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GROSS PRESENTATION AND HISTOMORPHOLOGICAL CHANGES OF PLACENTAE IN PATIENTS PRESENTING WITH INTRAUTERINE FOETAL DEATH AT KENYATTA NATIONAL HOSPITAL
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GROSS PRESENTATION AND HISTOMORPHOLOGICAL CHANGES OF PLACENTAE IN PATIENTS PRESENTING WITH INTRAUTERINE FOETAL DEATH AT KENYATTA NATIONAL HOSPITAL

A. OWINO, O. GACHUNO, H. TAMOOH and E. A. ROGENA

ABSTRACT

***Background*: There are 3.2 million annual stillbirths, at least 98% occur in low-/middle income countries, and on average, as many as two-thirds of these stillbirths are thought to occur antenatally, prior to labour. The most useful test towards a diagnosis after stillbirth is pathological examination of the placenta and the foetus. However, this pathological examination is done in less than half of the placentae after cases of stillbirth.**
***Objective*: To determine gross presentation and histomorphological changes of placentae in patients presenting with intrauterine foetal death as compared to live births.**
***Design*: A case control study.**
***Setting*: The Kenyatta National Hospital's labour ward and the Department of Human Pathology, University of Nairobi.**
***Subjects*: The cases were mothers who presented with IUFD at a gestation of 28 weeks and above. The controls were a comparative group of mothers who delivered live babies at the hospital and were matched for age.**
***Results*: Reduction of the mass of functioning villi was present in 11.8% of placenta in the stillbirth group compared to 2% in the live birth group (p-value 0.002). There was significant presence of other placental abnormalities in the stillbirth group (22.5%) compared to the live birth group (9.8%) (p-value-0.002).**
***Conclusion*: This study revealed that histological examination of placenta is useful in identifying some causes of stillbirths. This knowledge may lead to preventive measures which would lower perinatal mortality.**

INTRODUCTION

A stillbirth or intrauterine foetal death (IUFD) is defined as the complete expulsion from its mother, after at least 22 weeks' of pregnancy (or weighing more than 500 grams if the gestation period is unknown), of a product of conception in which, after such expulsion or extraction, there is no breathing, cardiac activity, pulsation of the umbilical cord or unmistakable movement of voluntary muscle. Stillbirths can be divided into early and late stillbirths. Early stillbirths occur before28 weeks gestation while late stillbirths occur at or later than 28 weeks gestation (1,2).

Of the world's 3.2 million annual stillbirths, at least 98% occur in low-/middle-income countries, and on average, as many as two-thirds of these stillbirths are thought to occur antenatally, prior to labour. Proportions of antenatal and intrapartum stillbirths may vary in different low- and middle-income country settings depending on the prevalence of risk factors and quality of antenatal and obstetric care (3,4).

While the highest absolute numbers of stillbirths occur in South Asia, driven by the large population size of that region, the incidence rates are highest in sub-Saharan Africa. Wide variations exist: in highincome countries, stillbirth rates are below 5 per 1000 births, compared to approximately 32 per 1000 in South Asia and sub-Saharan Africa (5). The prevalence of stillbirth at the community level is typically less than 1% in more developed parts of the world but could exceed 3% in less developed areas (6).A study done at Eldoret District Hospital in 1992 found that the stillbirth rate was 30.5/1000 (7). According to records at the Kenyatta National Hospital there were 1066 stillbirths out of a total of 17 881 deliveries in 2007 and 2008 combined, which gives a stillbirth rate

of 59.6/1000 (8).

Approximately half of stillbirths occur prior to 28 weeks of gestation and about 20 percent are at or near term (9). Intrauterine foetal death (IUFD) occurs in < 1 % of singleton pregnancies. The incidence in twin pregnancies varies between 0.5 and 6.8% (10). Despite stillbirths being one of the most common adverse pregnancy outcomes (11), the condition has not been well studied even though the disease burden approaches that of postnatal deaths (12,13).

The known causes of stillbirth include genetics (25-35% of stillborn infants undergoing autopsy have genetic abnormalities), infections (10-15% stillbirths have been associated with bacterial, protozoal and viral infections) and fetomaternal hemorrhage (3-14% of all stillbirths) (3,9). A study done in Italy showed that abnormal placental pathology in stillbirths ranged from 20.7% to 39.6% depending on the classification system used (11).

The most useful test towards a diagnosis after stillbirth is pathological examination of the placenta and the foetus (6,9).However, one study in Italy on stillbirths and deaths in infancy showed that the placenta was examined in only 44% of intrapartum stillbirths (6).

