Research Article

Polyhydramnios-Maternal and Foetal Outcomes in Pregnancy

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ABSTRACT

Introduction: Excessive accumulation of liquor amnii causing discomfort to the patient and/or when an imaging help is needed to substantiate the clinical diagnosis of the lie and presentation of the foetus is referred to as polyhydramnios[1]. It develops as a consequence of disturbed equilibrium between production, foetal resorption, and secretion of amniotic fluid[2]. Polyhydramnios is associated with maternal Diabetes Mellitus, increased rate of Caesarean sections due to malpresentations, postpartum haemorrhage, congenital anomalies in new-born, preterm deliveries, increased risk of NICU admissions and still births[3]. Hence our study is prompted to find out maternal and perinatal outcomes in women with polyhydramnios in pregnancy and to improve the maternal and perinatal outcome in polyhydramnios cases. **Aims and Objectives**: To study the risk factors associated with polyhydramnios affecting polyhydramnios in pregnancies >28wks of gestational age, its clinical presentation, mode of delivery and feto-maternal outcomes in polyhydramnios in a tertiary care centre. **Methods**: It was a prospective observational study conducted for 2 years from November 2020 to October 2022 at tertiary care centre. The data retrieved from the medical records on polyhydramnios included demographic data, complications of pregnancy, and maternal and neonatal outcomes. Appropriate Statistical analysis was performed. **Results**: 100 cases of polyhydramnios cases were studied. Majority cases were idiopathic (57%) and had mild polyhydramnios (54%) with AFI of 25-27cm. Most significant risk factor was congenital anomalies (19%). Most common maternal complication was preterm delivery (24%). Other maternal complications were premature rupture of membranes (16%), malpresentations (12%), cord prolapse (4%), cord presentation (4%) and post-partum haemorrhage (2%). 58% cases were delivered by Caesarean section. 19% neonates had various congenital anomalies and 4% babies with major congenital anomalies died. 12% babies were still born due to various causes such as major congenital anomalies in 4 cases, birth asphyxia in 4 cases, abruptio placentae in 2 mothers and cord prolapse intrapartum in 2 cases. Neonatal death rate was 35.29. **Conclusion**: Polyhydramnios has high maternal and neonatal complications, especially congenital anomalies and preterm delivery that increases risk of significant neonatal complications and a higher rate of Caesarean sections.

Key Words: *Polyhydramnios, Amniotic fluid index (AFI), congenital anomalies,preterm delivery, perinatal outcome.*

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INTRODUCTION

Polyhydramniosis defined as deepest vertical pool (DP) more than or equal than 8cm or amniotic fluid index (AFI) of equal or more than 24cm or AFI above the 95th percentile for gestational age on ultrasonography. While clinical definition states – Excessive accumulation of liquor amnii causing discomfort to the patient and/or when an imaging help is needed to substantiate the clinical diagnosis of the lie and presentation of the fetus[1].Incidence of this is reported to be around 1 to 2 % of total pregnancies, but the incidence has been reported to range from as low as 0.2 to as high as 3.9%.

It develops as a consequence of disturbed equilibrium between production, fetal resorption, and secretion of amniotic fluid[2].

The amniotic fluid volume (AFI) assessment is an integral part of the antepartum fetal surveillance because of its abnormality is an indicator of poor perinatal outcome[4]. On sonography, Amniotic fluid index (AFI) is measured by the vertical pocket (free of any fetal part) in four quadrants of abdomen in a pregnant womanof whose normal value is taken as 5-24 cm[5].

Perinatal morbidity and mortality are significantly increased when polyhydramnios is present at delivery[6]such as premature delivery and congenital anomalies like open neural tube defects, upper GI obstruction or malformations etc.

and both immunologic and non-immunologic forms of hydrops fetalis [7-10]. Hence an abnormally high level of amniotic fluid, polyhydramnios, alerts the clinician to possible fetal anomalies.

Other causes responsible for increased morbidity are gestational diabetes mellitus, placental abnormalities e.g., chorioangioma which is a placental tumor, isoimmunization and multiple gestation[11].

Maternal medical complications such as malpresentations, accidental hemorrhage (abruptio placentae) and cardiorespiratory embarrassment are very well-known complications during pregnancy and cord prolapse, uterine inertia, retained placenta and PPH are expected complications during labour.Idiopathic polyhydramnios is present in the absence of any risk factorand it is usually mild and does not present until the mid-third trimester and does not typically require treatment[12]. There is a higher rate of dysfunctional labor in the presence of polyhydramnios.

