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ORIGINAL ARTICLE

OUTCOME OF PRETERM TWINS COMPARED TO PRETERM SINGLETON NEONATES: A MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY IN ETHIOPIA

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ABSTRACT

Background: *In recent decades there has been a major increase in multiple birth rates, and the rate of twining vary from 6-9 per thousand life births to 20 per thousand live births across different areas of the world. Many studies have demonstrated higher neonatal and perinatal mortality and morbidity rates in twin deliveries compared to singleton births. This study was aimed to compare the outcomes of preterm twins and preterm singletons.*

Methods: *A prospective, observational multicenter study was conducted from July 2016 to May 2018 in five tertiary hospitals in Ethiopia. All preterm, liveborn infants born at or transferred at less than 7 days of life to one of the study hospitals with an estimated gestational age below 37 weeks were included.*

Results: *A total of 3,703 preterm neonates admitted to participating neonatal intensive care units were included in the study, of which 1171(31.6%) were twins. After adjusting for birth weight and gestational age, the mortality rate for preterm singletons of 31.0% was higher than the mortality rate for preterm twins of 24.8%, which was statistically significant (p-value = 0.001), OR of 1.37 (95% CI: 1.15 to 1.64). The study also identified an inverse relationship between birth weight and gestational age, and mortality. Male singletons were more likely to die than male twins (440 (32.4%) vs. 141 (23.4%); AOR 1.56 (95% CI: 1.22, 1.99); p=0.001)*

Conclusion: *Our study showed that the mortality of a singleton preterm infant was significantly higher than the mortality of a preterm twin infant.*

Keywords: Preterm; twins; singleton; neonatal intensive care units; multi-center

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Introduction

Globally, in recent decades, there has been a significant increase in multiple birth rates. For example, the rate of twinning varies from 6-9 births per thousand live births in South and South-East Asia while it is above 20 per thousand live births in Central African countries (1,2,4). The amount and quality of research on twins vary greatly, with the majority of epidemiolog-ical evidence coming from high-income countries. Fewer reports originated in developing countries. (3) A large review has found high rates of multiple birth in African LMIC compared to Asia. The twinning champion was believed to be Nigeria with rates above 18 per 1000 livebirths but several sub-Saharan and Central-African countries have rates above 15 per 1000(4). According to a demographic and health survey conducted in Ethiopia, the rate of multiple births from 2008 -2013 was 11.1 per 1000 births (5).

Many studies have demonstrated higher neonatal and perinatal mortality and morbidity rates in term twin deliveries compared to singleton births (6, 7). However, these studies have focused mainly on term neonates (6, 7). Reasons for higher mortality risk for twins include the higher rate of antepartum complications associated with preterm birth, as well as intrauterine growth restriction and uteroplacental insufficiency (8, 9). Among twins, the second twin is generally considered at higher risk of severe morbidity and mortality because of obstetric complications that may occur following the delivery of the first twin. These complications may include placental separation, cord prolapse, uterine atony, long delivery interval and cervical spasm (10,11).

Twinning rate is increasing in developing countries, and its impact on mortality and morbidity patterns is significant. In low-resource countries such as Ethiopia, there is a paucity of reliable data for such analyses. To our knowledge, little is known about whether the outcome of preterm twin neonates is different than preterm singleton neonates. The current study aims at assessing the mortality outcome of twins compared to singletons as well as twin A with twin B. Our rationale to study on preterm is to see the outcome of preterm twins and preterm singleton in developing country where the health system is very weak to manage these neonates, to our knowledge most of the studies were conducted on term twins and term singleton(6,7), and handling preterm and twin babies in developing countries is a challenge.

Methods

Study design

A sub-analysis of a prospective multi-centre observational study conducted from July 2016 to May 2018 to assess outcomes of twins and singletons preterm within a broader study on causes of mortality in preterm neonates (12). Socio-demographic and clinical maternal and neonatal characteristics were recorded.

We compared maternal and neonatal demographics as well as morbidity patterns and survival at 28 days postnatal between twin and singletons. We also compared survival

between twin A (first born twin) and twin B (second born twin) and assessed early neonatal deaths (neonatal deaths in first week of life) and late neonatal deaths (neonatal deaths occurring after seven days postpartum and within 28 days of life).