Detection of causes of stillbirths is important to identify deficiencies in the provision of care, to focus attention on areas in which improvements are possible, and to indicate areas in which new developments or knowledge may be expected to lead to preventive measures to lower perinatal mortality (11). In the field of litigation process, placental morphology may be very important for the demonstration that, for example, brain damage or intrauterine death resulted from placental or umbilical cord pathology and not from physician failure (14).

This study was designed to determine the gross and histological morphology of placentae, membranes and umbilical cords from mothers who presented with IUFD and compared with those mothers who delivered live babies.

MATERIALS AND METHODS

Study Design: Case control hospital based study.

Cases: Mothers who presented with IUFD at a gestation of 28 weeks and above constituted the cases. These mothers were chosen after confirmation of IUFD by ultrasound.

Controls: Comparative group of mothers who delivered live babies at the hospital were matched for age and comprised the controls.

Inclusion criteria

Cases: Mothers 18 year and above who presented with IUFD at a gestation of 28 weeks and above and consented.

Controls: Consenting mothers equal or above 18 years of age who delivered live babies at a gestation of 28 weeks and above.

Exclusion criteria: Mothers who presented with abdominal pregnancies

Study site: The study was conducted at the Kenyatta National Hospital's labour ward and the Department of Human Pathology, University of Nairobi.

Sample Size Calculation and Sampling Procedure

Sample size calculation: According to literature, abnormal placental pathology is present in between 20 and 40% of IUFD (11,14).Assumption was made that the proportion of women with abnormal placental histology were (p1=35%) in the group with intrauterine foetal death and (p2=10%) in the group with live births. At 80% power (β=0.2) and 95% (z=1.96) confidence interval using the formula below (Fleiss JL 1981) for comparing proportions, 51 participants in each group were included.

For unequal groups of size n_1 and n_2 where $r=n_2/n_1$, is

$$n_1 = \frac{\left\{Z/_{\alpha/2}\sqrt{(r+1)\,pq} + Z_{\beta}\sqrt{rp_1q_1+p_2q_2}\right\}}{rd^2}$$

where $p = \frac{p_1 + rp^2}{r+1}$ and $n_2 = rn_1$

For small samples, employ a "continuity correction"

$$n_1 = \frac{n_1}{1}\left(1+\sqrt{1+\frac{2(r+1)}{n^1{}_1 r|d|}}\right)$$

n is the number of subjects

p is the proportions in each category

$d = p_1 - p_2$

p_1. (bar) = $1 - p_1$

p_2. (bar) = $1 - p_2$

z = value of standard level for 95% confidence interval

Sampling procedure: Every woman presenting with IUFD at labour ward during the study duration and meeting the inclusion criteria was recruited after confirmation of foetal demise by ultrasound. The recruitment was done until the desired sample size was achieved. A similar number of women with live births was also recruited and matched for age.

Data collection: The principal investigator and the assistants (midwives)approached a client who presented with IUFD (or a client with live foetus in case of controls) at the labour ward and who met the inclusion criteria. The purpose and nature as well as the benefits of the study were explained to the client

by the principal investigator and/or the assistants. An informed consent was sought. Once informed consent was given, information was obtained from the client and the ANC card if available.

After delivery, the baby was examined. The placenta, the foetal membranes and umbilical cord were collected, weighed and put in a container with 10% formalin at a volume ratio of placenta to formalin of 1:10. The information from the client, ANC card, baby, placenta, foetal membranes and umbilical cord was entered into a pre-formatted questionnaire. The placenta, foetal membranes and umbilical cord were then taken to the histology laboratory with a request form which had a corresponding serial number as the questionnaire of the client from whom the placenta was obtained.

The placenta, foetal membranes and umbilical cord were examined soon afterdelivery to the laboratory. The histological processing, sectioning, staining and mounting was subsequently done. The slides were examined by a pathologist and quality control was carried out by review of randomly selected slides by a blinded pathologist. The findings were entered into the questionnaire.

The study instrument was a pre-formatted questionnaire which consisted of eight sections covering socio-demographic data, past obstetric history, history of index pregnancy, medical history, gross examination of foetus, examination of umbilical cord and examination of placenta.

Data Management: All participant data did not bear the names of the participant but rather a serial number. Data forms were kept in a secure lockable cabinet only accessible by the principal investigator and the statistician. Data were entered into a password protected Ms Access database prepared by the statistician. The investigator upon completion of data entry checked all the entered data against the hard copy forms.

Statistical analysis: Data analysis was performed using Statistical Package for Social Scientists (SPSS Version 17.0). The proportion of women presenting with IUFD and those with abnormal placental histology was estimated using simple frequencies. At the univariate analysis level, correlates associated with these factors were performed using chi-squared and Fishers exact test for categorical data and and t-tests for continuous variables.

