Original Article

Gender-Specific Reference Charts of Fetal Head Circumference in a Singaporean Population

George SH Yeo, [1]*MBBS, FRCOG, FAMS*, Maili Qi, [2]*MD, MRCOG, FACOG*, Ruochen Du, [1]*BSc (Hons)*, Padma Lata Mahavadi, [1]*BE, GDipSA*, Chee Fu Yung, [3]*MBChB, MSt (Camb), FFPH*, Koh Cheng Thoon, [3]*MBBS, MMed (Paeds), MRCPCH*, Edwin WH Thia, [1]*MBBS, MRCOG, MMed (O&G)*, Kai Lit Tan, [1]*MBBS, MRCOG, MMed (O&G)*, Fon Min Lai, [1]*FRCOG, FRANZCOG, MMed (O&G)*, Nicole KL Lee,[1]*PhD*

Abstract

Introduction: With the global outbreak of Zika virus and its association with microcephaly, an up-to-date fetal head circumference (HC) nomogram is crucial to offer a reference standard in order to make an accurate diagnosis. This study was conducted to revise the local fetal HC nomogram. **Materials and Methods:** In this retrospective study, ultrasound data was used for construction of the fetal HC nomogram from a total of 6155 pregnancies in the ethnic Chinese population with low risk profile at KK Women's and Children's Hospital over a 10-year period. Regression model was fitted to calculate the mean and standard deviation of HC at each gestational age (GA). Comparison of HC between ethnic groups (no significant differences) and genders were made. The revised chart was compared with another commonly used reference chart (Hadlock). In an independent test population, different reference charts were used to estimate number of cases with microcephaly. **Results:** A statistically significant difference of HC between the genders was observed across all gestational ages. Gender-specific reference charts and equation were computed. Our revised fetal HC chart showed a different distribution from the Hadlock chart. Compared with the gender-specific charts, the Hadlock HC chart would significantly under-report microcephaly cases in male fetuses, and tend to over-report in female fetuses. **Conclusion:** This study provides a new set of gender-specific fetal HC charts in the Singaporean population for antenatal ultrasound surveillance of microcephaly.

Ann Acad Med Singapore 2017;46:367-73

Key words: Microcephaly, Nomogram, Zika

Introduction

On 1 February 2016, the World Health Organization (WHO) declared the current Zika outbreak a Public Health Emergency of International Concern (PHEIC). On 31 March 2016, WHO announced that based on a growing body of research, there is scientific consensus that Zika virus is a cause of microcephaly and Guillain-Barre syndrome (GBS). More recently, the infection has also been associated with other clinical conditions and neuroimaging findings mainly relating to the central nervous system, including brain abnormalities, epilepsy, hearing and visual impairment, impairment of psychomotor development, and defects of the bones and joints. With a wide range of congenital abnormalities observed to be linked to Zika virus infection, WHO suggested the presence of new congenital syndrome and termed it Congenital Zika Syndrome.[1] Modelling analysis by Cauchemez et al and Johansson et al suggested that the estimated risk of microcephaly associated with maternal infection with the Zika virus is between 0.88% to 13.2%.[2,3] In Singapore, as of 13 November 2016, more than 400 cases of locally transmitted Zika have been confirmed, including a few who were pregnant women. To date, there is no published case of an affected fetus in Singapore.

Microcephaly is defined by WHO as an occipito-frontal head circumference (HC) ≥ 2 standard deviations below the mean for age and sex. Early diagnosis of microcephaly can be made by fetal ultrasound antenatally. WHO recommends an ultrasound of the fetus in the late second or early third trimester (preferably between 28 and 30 weeks) to identify fetal microcephaly and/or other brain abnormalities.[4] To diagnose microcephaly accurately, an appropriate reference standard of HC is of crucial importance. There being

[1]Department of Maternal Fetal Medicine, KK Women's and Children's Hospital, Singapore
[2]Department of Obstetrics and Gynaecology, KK Women's and Children's Hospital, Singapore
[3]Infectious Disease Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore
Address for Correspondence: Prof George Yeo Seow Heong, Department of Maternal Fetal Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899.
Email: dr.george.sh.yeo@gmail.com

numerous different international and local fetal HC reference charts in use in different ultrasound machines, the number of microcephaly cases detected could theoretically vary depending on the charts used. Over- and under-detection of microcephaly during antenatal screening have important implications for clinical and public health response in view of the proven link between Zika virus and microcephaly. The current Singapore HC chart was published more than 2 decades ago.[5]

The primary aim of this paper was to update our local fetal HC nomogram to offer a national reference standard. We also investigated possible gender-specific and ethnicity-specific fetal HC nomogram. The secondary aim was to compare the updated fetal HC nomogram with another published reference chart.