There are good radiology facilities for timely and accurate diagnosis of polyhydramnios in our tertiary care centre and there are approximately 19,000 deliveries with a well- equipped labour room and NICU facilities available 24x7. Also, we encounter many patients with abnormal liquor and there are complications associated with polyhydramnios pregnancies. Hence this study was undertaken to assess the feto-maternal complications of polyhydramnios in this institution.

AIMS AND OBJECTIVES
1. To study the risk factors associated with polyhydramnios affecting polyhydramnios in pregnancies >28wks of gestational age
2. To study clinical presentation of polyhydramnios
3. To study the mode of delivery in polyhydramnios
4. To study feto-maternal outcomes in polyhydramnios

INCLUSION CRITERIA
1. All pregnant women >28wks having USG obstetric report showing AFI >25cm
2. All pregnant women >28wks clinically having polyhydramnios
3. Single or multiple pregnancy
4. Mother with any pre-existing medical history i.e., diabetes mellitus, gestational diabetes mellitus, Rh isoimmunization, hypertension.
5. Fetal malformations with polyhydramnios

EXCLUSION CRITERIA
1. 1 AFI <25cm
2. 2 Gestational age < 28 weeks
3. 3 Not willing to participate in study

METHODOLOGY
This study included prospective analysis of pregnancies with polyhydramnios at a gestational age of more than 28 weeks, at the Obstetric department of a tertiary care centre over a study period of 24 months. Pregnant women >28wks with USG Obstetric report showing AFI >25cm and clinically having polyhydramnios attending OPD/IPD of Tertiary care Hospital who fulfilled the inclusion criteria and exclusion criteria who were willing to participate in the study were included. Institutional ethical committee approval was taken for the present study. After approval from the Institutional Ethics Committee, a valid informed consent was taken. Once the cases were enrolled for the study, a thorough history and physical examination was done as per proforma and information was filled in the prescribed validated proforma.

All investigations were done: Hemoglobin, Blood group & Rh typing, Urine routine microscopy, TSH, GCT, HIV, HbsAg, HCV and latest obstetric USG for AFI and anomalies. Additional investigations were done for Diabetes Mellitus as it is associated in cases with raised GCT along with polyhydramnios. They were Fasting BSL, Post Prandial BSL, HBA1C (whenever possible) and accordingly the cases were treated with antidiabetic medications. Also, BP monitoring in cases of hypertension and management of the same were done.

Evaluation of fetal wellbeing was done with the help of NST (nonstress test) and USG based on severity.Incidence of premature rupture of membranes (PROM) and malpresentations were recorded. Theadministration of corticosteroid to the mother was done to enhance fetal lung maturity in cases of preterm delivery. Women not in labour and away from term were managed conservatively with rest, hydration, left lateral position, steroid cover and control of etiological factors was done if possible. Women in labour were watched for their progress of labour and mode of delivery i.e.,Vaginal or Caesarean section, perinatal outcome and maternal outcome were recorded. Caesarean Sections were done for Obstetric indications only. Maternal outcome in terms of PPH, preterm delivery, malpresentations, premature rupture of membranes, any accidental hemorrhage or cord prolapse. Perinatal outcome in terms of weight of baby, APGAR score, IUFD, stillbirths, NICU admissions and early neonatal deaths were also noted. All findings were noted in

Microsoft excel sheet and statistical analysis was done using descriptive statistics.

RESULTS

During the study period, after considering inclusion and exclusion criteria, 100 cases were eligible for the study.