Ethical Considerations: This study was approved by the Kenyatta National Hospital Ethics and Research Committee. Informed consent was obtained from the client before being recruited.

The contact address of the investigator was given to the client incase she may have required further details about the study or may have wished to withdraw from the study. The information was communicated both verbally and in writing. Refusal to participate in the study did not deny the patient the appropriate management. The client did not bear the cost of the histology examination done on the placenta and the histology report was communicated to the client and the clinical team that managed the client.

Study Limitations: Determination of causes of IUFD would have been more complete if autopsy of the stillbirth would have been performed in addition to the placental pathology. Autopsy of the stillbirth was not performed in this study. This was not possible because of financial constraints.

No bacterial cultures or special stains were performed on the placenta, therefore, microorganisms could not be identified in cases where there were histological signs of infection.

RESULTS

This study was carried out over four months between June and September 2010. During this period 102 mothers were enrolled in the study. There were 51 mothers in the live births group and 51 mothers in the IUFD group.

Table 1
Socio-demographic characteristics of the participants by pregnancy outcome

Characteristic	Pregnancy outcome			
	Live	Stillbirth	Total	P-value
	No%	No%	No%	
Religion				
1 Catholic	19(37.3)	15(29.4)	34(33.3)	0.465
2 Protestant	31(60.8)	33(64.7)	64(62.7)	
3 Muslim	1(2.0)	3(5.9)	4(3.9)	
Education Level				
1 None	2(3.9)	3(5.9)	5(9.8)	0.117
2 Primary	7(13.7)	17(33.3)	24(23.5)	
3 Secondary	23(45.1)	21(41.2)	44(43.1)	
4 College/University	19(37.3)	10(19.6)	29(28.4)	
Employment status				
1 Salaried job	13(25.5)	10(19.6)	23(22.5)	0.098
2 Self-employed	13(25.5)	6(11.8)	19(18.6)	
3 Unemployed	25(49.0)	35(68.6)	60(58.8)	
Marital status				
1 Single	4(7.8)	5(9.8)	9(8.8)	0.547
2 Married	46(90.2)	45(88.2)	91(89.2)	
4 Divorced	0(0)	1(2.0)	1(1.0)	
5 Separated	1(2.0)	0(0)	1(1.0)	
Current smoker				
None	51(100.0)	51(100.0)	102(100.0)	-
Alcohol consumption				
No	51(100.0)	50(98.0)	101(99.0)	0.315
Yes	0(0)	1(2.0)	1(1.0)	

Table 2
Gross examination of foetus by pregnancy outcome

Gross examination of the foetus	Pregnancy Outcome			
	Live	Stillbirth	Total	P- Value
	No%	No%	No%	
Sex of foetus				
Female	26(50.9)	22(43.1)	48(47.1)	0.713
Male	25(49.1)	29(56.9)	54(52.9)	
Condition of foetus				
Fresh	0(0)	12(23.5)	12(11.8)	<0.001
Live	51(100.0)	0(0)	51(50.0)	
Macerated	0(0)	39(76.5)	39(38.2)	
Foetus outcome				

Live	51(100.0)	0(0)	51(50.0)	<0.001
Still Birth	0(0)	51(100.0)	51(50.0)	
Gross foetal malformations	(spina bifida)			
No	50(98.0)	50(98.0)	100(98.0)	1.000
Yes	1(2.0)	1(2.0)	2(2.0)	

There were more babies who were macerated (38% of the total) in the stillbirth group compared to those who were fresh (11.8%) (Table 2).

Table 3
Examination of the umbilical cord by pregnancy outcome

Examination of the umbilical cord	Pregnancy Outcome Live No%	Stillbirth No%	Total No%	P-value
Attachment of cord to placenta				
Central insertion	47(92.2)	44(86.3)	91(89.2)	0.473
Marginal insertion	4(7.8)	6(11.8)	10(9.8)	0(0)
Velamentous insertion	1(2.0)	1(1.0)		
Presence of umbilical knots				
No	51(100.0)	50(98.0)	101(99.0)	0.315
Yes	0(0)	1(2.0)	1(1.0)	
No of umbilical cord veins				
1	51(100.0)	51(100.0)	102(100.0)	-
No of umbilical cord arteries				
2	51(100.0)	51(100.0)	102(100.0)	-

There were no significant differences in attachment of the cord between the two groups (Table 3).