Materials and Methods

Ultrasound data of fetal HC from ethnic Chinese women seen at the KK Women's and Children's Hospital (KKWCH) between 1 January 2005 and 31 December 2015 was selected from an existing fetal ultrasound database. One data point of any subject's multiple scans across the whole range of gestation between 11^{+0} and 39^{+6} weeks was randomly selected and used in this project. All included subjects were spontaneous singleton pregnancies, had first-trimester dating scan based on crown-rump length (CRL) and had term live births between 37-42 weeks. The exclusion criteria included abnormal fetal karyotype, congenital malformations, and maternal diseases that would affect the growth of the fetus (pre-eclampsia/eclampsia, diabetes mellitus, renal disease, and etc.). When there were excessive numbers of cases in a particular gestation week, subjects were randomly selected to ensure similar distribution throughout gestations (280-400 cases/week).

HC was measured by trained sonographers as previously published.[6-8] An intra- and inter-operator reproducibility study was conducted to establish the intra- and inter-observer variance (using technical error of measurement, TEM) in this centre. In the first trimester, electronic linear callipers should be used to measure the fetus in a neutral position. The biparietal diameter (BPD) and HC were measured on the largest true symmetrical axial view of the fetal head. From the second trimester onwards, fetal head was measured at the axial plane at the level where the continuous midline echo is broken by the cavum septum pellucidum in the anterior third. At this level, the anterior horns, the thalamus and posterior horns with the choroid plexus were visible. BPD was then measured from the proximal echo of the fetal skull to the distal side of the border deep to the ultrasound beam (outer-to-outer). The occipital-frontal diameter (OFD) was measured in the same plane between the leading edge of the frontal bone and the outer border of the occiput. The HC was calculated from the BPD and OFD measurements using the following formula:

HC = (BPD + OFD) x 0.5 x 3.14

Statistical analyses were performed using the R software and the data were analysed as recommended.[9] In brief, polynomial regression model was fitted to the measurement of HC as a function of gestational age (GA). The selected model was chosen based on adjusted r^2 value. Since the residuals were also dependent on GA, a polynomial regression analysis was performed between the absolute residuals and GA. The fitted values of this regression model were multiplied by $\sqrt{(\pi/2)}$ (= 1.253), to give gestation-specific standard deviations. Centiles were calculated using the formula: centile = mean + K x SD, where K is ±1.88 for 3rd and 97th centiles.

The differences of HC between the genders and a gender-specific equation were tested by applying multivariate regression fitted to the HC measurements, with GA as numeral variable and sex as categorical explanatory variable. The gender-specific equation was then used to estimate the number of microcephaly cases in a test population that comprised of all pregnant women seen in KKWCH from January to September 2016, and compared to the above unisex equation.

Another separate sample population comprised of ethnic Chinese (n = 2198), Indian (n = 1923) and Malay (n = 2668) were acquired, using the same criteria as the study population, from women seen at our centre from 2011 to 2015. Using this sample population, we compared differences of HC between the 3 ethnic groups, by applying multivariate regression fitted to the HC measurements, with GA as numeral variable, and race as categorical explanatory variable.

Comparison was also made between our updated charts with another published reference chart (Hadlock et al[10]). The study was approved by SingHealth Centralised Institutional Review Board (CIRB) on 16 October 2015 with reference number of 2015/2613.

Results

A total of 6155 low-risk pregnancies in ethnic Chinese population were evaluated between 11^{+0} and 39^{+6} weeks of gestation. In the intra- and inter-observer variability study, we established that the intra- and inter-observer variance (TEM) in this centre were 4.85 mm and 6.95 mm, respectively, with an inter-class correlation efficient (ICC) of 0.997 and 0.989.