Table No 1: Distribution according to Sociodemographic Variables

PARAMETERS AGE	Frequency N=100	Percentage (%)
18-20 years	17	17
21-25 years	57	57
26-30 years	18	18
31-35 years	2	2
>35 years	6	6
BOOKING STATUS		
Booked	52	52
Un-booked	48	48
SOCIOECONOMIC CLASS		
Upper (I)	0	0
Upper Middle (II)	5	5
Lower Middle (III)	22	22
Upper lower (IV)	60	60
Lower (V)	13	13
RESIDENCE		
Urban	65	65
Rural	35	35
EDUCATION		
Illiterate	3	3
Primary (upto 5th STD)	22	22
SSC (6th to 10th STD)	44	44
HSC (11th and 12th STD)	27	27
Graduation	4	4

Out of 100 cases, 57 (57%) belonged to 21-25 years of age group followed by 18 (18%) in 26 to 30 years of age group. 18-20 years age had 17 (17 %), more than 35 years were 6 (6%) and 2(2%) were in age group of 31-35 years. 52 (52%) cases were booked and 48 (48%) cases were un-booked. Out of 100 cases, maximum that is 60 % pregnant women belong to class IV (upper lower class) of Modified Kuppuswamy Classification for socioeconomic status and 22% are class III (Lower Middle), 13 % are in class V (Lower) and 5 are in class II (Upper Middle) and 0 % are in Class 5 (Upper).65 % pregnant women were residing in Urban area and 35 % pregnant women were in rural area.44 (44%) studied till SSC while 27 (27%) studied till HSC and 22 (22%) cases were educated up to primary level. 3% cases were illiterate. 4 (4%) cases were graduates but were nonworking.

Table No 2: Distribution according to gravidity, risk factors and severity

GRAVIDA (G)	Frequency N=100	Percentage (%)
G1	46	46
G2	41	41
G3	10	10
G4	3	3
>G5	0	0

RISK FACTORS		
Idiopathic	57	57
Rh isoimmunization	6	6
Gestational hypertension	8	8
Preeclampsia	2	2
GDM	6	6
Twin gestation	2	2
*Congenital anomalies	19	19

AMNIOTIC FLUID INDEX (AFI)		
25-27 cm (Mild)	54	54
28-31 cm (Moderate)	44	44
32-35 cm (Severe)	2	2

Out of 100 cases, 46 (46%) cases were primigravida, 41 (41%) cases were second gravida, 10 (10%) cases were third gravida and 3 (3%) cases were fourth gravida. Out of 100 cases, 57 (57%) were idiopathic, 19 (19%) had congenital anomalies, 8 (8%) cases had gestational hypertension, 6 (6%) had GDM and Rh isoimmunization, 2 (2%) had preeclampsia and twin gestation. *Congenital anomalies (19%) found in our study were esophageal atresia (3%), pulmonary atresia with pulmonary stenosis (1%), cleft lip with cleft palate (5%), congenital diaphragmatic hernia (2%), duodenal atresia (1%), neural tube defects like myelomeningocele, syringomyelia, holoprosencephaly (3%), renal tumor that is mesoblastic nephroma (1%), tortuous ductus arteriosus (1%), cerebral ventriculomegaly (1%), single umbilical artery (1%).Out of 100 cases, 54 (54%) cases were having mild polyhydramnios (AFI 25-27cm), 44 (44%) cases moderate polyhydramnios (28-31cm) followed by 2 (2%) cases having severe polyhydramnios (AFI 32-35cm).

Table No 3: Distribution according to weeks of gestation during admission and deliveries

	Gestational Age	Frequency N=100	Percentage (%)
Preterm admissions (<37weeks) 31%	28-34 weeks	16	16
	34.1-36.6 weeks	15	15
Term admissions (>37weeks) 69%	37-40 weeks	67	67
	40.1-41weeks	2	2
Preterm deliveries (<37weeks) 24%	28-34 weeks	8	8
	34.1-36.6 weeks	16	16
Term deliveries (>37weeks) 76%	37-40 weeks	74	74
	40.1-41weeks	2	2

Out of 100 cases, 67 (67%) were between 37-40 weeks of gestational age, 16 (16%) between 28 to 34 weeks, 15 (15%) were between 34.1 to 36.6 weeks followed by 2 (2%) were between 40.1 to 41 weeks. 74 (74%) cases delivered at 37-40 weeks, 16 (16%) delivered at 34.1-36.6 weeks, 8 (8%) delivered at 28-34 weeks followed by 2 (2%) cases at 40.1 to 41 weeks. 24 (24%) cases underwent induction of labour and the method of induction was Dinoprostone gel (PGE2) method for various indications. Out of 24 cases 14 % PROM, 2 % Severe preeclampsia, 8% Gestational hypertension at 39wks of gestational age were induced with Dinoprostone gel. Out of 100 cases, 42 (42%) delivered vaginally and 58 cases (58%) underwent Caesarean section.