Study settings

This multi-center study was conducted in five tertiary hospitals in Ethiopia with the intention to obtain a geographical representation across regions in the country. The centers were located in the northwest (University of Gondar Hospital), Southwest (Jimma University Hospital), and three hospitals within the Addis Ababa region (Ghandi Memorial Hospital, St. Paul's Hospital Millennium Medical College, and Tikur Anbessa Hospital). These centers were selected because of high annual case load. All centers are academic and referral hospitals and provide care to both inborn (babies born in the study health facilities) and out-born neonates (babies born outside of study health facilities) by pediatric residents, pediatricians and in 3 sites, neonatologists.

Study participants

All preterm, liveborn infants born at or transferred at less than 7 days of life to one of the study hospitals with an estimated gestational age below 37 weeks were approached for parental consent by the study staffs. Gestational age was determined according to hierarchical algorithm: (1) ultrasound before 28 weeks of gestation; (2) ultrasound at or after 28 weeks of gestation and agreement with a reliable last menstrual period or New Ballard Score; (3) reliable last menstrual period and the New Ballard Score (13) (4). If the discrepancy between last menstrual period and the New Ballard Score was greater than 2 weeks, the last menstrual period date was used; and (5) without reliable ultrasound and last menstrual period estimate, the New Ballard Score alone was used. Excluded were neonates who withdrew from the study and higher order multiples (see study flow diagram).