Table 4
Gross and microscopic examination of the foetal membranes by pregnancy outcome

Gross and microscopic examination of foetal membranes	Pregnancy Outcome Live No%	Stillbirth No%	Total No%	P-value
Gross examination of membranes				
Presence of pus	0(0)	3(5.9)	3(2.9)	0.198
Meconium stained	10(19.6)	8(15.7)	18(17.6)	
Fresh	41(80.4)	40(78.4)	81(79.4)	
Microscopic examination of membranes				
Presence of amnion nodosum				
No	48(94.1)	51(100.0)	99(97.1)	0.079
Yes	3(5.9)	0(.0)	3(2.9)	
Presence of amniotic strands				
No	51(100.0)	51(100.0)	102(100.0)	-
Presence of histological chorioamnionitis				
No	36(70.6)	25(49.0)	61(59.8)	0.026
Yes	15(29.4)	26(51.0)	41(40.2)	

Histological chorioamnionitis was more marked in the stillbirth group compared to the live birth group. There was chorioamnionitis in 51% of the membranes in the stillbirth group compared to 29.4% in live birth group (p-value 0.026) (Table 4).

Table 5

Weight and diameter of the placenta by pregnancy outcome

Gross examination of placenta	Mean	Std. Deviation	Minimum	Maximum	p-values
Weight of placenta (gms)					
Live (N = 51)	486.27	119.114	180	750	0.001
Still Birth (N = 51)	390.90	155.600	100	700	
Total (N = 102)	438.59	145.966	100	750	
Diameter of placenta (cm)					
Live (N = 51)	14.31	3.530	8	30	0.004
Still Birth (N = 51)	12.41	2.968	8	20	
Total (N = 102)	13.36	3.383	8	30	

There was a statistically significant difference in the mean weight (p-value 0.001) and diameter (p-value 0.004) of the placenta in the live birth group compared to the stillbirth group (Table 5).

Table 6

Microscopic examination of placenta by pregnancy outcome

Microscopic examination of placenta	Pregnancy Outcome			
	Live No%	Stillbirth No%	Total No%	P-value
Presence of developmental abnormalities				
No	51(100.0)	51(100.0)	102(100.0)	-
Presence of lesions which reduce the mass of functioning villi				
No	50(98.0)	40(78.4)	90(88.2)	0.002
Yes	1(2.0)	11(21.6)	12(11.8)	
Presence of haematomas and thrombi				
No	47(92.2)	38(74.5)	85(83.3)	0.017
Yes	4(7.8%)	13(25.5)	17(16.7)	
Presence of villous abnormalities				
No	47(92.2)	25(49.0)	72(70.6)	<0.001
Yes	4(7.8)	26(51.0)	30(29.4)	
Presence of abnormalities of fetal stem arteries				
No	50(98.0)	49(96.1)	99(97.1)	0.558
Yes	1(2.0)	2(3.9)	3(2.9)	
Presence of other placental abnormalities				
No	46(90.2)	33(64.7)	79(77.5)	0.002
Yes	5(9.8)	18(35.3)	23(22.5)	

There was more pathology in the placenta from stillbirths compared to the live births (Table 6). Reduction in the mass of functioning villi was present in 11.8% of placenta in the stillbirth group compared to 2% in the live birth group (p-value 0.002).

In the stillbirth group, 16.7% of placenta had

haematomas and/or thrombi compared to 7.8% in the live birth group (p-value 0.017). There was significant presence of other placental abnormalities in the stillbirth group (22.5%) compared to the live birth group (9.8%) (p-value 0.002). These were mainly placental infarcts.

However, there were no significant differences between the two groups involving abnormalities of fetal stem arteries (p-value 0.558).

DISCUSSION

Histological changes were present significantly in the placentae from mothers who had IUFD compared with those who had live births.

In this study there was no significant presence of amnion nodosum in the foetal membranes. Studies elsewhere have shown that the presence of amnion nodosum dominates the morphological picture of the amniotic surface in most cases of oligohydramnios (18).

This study showed significant presence of histological chorioamnionitis among membranes from stillbirths. A majority of these were acute chorioamnionitis with abscesses which is suggestive of bacterial infection. Where the cause of stillbirth is known, 10-15% of the causes are associated with infections (3,9). Infection may cause stillbirth by several mechanisms, including direct infection to the foetus, placental damage and severe maternal illness (16).Inflammatory lesions occur 2.6 times more often in stillbirths (14).

This study did not show any significance in babies who had gross congenital anomalies. A number of life threatening congenital malformations can cause IUFD (17). Some of these would have required autopsy to discover. However, autopsies were not carried out in this study hence limiting interpretation on the prevalence.