Table No 4: Distribution according to indication of LSCS
Following cases were delivered by caesarean section (table 4).

Indications	Number	%
Previous LSCS with impending scar dehiscence	17	29.3
Fetal distress	11	18.9
CPD in labour	6	10.2
Primi breech in labour	6	10.2
Twin gestation with 1st twin breech	2	3.4
Cord presentation	4	6.8
Failure of induction	2	3.4
Previous LSCS with PROM	2	3.4
Transverse lie	2	3.4
Oblique lie	2	3.4
Cord Prolapse	2	3.4
Multi Breech with unfavorable cervix	2	3.4
Total	58	100

58 (58%) cases delivered by Caesarean section and 42 (42%) delivered by vaginal route. Out of 58 cases, 32.7% cases due to previous scar and 13.6% cases underwent LSCS due to malpresentations (table1). Fetal distress was seen in 18.9% while 3.4% did not progress.

Caesarean sections were done only for Obstetric indications.

Table No 5: Distribution according to Maternal Complications

Maternal complication*	Frequency N=100	Percentage (%)
INTRAPARTUM COMPLICATIONS		
Preterm labour/delivery	24	24
Premature Rupture of Membranes	16	16
Cord Presentation	4	4
Cord Prolapse	4	4
Meconium-stained liquor	11	11
Placental Abruption	2	2
Malpresentation	12	12
POST PARTUM COMPLICATIONS		
Post-partum Hemorrhage (PPH)	2	2

Maternal complications like preterm labour (24%), PROM (16%), malpresentation (12%), meconium-stained liquor (11%), cord presentation (4%), cord prolapse (4%), APH i.e., placental abruption (2%) was seen. 2% cases had atonic post-partum hemorrhage.

*(Note - One pregnant woman may have had more than one complication.)

Table No 6: Distribution according to perinatal outcome

PERINATAL OUTCOME	Frequency N=102	Percentage (%)
BIRTH WEIGHT		
1-2kg	22	21.56
2-2.499kg	32	31.37
2.5-2.999kg	40	39.21
3-3.499kg	6	5.88
3.5-4 kg	2	1.96
PERINATAL OUTCOME		
LIVE BIRTH	90	88.23
STILL BIRTH	12	11.76
OUTCOME OF NICU admitted babies	**Frequency of NICU admissions (n=17)**	**Percentage (%)**
Discharged within 7 days	3	17.64
Death within 7 days	6	35.29
Duration of NICU stay more than 7 days (for weight gain)	8	47.05
Total	17	100

40 (40%) babies were of 2.5 to 2.999 kg, 32 (32%) between 2-2.499 kg, 22 (22%) between 1-2 kg, 6(6%) were between 3-3.499kg and 2(2%) were of 3.5-4kg. Out of 102 babies, 90 (88.23%) were born alive and 12 (11.76%) were still born. Out of 90 live births, 17 (18.89%) required NICU admission. Out of 17 babies who required NICU admission, 10 (58.82%) required due to prematurity, 5 (29.4%) for RDS and 2 (11.7%) for birth asphyxia. Among 12 still born babies, 4 (33.33%) were having major congenital anomalies, 4(33.33%) babies had severe birth asphyxia, 2 (16.67%) had intrapartum cord prolapse and 2 (16.67%) had abruptio placentae in mother. Out of 6 neonatal deaths from NICU, 4 (66.66%) died because of respiratory distress syndrome, 2 (33.3%) due to severe sepsis.

DISCUSSION

Our study is a hospital based prospective, observational study conduction with 100 cases to evaluate clinical study of polyhydramnios after 28 weeks of pregnancies.

In our study 57% cases belonged to 21 to 25 years of age which was comparable with Reshita D et al[13] 2015 (90%) in age group of 21 to 30 yrs, Lalchan Set al[14] 2018 (65.8%) in age group 21-30years and Gurung SD et al[15](58.3%).In our study most common age group was 21 years to 25 years because in India women get married between 21 to 25 years of age group so maximum cases detected in this age group. 46% cases were primigravida which is comparable to the studies of Rajgire AA et al[16] (40.1%), Reshita et al[13](36.7%), Bakhsh et al[17] (24.9%) and Gurung SD et al[15] (20.8%). Hence overall in all studies, polyhydramnios is more commonly seen in multigravida cases.