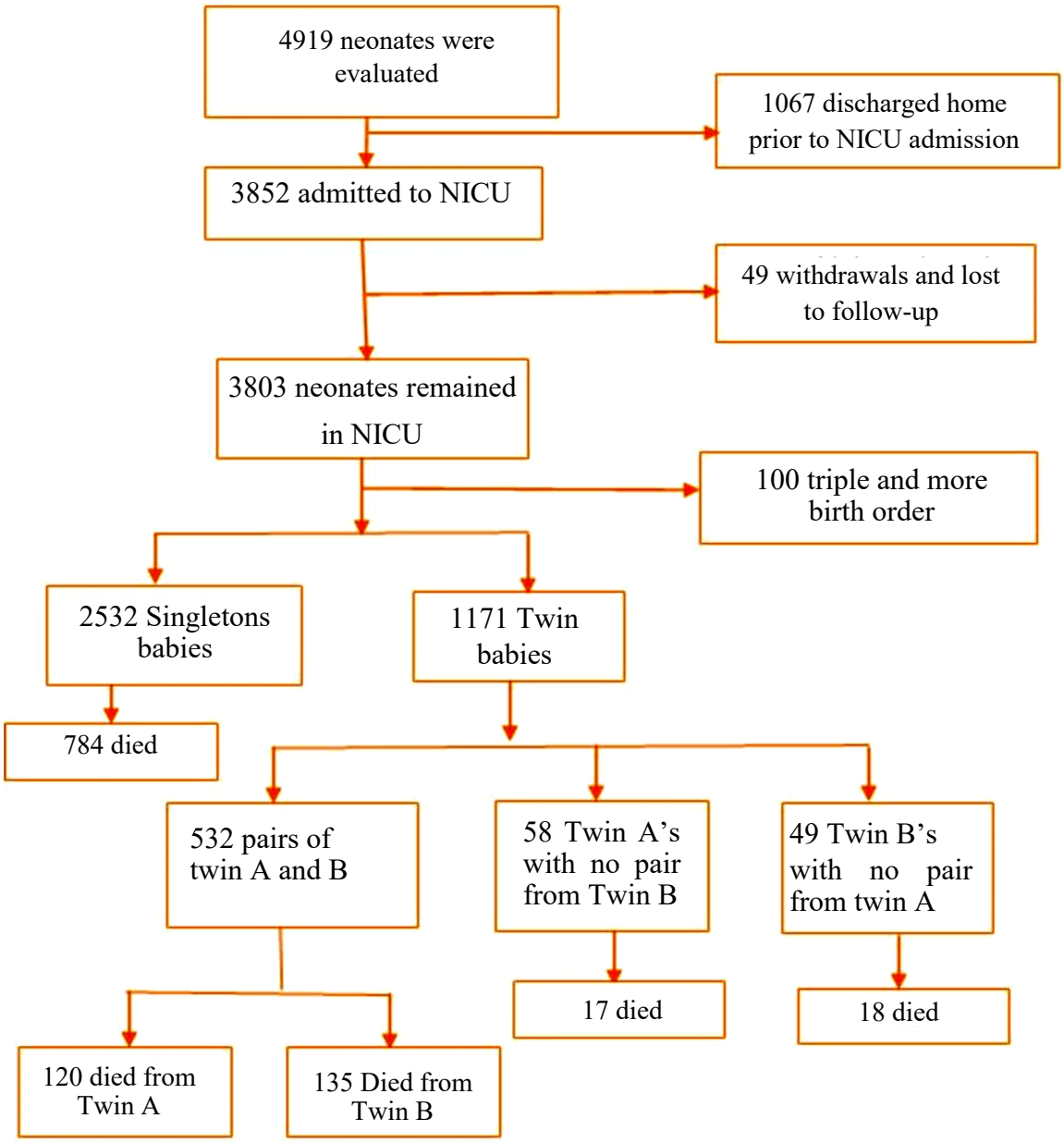

Figure 1, Study flow diagram; newborn intensive care unit

Data collection and analysis

Specific case report forms were filled for eligibility, socioeconomic status, maternal and obstetric history, and neonatal admission information including physical examination and laboratory findings, as well as admission and discharge diagnosis, and survival at 28 days. Imaging, laboratory results and microbiology of blood and cerebrospinal fluid specimens were limited, it was not done for all. We defined polycythemia when the venous hematocrit was greater than or equal to 65% and hypoglycemia when the blood sugar level was below 45 mg/dl (14). In the present study, radiologic examination including X-ray was performed inconsistently. However, for the diagnosis of RDS, clinical criteria such as rapid labored breathing, grunting, presence of

subcostal retraction, cyanosis, and decreased air entry in bilateral lung field plus or minus chest X-ray findings immediately or within a few hours of delivery were used. Neonatal sepsis was defined after assessing the risk factors for infection including maternal infection during labor, clinical signs and symptoms suggestive of infection, hematologic profile, and C-reactive protein.

Statistical assessment

The mean, median and standard deviations were analyzed, and descriptive statistics calculated with Stata version 14.2 (2017 package).

Patient and public Involvement

Informed and written consent was obtained from parents or caretakers prior to the infants' participation in the study. Consent information was available in English, Amharic or Oromifa languages, as appropriate.

Ethical approval and informed consent

The Institutional Review Board of Addis Ababa University, Jimma University and the University of Gondar approved the primary study protocol (IRB approval number: AAUMF 03-008). Informed, written consent was obtained from all participants' parents. Confidentiality of the information was guaranteed.

The study was supported by a grant from the Bill & Melinda Gates Foundation.

Data sharing

No additional data available.

Results

Of 4,919 preterm newborns screened during the study period, 3,852 were admitted and 1,067 were discharged home. As shown in figure 1, after exclusion of higher multiple babies (triplets, quadruplets) (2.6%) and some withdrawal (1.3%), a total of 3,703 preterm neonates were included for analysis

Demographics

Overall, 31.6% of admitted preterm neonates were twins. The male to female ratio was 1.15 and the singleton to twin birth ratio was 2.16. The overall mean birth weight was 1,728 grams for singletons and 1,701 grams for twins; 33.0% and 26.8% had a birth weight over 2000 gram, respectively. Table 1 details demographics and the main outcomes, early and late neonatal death. In terms of geographic differences in twining within various parts of Ethiopia, the University of Gondar had a higher rate with 314 (36.9%) twins compared to the rest of study sites, followed by Ghandi memorial hospital 128 (35.1%). The mean gestational age for both preterm singletons and preterm twins is 32.9 weeks, and the mean birth weight is 1728 gram for preterm singleton and 1701 gram for preterm twins. When we look at the gender 1357 (54.4%) of singleton are male and 602(51.9%) of twins are male. One thousand thirty-nine (41%) of preterm singleton and 541(46.2%) of preterm twins gestational age is 32-34 weeks. Early neonatal death was identified in 599(76.4%) of preterm singleton and 214(73.8%) of preterm twins.

