796	17.928	0.086	86
21	15.14	6.605	11.105	0.073	73
22	18.20	5.494	14.379	0.79	79
23	22.10	4.525	15.911	0.072	72
24	16.50	6.061	16.334	0.099	99
25	21.80	4.587	18.312	0.084	84

For cases-

Mean- 83.12

SD-24.1407,S.error- 4.8

p value – 0.31575(groups are matched).[not significant]

DISCUSSION

Various means are adopted to determine the calcification of placenta. Macroscopic, Microscopic, histological and Radiological estimation of calcium content of placenta, has been done by many workers [4,5,6,7,8]. and the result varies due to employment of different technique and different standards which are considered for significant calcification. A very few studies are available in quantitative estimation of calcium content of placenta. The quantity of calcium of various grades examined by the Large section Technique was expressed in mg/sq cm varied from 0.02 to 0.36 [9]. Placental insufficiency due to calcification which was regarded as one of the factors for placental degeneration is now of growing interest for better obstetric management and foetal care.[4].

Mischel (1958)[10] and Einbrodt et al (1962)[11] found chemically an increased calcium content in toxaemic placenta. But on the contrary Fox, H. [9]himself found low incidence of calcification in the toxaemic group. No correlation was found between placental calcification and antepartum haemorrhage by Tindal[4], Wentworth[6].It was thought that calcification was not found in any pathological state which was in agreement with Hassler, O[5]. According to Tindal [4] calcification does not interfere with foetal growth. The lack of any significant variation of the calcium content of both the groups in our study, it can be stated that placental calcification is not the sole cause of placental degeneration which may be alarming in obstetric management and this in agreement with the findings of Tindal and Fox H.[4,9].

CONCLUSION

Placenta the vital organ for the survival of the growing foetus is of growing interest in the field of research. In our study it is seen by quantitative estimation of calcium that there is no significant difference in the calcium content of the placentae between normal gestational group and those with history of antepartum haemarrhage. This is in agreement with the findings of Tindal [4] and Hasler. O [5]. Both of them concluded that calcification was a physiological process rather than pathological. An extended study using a combination of histological chemical and radiological method will be more conclusive with a larger sample size.

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Photo 1) Placenta Grinded in a mixer grinder to get a homogenized mass.

Photo 2) A small amount was taken with a spatula in a crucible, weighed and charred in a electric heater for about 1 hour.

Photo 3) Ashed in a muffle furnace at 500^0c for 2-3 hours.

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