Out of 100 cases, majority of the cases admitted (67%) were at 37-40 weeks of gestational age followed by 28-34 weeks (16%), 34.1-36.6 weeks (15%) and 40.1-41 weeks (2%). Rajgire AA et al[16](90% at 37-41 weeks), Reshita et al[13] (50% at 37-40weeks) and Gurung SD et al[15] (50% at 37-41weeks) noted similar observations in their studies. Gupta et al[18] study shows only 7.14% cases with 37-40wks of gestational age. The cases between 40-42 wks of pregnancy in all above studies were far less than those between 37-40 wks of gestational age. The reason being that amniotic fluid volume decreases as the gestational age advances and present study shows mild cases of polyhydramnios (AFI between 25-27cm) in greater number leading to decreasing trend of liquor in later gestational ages.

Out of 100 cases, 54 (54%) cases were having mild polyhydramnios (AFI 25-27cm), 44 (44%) cases moderate polyhydramnios (28-31cm) followed by 2 (2%) cases having severe polyhydramnios (AFI 32-35cm) This is concordant to the studies of Gurung SD et al[15] (87.5%), Malas NOM et al [19](84%), Lalchan et al[14] (71.1%) and Gupta et al[18](57.14%) where majority of cases were of mild polyhydramnios.

Out of 100 cases, 57 (57%) were idiopathic. This is concordant to the studies of Gurung et al[15] (70.9%), Rajgire AA et al[16] (41.6%), Bakhsh et al[17] (43.7%), Gupta et al[18] (54.28%), Malas et al[19] (67%) and Kaur EJ et al[20] (57%). 19% cases had congenital anomalies as a risk factor similarly observed in Kaur EJ et al[20](30%), Rajgire AA et al[16] (27%), Gupta et al[18] (24.27%) and Gurung et al[15](12.5%).

74 (74%) cases delivered at 37-40 weeks, 16 (16%) delivered at 34.1-36.6 weeks, 8 (8%) delivered at 28-34 weeks followed by 2 (2%) cases at 40.1 to 41 weeks. This is concordant to the studies of Kaur EJ et al[20] where 68% delivered at 37-40weeks, Bakhsh at al[17] where 86.1% delivered at 37-40 weeks, Gupta et al[18] (54.28%) at 37-41weeks and Gurung SD et al[15] (50%) delivered at 37-41weeks. Majority cases in our study were delivered at 37-40weeks of gestational age as polyhydramnios was mild and hence there was no sudden onset of labour or maternal respiratory distress so much that delivery immediately needed to be conducted.

LSCS was the mode of delivery in 58% cases in our study and it was comparable to the studies of Gurung SD et al[15](75%), Bakhsh et al[17](71.4%) and Kaur EJ et al[20](54%). In other studies route of delivery was vaginal in Punithavathi et al[21](72%) and Gupta et al [18] (68.6%). The high rate of Caesarean sections in present study was due to Obstetric indications of LSCS. Caesarean sections were not done for polyhydramnios per se, so the high rate of Caesarean sections was not attributed to polyhydramnios.

Preterm delivery was seen in 24% women, this was higher than reported byPunithavathi et al[21](14%), Gupta et al[18] (10%), Kaur EJ et al[20] (6%) and was much higher than what is reported by Rajgire et al[16] (5%). Malpresentations occurred in about 12% cases of polyhydramnios in our study which was comparable with Kaur EJ et al[20] (13%) and Rajgire et al[16] (15.17%). There were no complications in 49% cases which was comparable to Punithavathi et al[21] (56%), Gupta et al[18] (57.1%), Kaur EJ et al[20] (57 %) and Rajgire et al[16] (59.97 %) studies. In our study premature rupture of membranes occurred in 16% cases. A higher incidence of premature rupture of membranes 20% and14% was reported by Punithavathi et al[21] and Kaur EJ et al[20] respectively. Postpartum hemorrhage occurred in 2% of cases. Our observation was comparable with study done by Punithavathiet al[21] (4%), Gupta et al[18] (4.28%), Kaur EJ et al[20] (7%) and Rajgire et al[16](3.3%) studies.