Table 1. Neonatal demographics and outcomes of preterm singletons vs. twins admitted to study sites in Ethiopia from July 2016 to May 2018

	Singletons n=2,532 (%)	**Twins n=1,171 (%)**
Mean gestational Age	32.9	32.9
Mean birth Weight	1728	1701
Gender	2,496	1,160
Female	1139 (45.6)	558 (48.1)
Male	1357 (54.4)	602 (51.9)
Birth Weight (grams)	2,481	1,152
<1000	120 (4.8)	35 (3.0)
1000-1499	638 (25.7)	286 (24.8)
1500-1999	907 (36.6)	522 (45.3)
≥2000	816 (33.0)	309 (26.8)
Gestational age (weeks)		
<28	78 (3.1)	23 (2.0)
28-31	611 (24.1)	277 (23.7)
32-34	1039 (41.0)	541 (46.2)
35-37	804 (31.8)	330 (28.2)
Deaths	784/2,532 (31.0)	290/1,171 (24.8)
Early Neonatal death (<7days)	599 (76.4)	214 (73.8)
Late Neonatal death (7-28 days)	185 (23.6)	76 (26.2)

Maternal clinical characteristics related with neonatal mortality

Overall, there was no statistical difference between maternal characteristics of deceased singletons and twins. Most maternal morbidities such as hypertension and antepartum hemorrhage occurred more frequently among mothers of singletons, but none of these differences were statistically significant (Table 2). Antepartum hemorrhage is more seen in mother of singleton 297(35.7%) than twins 62 (29%) with AOR of 1.30 (95% CI: 0.66,2.57) and p value of 0.447. Eclampsia is more seen in mother of singleton 68(27.9%) than twins 16(6.3%) with AOR of 5.73 (95% CI: 0.48,8.12) and p value of 0.167. Cardiac disease is more seen in mother of singleton 34 (23.8%) than twins 8(12.5%) with AOR of 2.54 (95% CI: 0.14,5.16) and p value of 0.527. When we look at the mode of delivery C-section is more done for mothers of singleton 958 (31.1%) than twins 436(21.3%) with

AOR of 1.36 (95% CI: 0.99,1.85) and p value of 0.052. Mothers whose age is between 30-39 gave birth more to preterm singleton 636 (31.5%) than preterm twins 312(23.7%) with AOR of 1.37 (95% CI:0.96,1.95) and p value of 0.082.

Table 2. Comparison of maternal characteristics of deceased preterm singleton and twins admitted to study hospitals with adjusted odds ratio for birth weight and gestational age

	Total	Singleton	Twin		Adjusted for birthweight and gestational age		
		N (% died)	N (% died)	COR	AOR	95% CI	p-value
HIV*	95	69 (21.7%)	26 (30.8%)	0.63	0.58	0.18, 1.93	0.378
Hypertension	984	769 (32.9%)	215 (24.2%)	1.54	1.13	0.76, 1.68	0.534
Urinary tract infection	164	116 (36.2%)	48 (29.2%)	1.38	1.54	0.69, 3.44	0.292
Antepartum hemorrhage	359	297 (35.7%)	62 (29.0%)	1.36	1.30	0.66, 2.57	0.447
Preeclampsia	836	647 (33.1%)	189 (25.4)	1.45	1.07	0.71, 1.62	0.749
Eclampsia	84	68 (27.9%)	16 (6.3%)	5.76	5.73	0.48, 8.12	0.167
Cardiac disease	42	34 (23.5%)	8 (12.5%)	2.15	2.54	0.14, 5.16	0.527
Gestational diabetes	47	45 (24.4%)	2 (0.0%)	NA	NA	NA	NA
Mode of Delivery							
C-Section	1394	958 (31.1%)	436 (21.3%)	1.67	1.36	0.99, 1.85	0.052
Vaginal	2231	1523 (30.5%)	708 (27.3%)	1.17	1.53	1.89, 1.25	0.001
Maternal Age							
<20	269	213 (31.5%)	56 (39.3%)	0.71	0.67	0.34, 1.34	0.259
20-29	2431	1643 (30.7%)	788 (24.2%)	1.39	1.44	1.16, 1.80	0.001
30- 39	948	636 (31.5%)	312 (23.7%)	1.48	1.37	0.96, 1.95	0.082
≥40	41	32 (31.3%)	9 (22.2%)	1.59	1.87	0.26, 3.56	0.534