Abnormal placental pathology in stillbirths range from 20.7% to 39.6% depending on the classification system used (11,14). There were significant differences in the placental pathology between the two groups. All the different aspects of abnormal placental pathology were significantly present in the IUFD group compared to the live birth group.The most common histological abnormalities are: infarction decreased villous vascularity, peri-villous fibrin deposition and leukocyte infiltration (18).Villous dysmaturity as well as villous and uteroplacental vascular pathology may result in chronic and acute placental insufficiency which represents the cause for intrauterine death (14,19).

In conclusion, this study revealed that abnormal placental pathology was significantly present in placentae from stillbirths. Therefore histological examination of the placenta in cases of IUFD is important since useful information can be obtained. The study also showed that histological examination of placenta may help in identifying some causes of stillbirths. This knowledge may lead to preventive measures which would lower perinatal mortality.

We recommend that all stillbirths should have their placentae examined histologically since this provides useful information as to the cause of death. More research on causes of IUFD in Kenya focusing on autopsies will help add more information.

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REFERENCES

1. Chalumeau, M., Bouvier-Colle, M. H., Breart, G., *et al*. Can clinical risk factors for late stillbirth in West Africa be detected during antenatal care or only during labour? *Intern. J. Epidemiol*. **31**: 661-668.
2. Parast, M. M., Crum, C. P. and Boyd, T. K. Placental histologic criteria for umbilical blood flow restriction in unexpected stillbirth. *Human Pathol*. **39**: 948-953.
3. M enezes, E. V., Yakoob, M. Y., Soomro, *et al*. Reducing stillbirths: prevention and management of medical disorders and infections during pregnancy. *BMC pregnancy and Childbirth*. *9 (suppl 1)*: S4.
4. Flenady, V., Frøen, J. F., Pinar, H., *et al*. Causes of death and associated conditions (Codac)-a utilitarian approach to the classification of perinatal deaths. *BMC Pregnancy and Childbirth*. **9**: 22.
5. Lawn, J. E., Yakoob, M. Y., Haws, R. A., Soomro, T., *et al*. 3.2 million stillbirths: epidemiology and overview of the evidence review. *BMC Pregnancy and Childbirth*. **9 (suppl 1)**:S2.
6. Marchetti, D., Belviso, M. and Fulcheri, E. A case of stillbirth: The importance of placental investigations in medico-legal practice. *Am. J. Forensic Med. Pathol*. **30**: 64–68.
7. Were, E. O. Stillbirths at EldoretDistrictHospital: A retrospective study. *East Afr. Med. J.* **71** : 607-610.
8. KenyattaNationalHospital, Department of Medical Records, 2009. Unpublished
9. Silver, R. M., Varner, M. W., Reddy, U. *et al*. Work-up of stillbirth: a review of the evidence. *Am. J. Obstet. Gynecol*. **196**: 433-444.
10. Maslow, A. D., Breen, T. W., Sarna, M. C., *et al*. Prevalence of coagulation abnormalities associated with intrauterine fetal death. *Can. J. Anaesth*. **43**: 1237-1243.
11. Vergani, P., Sabrina, C., Pozzi, E., *et al*. Identifying the causes of stillbirth: a comparison of four classification systems. *Am. J. Obstet. Gynecol*. **99**: 319.e1-319.e4.
12. Spector, J. M. and Daga, S. Preventing those so-called stillbirths. *Bulletin of WHO*. **86**: 315-316.

13. Headley, E., Gordon, A. and Jeffery, H. Reclassification of unexplained stillbirths using clinical practice guidelines. Australian and New Zealand *J. Obstet. and Gynaecol*. **49**: 285-289.
14. Horn, L. C., Langer, A., Stiehl, P., *et al*. Identification of the causes of intrauterine death during 310 consecutive autopsies. *European J. Obstetrics & Gynecol. and Reprod. Biol*. **13**: 134-138.
15. McClure, E. M. and Goldenberg, R. L. Infection and stillbirth. *Semin Fetal Neonatal Med*. **14**: 182-189.
16. F ox, H. and Wells, M. Haines and Taylor: Obstetrical and Gynaecological Pathology, 4th ed. (vol 2). Churchill Livingstone, 1995.
17. Heazell, A. E. P. and Martindale, E. A. Can postmortem examination of the placenta help determine the cause of stillbirth? *J. Obstetrics and Gynaecol.* **3**: 225–228.
18. Gagnon, R. Placental insufficiency and its consequences. *European J. Obstetrics & Gynecol. and Reprod. Biol.* **110**: S99-S107.
19. Korteweg, F. J., Gordijn, S. J., Timmer, A., *et al.* A placental cause of intrauterine fetal death depends on the perinatal mortality classification system used. *Placenta* 2008; **29**:71-80.