Birth weight was between 2.5-2.999kg in 39.21% babies, 2-2.499kg in 31.37% babies, <2 kg in 21.56%, 3-3.499kg in 5.88% babies and 3.5-4kg in only 1.96% babies. Gupta et al[18] study shows that 71.42% babies were above 2.5kg but below 3.5kg, 21.43% were below 2.5kg and there were 7.14% babies whose birth weight was >4kg and it is similar to Bakhsh et al[17] study which shows 85.7% babies who were from 2.5kg to 3.5kg. There is no study similar to present study where all other studies Punithavathi et al [21] 38.46%, Reshita et al[13] 36.7% and Gurung SD et al[15]37.5% show highest birth weight in the range of 3-3.5kg while present study has highest birth weight in range of 2.5-3kg. In present study we observed that number of deliveries were highest at 37-40wks of gestational age. Also, the cases were from poor socioeconomic class and were nutritionally poor. Hence birth weight of 2.5-2.999kg was more commonly (39.21%) observed in present study.

Out of 102 babies (2 were twin gestation), 12 (11.76%) cases were still born. Similar findings were observed by Rajgire et al[16], Gupta et al[18], Kaur EJ et al[20] and Gurung SD et al [15]. Punithavathi et al[21] study showed that 28% neonates were still born. The causes of still birth are not known in the above studies.12 babies in present study had stillbirth as mother had developed abruptio placentae in 2 cases, 2 mothers had cord prolapse intrapartum (these delivered vaginally) and 4 babies had major congenital anomalies and 4 babies with IUGR with very low birth weight had still birth due to birth asphyxia in the gestational age between 28-34 weeks.1baby in the gestational age 34.1-37 weeks and 1 in 37-40 weeks had stillbirth as the mother developed abruptio placenta.Congenital anomalies where baby did not survive were Holoprosencephaly, major congenital anomalies (Oesophageal atresia with suspected ASD with gross ascites), pulmonary atresia with pulmonary stenosis and sudden death in GDM mother.As major congenital anomalies were observed in 4 cases, corrected still birth rate is 7.84%.in present study.

APGAR score at 5 minutes was <7 in 18.89% and >7 for 81.11% neonates respectively. Kaur EJ et al[20]and Punithavathi et al[21]noted similar observations in their study. However, Malas et al[20], Bakhsh et al[17] and Reshita et al[13]showed different results where APGAR score was more than 7 in all these studies. As preterm deliveries were higher in number that is 24%, more cases had poor APGAR score even though rate of Caesarean section was increased (58%) due to polyhydramnios in our study. Rest of the cases had congenital anomalies for which NICU admissions were required for >7 days (8 cases in our study).

Babies required NICU admission in 18.89% cases in present study which was comparable with Bakhsh et al[17] (35.7%), Punithavathi et al[21] (28%) and Kaur EJ et al[20] (21%) and was much lower in Reshita et al[13] (3.3%).

When neonatal outcomes were analyzed, respiratory distress syndrome (66.67%) was the most common cause for neonatal mortality followed by septicemia (33.33%).

CONCLUSION
Polyhydramnios is a high-risk pregnancy, associated with both maternal and perinatal morbidity and mortality. Majority of cases of polyhydramnios are idiopathic. However, congenital anomalies are the most common risk factor associated with polyhydramnios. Cases with polyhydramnios have malposition and malpresentations hence they add to higher rates of Caesarean sections. Inspite of thorough possible investigations, it is unfortunate that in large number of cases the cause for polyhydramnios is left unknown (idiopathic). We recommend study with large number of cases as we had studied on a limited number of cases.

ABBREVIATIONS: AFI-Amniotic Fluid Index, PROM- Premature Rupture of membranes, LSCS-Lower Segment Caesarean Section, NICU- Neonatal intensive care unit, GDM-Gestational Diabetes Mellitus, RDS- Respiratory Distress Syndrome, APH- Antepartum Hemorrhage, IUFD- Intrauterine Fetal Death, PPH- Post Partum Hemorrhage.

Authors' contributions:
Dr. ARK developed the study proposal, managed the research implementation, data collection, analyzed data and wrote the manuscript. Dr. VYK developed the study proposal, assisted with data analysis and reviewed the manuscript. Dr. BVK participated in development of the study proposal, participated in research team meetings to monitor study progress, reviewed preliminary results and reviewed the manuscript. Dr. SNG assisted with development of the study proposal, reviewed preliminary results and reviewed the final manuscript. All authors have read and approved the manuscript.

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