Clinical outcome and mortality of preterm singletons and twins

Overall, the most common discharge diagnosis was respiratory distress syndrome (RDS) but there was no statistical difference between singletons (1,163; 47.1%) and twins (511; 45.1%) with an AOR 1.07 (95% CI: 0.91 to 1.24); p=0.3.Polycythemia, hypoglycemia, hyperbilirubinemia, and hypothermia were significantly more often associated with twins, and congenital malformations and sepsis with singletons (table 3). Polycythemia is more seen in preterm twins 63(5.6%) than preterm singleton 86(3.5%) with AOR of 0.62 (95% CI:0.44,0.88); p=0.006. Hyperbilirubinemia is more seen in preterm twins 391(34.3%) than preterm singleton 740(29.8%) with AOR of 0.83 (95% CI:0.72,0.97); p=0.020. Hypoglycemia is more seen in preterm twins 348 (31.1%) than preterm singleton 550(22.5%) with AOR of 0.67 (95% CI:0.57,0.78); p=0.001. . Sepsis is more seen in preterm

singleton 943(39.7%) than preterm twins 404 (35.8%) with AOR of 1.21 (95% CI:1.04,1.40); p=0.012. Hypothermia is more seen in preterm twins 724(63.9%) than preterm singleton 1373(55.5%) with AOR of 0.71 (95% CI:0.61,0.82); p=0.001. Female death is more seen in preterm singleton 332 (29.2%) than preterm twins 145(26%) with AOR of 1.15 (95% CI:0.89,1.50); p=0.292.

Table 3. Morbidity and mortality between singleton and twin preterm neonates admitted to study sites.

	Single	Twin	Adjusted for gestational age and birth weight		
Variable	**n=2,532**	**n=1,171**	**AOR**	**95% CI**	**P-value**
Respiratory distress syndrome	1163 (47.1)	511 (45.1)	1.07	0.91, 1.24	0.431
Polycythemia	86 (3.5)	63 (5.6)	0.62	0.44, 0.88	0.006
Hyperbilirubinemia	740 (29.8)	391(34.3)	0.83	0.72, 0.97	0.020
Hypoglycemia	550 (22.5)	348 (31.1)	0.67	0.57, 0.78	0.001
Congenital malformation	99 (4.0)	9 (0.8)	5.09	2.56, 10.1	0.001
Sepsis	943 (39.7)	404 (35.8)	1.21	1.04, 1.40	0.012
Hypothermia	1373 (55.5)	724 (63.9)	0.71	0.61, 0.82	0.001
Male Death	440 (32.4)	141 (23.4)	1.56	1.22, 1.99	0.001
Female Death	332 (29.2)	145 (26.0)	1.15	0.89, 1.50	0.292
Overall Death	784 (31.0)	290 (24.8)	1.37	1.15, 1.64	0.001

After adjusting for birthweight and gestational age, the 7-day mortality in singletons (599; 23.7%) was significantly higher than in twins (214; 18.3%; p=0.001) with an AOR of 1.37 (95% CI: 1.15 to 1.64) and the 28-day mortality rate in singletons was higher than in twins (784; 31.0% vs 290; 24.8%; AOR 1.37 (95% CI: 1.15 to- 1.64). The majority of singletons 599 (76.4%) and twins 214 (73.8%) died within the first 7 days (Table 1).

For both twins and singletons, we confirmed the inverse relationship between birth weight and gestational age, and mortality (Table 3). Male singletons were more likely to die than male twins (440 (32.4%) vs. 141 (23.4%); AOR 1.56 (95% CI: 1.22, 1.99); p=0.001) (Table 4).

Eighty-seven percent of preterm twins and 85.9% of preterm singleton born at gestational age less than 28 weeks have died. When we look at the gender, 29.2% of female preterm singleton and 26% of preterm twins have died. Mode of delivery, 31.1% of preterm singletons born by cesarean section have died while 21.3% of preterm twin with the same mode of delivery have died.

Table 4. Mortality of preterm neonates admitted to study sites by demographics.

	Categories	Singleton		Twin	
		Total Birth	Percent Died	Total Birth	Percent Died
Gestational age (N=2,532)	<28	78	85.9	23	87.0
	28-31	611	57.9	277	50.2
	32-34	1039	23.4	541	17.7
	35-37	804	14.9	330	10.6
Infant gender (N=3,656)	Female	1139	29.2	558	26.0
	Male	1357	32.4	602	23.4
Birthweight (N=3,633)	<1000	120	82.5	35	82.9
	1000-1499	638	50.8	286	48.6
	1500-1999	907	23.9	522	17.4
	>=2000	816	15.7	309	8.1
Maternal age (N=3,689)	<20	213	31.5	56	39.3
	20-29	1643	30.7	788	24.2
	30-39	636	31.5	312	23.7
	>40	32	31.3	9	22.2
Mode of delivery (N=3,663)	Caesarean section	958	31.1	436	21.3
	Other	1551	30.7	718	27.0

Clinical differences between twin A (first born) and twin B (second born)

First-born twins (twin A) were slightly more often girls and had a higher birthweight. Their morbidity was generally lower than for the second twin (twin B) except for necrotizing enterocolitis, but this was not statistically significant. Twin A had significantly less asphyxia with organ failure with an COR 0.34 (95% CI: 0.16, 0.70), and also respiratory distress, anemia and polycythemia, but no difference in overall mortality was noted (table 5). When we look at the weight difference between twin A and B; 251(48%) of Twin A weigh 1500-1999 gram compared to twin B 231(44.1%) COR 1.17(95% CI:092,1.49). RDS is diagnosed more on twin B 237(46%) than twin B 222(43.1%) with COR of 0.89 (95% CI:0.70,1.14). Asphyxia with organ failure is diagnosed more twin B 29(5.7%) than twin A 10(2%) with COR of 0.34 (95% CI:0.16,0.70). Hypoglycemia is diagnosed more twin A 161(31.6%) than twin B 154 (30.3%) with COR of 1.06 (95% CI:0.81,1.38). Sepsis is diagnosed more twin B 189(36.9%) than twin A 180(35.1%) with COR of 0.92 (95% CI:0.71,1.19). Polycythemia is diagnosed more twin B 32(6.9%) than twin A 24(4.7%) with COR of 0.75 (95% CI:0.44,1.29). More twin B 135(25.4%) died than twin A 120(22.6%) with COR of 0.86 (95% CI:0.65,1.14).

Table 5. Clinical differences between twin A (first born) and twin B (second born) of preterm twin pairs admitted to study sites. Single twin admissions were omitted.

	Twin A N (%)	Twin B N (%)	COR	95% CI
Gender female	262/528 (49.6)	244/527 (46.3)	1.14	0.89, 1.45
Birthweight (grams)	N=524	N=524		
<1000	16 (3.1)	17 (3.2)	0.97	0.48, 1.94
1000-1499	115 (22.0)	142 (27.1)	0.76	0.57, 1.01
1500-1999	251 (48.0)	231 (44.1)	1.17	0.92, 1.49
≥2000	142 (27.1)	134 (25.6)	1.08	0.82, 1.42
Respiratory distress syndrome	222 (43.1)	237 (46.0)	0.89	0.70, 1.14
Asphyxia with organ failure	10 (2.0)	29 (5.7)	0.34	0.16, 0.70
Hypoglycemia	161 (31.6)	154(30.3)	1.06	0.81, 1.38
Sepsis	180 (35.1)	189 (36.9)	0.92	0.71, 1.19
Necrotizing enterocolitis	25 (4.9)	22 (4.3)	1.15	0.64, 2.07
Anemia	59 (11.5)	50 (9.7)	1.21	0.81, 1.80
Polycythemia	24 (4.7)	32 (6.2)	0.75	0.44, 1.29
Died	120 (22.6)	135 (25.4)	0.86	0.65, 1.14

Discussion

Worldwide the largest proportion of under five deaths are newborns of which prematurely born demand the highest death toll (22). Twinning is a frequent cause of premature delivery and although twins make for 3-15% of all newborns with large regional differences, they generally use a disproportionate high number of hospital beds compared to singletons. In our study more than 30% of admitted preterm neonates were twins (23).

Both preterm singleton and twin deliveries often require special care during pregnancy and after delivery. Comparing the mortality patterns between preterm twin neonates and preterm singleton neonates will have significant implications for allocation of healthcare resources, especially in developing countries. In this large multi-center study, although the mortality in preterm neonates was high, preterm singleton neonates were more likely to die than preterm twin neonates were.

In our study, while the mortality of both groups was very high, the mortality rate of singletons 784 (31.0%) was higher than that of twins 290 (24.8%). This finding was similar to the study done in Northern Belgium, in which twins compared to singletons had lower or comparable neonatal mortality rates (15). For example, in one study done in Korea, the neonatal mortality rate was lower in twin pregnancies than in singleton pregnancies for those with gestational ages > 29 weeks (16). Contrary to our findings, most studies have shown an

overall higher mortality rate among twins than singletons, but these were usually not restricted to preterm infants (17,18). We interpret these findings such that twins are more likely to be delivered preterm and since preterm infants have a higher mortality than term infants the mortality in twins if all births are considered will be higher. However, in our study, where only preterm infants were included, among those infants, the mortality was lower.

There are a number of potential explanations for the lower risk of mortality among preterm twins compared to preterm singletons. First, the increased mortality of preterm singletons could be explained by the belief that twin pregnancies are considered as high risk and might be monitored more closely than preterm singletons. Gestational age and birth weight affects the outcome of neonates and existing literature demonstrates that, in singletons, the highest growth peak occurs between 36 and 38 weeks, whereas in twins, this peak occurs between 32 and 36 weeks perhaps increasing the organ system maturation resulting in better adaptation and survival (19,20).

The cause of preterm in twins is less pathological than the cause of preterm in singletons', twins are programmed to be born preterm, not because of an underlying pathology but because of a limited uterine environment (19). Additional possible explanations could be that in preterm twins beyond 31 weeks' gestation, the lungs undergo an earlier in utero maturation than those of singleton fetuses. The difference in mortality could also be explained by increased rate of lung maturity (22). In our study, we also noted a higher rate of RDS in singletons than twins, with RDS also being the leading cause of mortality (23). The other possible reason could be increased rate of congenital malformations found in singletons than twins which could contribute to the mortality of singleton babies. Finally, it is possible that in this study sicker singleton infants were admitted to the NICUs since it is probable that the healthier singletons were discharged home while the healthy twins were more likely to be observed in the NICUs.

In the current study, we identified an inverse relationship between gestational age and birth weight with mortality in both preterm twins and preterm singletons; similar findings were reported from studies in Japan (24). The immaturity in several organ systems to support adaptation in extrauterine environment increases the risk of mortality in very preterm and low birth weight infants (25, 26).

In this study, we noticed more male singletons die than twins. Similar results were reported in a study done in Asia. The underlying mechanisms contributing to this are not well understood but some hypotheses have been proposed including genetic and endocrine differences. Currently, pulmonary disease and its complications remain predominant causes of early death, because of the inhibitory effects of androgens on lung development (28,29).

Our study was a large, prospective study, involving multiple centers in a low-resource setting with a high neonatal mortality (30,31).

The current finding will help to understand the increased risk of mortality found in preterm singletons compared to preterm twins. The finding of this study should be interpreted with caution because of limitations including the lack of data on zygosity and placental chorionicity.

Strengths of the study

Our study was a large, prospective study, involving multiple centers in a low-resource setting with a high neonatal mortality

Limitations of the study

Our study has one important limitation which is that not all of the study participants had ultrasound done during the first trimester of pregnancy to determine gestational age which is needed to validate that they are truly preterm infants. We used last menstrual period method or the New Ballard method to determine prematurity.

Conclusions

We conclude that there is a higher mortality among preterm singletons compared to preterm twins; additionally, gestational age and birth weight have an inverse relation with mortality in both preterm singletons and twins.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the research, authorship and/or publication of this article.

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Contributions of authors

AGD: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

ZTK: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

YHM: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

GMA: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

ZTB: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

AD: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and

agrees to be accountable for all aspects of work; insuring integrity and accuracy.

SA: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

NWG: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

EMM: contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted the manuscript critically revised the manuscript; gave final approval and agrees to be accountable for all aspects of work; insuring integrity and accuracy.

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