The raw data of HC was fitted to the GA in weeks satisfactorily with a cubic polynomial model (Fig. 1). The corresponding formula for the regression model is as follows (with GA in weeks):
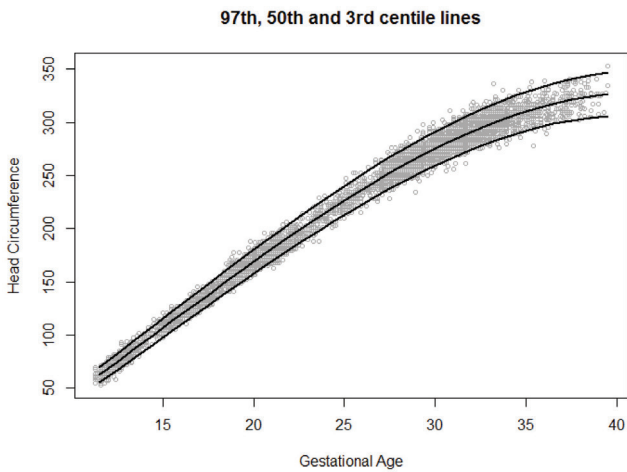
97th, 50th and 3rd centile lines

Fig. 1. The updated fetal head circumference (HC) by gestational age chart. The top line represents 97th percentile, middle line represents 50th percentile, and bottom line represents 3rd percentile.

$$Mean = -63.21 + 8.611*GA + 0.2072*GA^2 - 0.006036*GA^3$$

The absolute residuals for HC measurement across GA were fitted satisfactorily using a simple linear fit. The equation for the standard deviation (SD) is as follows (with GA in weeks):

$$SD = 0.988062 + 0.249153*GA$$

Table 1 shows the 3rd, 10th, 50th, 90th, and 97th percentile values and standard deviations as a function of GA for fetal HC.

Small but statistically significant differences in HC across all GAs (11^{+0} to 39^{+6} weeks) were observed between the genders. The HC of male fetuses is consistently larger than females, and the difference increases with GA. A gender-specific equation was computed as below,

$$Mean = -63.16 + 8.528*GA + 0.2711*GA^2 - 0.006051*GA^3 + 0.1309*sex*GA$$

Table 1. Revised Fetal Head Circumference (HC) Percentile Values by Gestational Age

Gestational Age (Week)	Head Circumference, mm					Standard Deviation
	3rd Centile	10th Centile	50th Centile	90th Centile	97th Centile	
$11^{+0} - 11^{+6}$	55.12	57.43	62.37	67.31	69.62	3.85
$12^{+0} - 12^{+6}$	67.14	69.60	74.86	80.11	82.57	4.10
$13^{+0} - 13^{+6}$	79.25	81.85	87.43	93.01	95.62	4.35
$14^{+0} - 14^{+6}$	91.40	94.16	100.06	105.95	108.71	4.60
$15^{+0} - 15^{+6}$	103.58	106.48	112.70	118.91	121.82	4.85
$16^{+0} - 16^{+6}$	115.73	118.78	125.32	131.85	134.91	5.10
$17^{+0} - 17^{+6}$	127.82	131.03	137.88	144.74	147.94	5.35
$18^{+0} - 18^{+6}$	139.82	143.18	150.35	157.53	160.88	5.60
$19^{+0} - 19^{+6}$	151.70	155.20	162.69	170.18	173.69	5.85
$20^{+0} - 20^{+6}$	163.40	167.05	174.87	182.68	186.33	6.10
$21^{+0} - 21^{+6}$	174.91	178.71	186.84	194.97	198.77	6.34
$22^{+0} - 22^{+6}$	186.17	190.12	198.57	207.02	210.97	6.59
$23^{+0} - 23^{+6}$	197.16	201.26	210.03	218.80	222.90	6.84
$24^{+0} - 24^{+6}$	207.84	212.09	221.18	230.27	234.52	7.09
$25^{+0} - 25^{+6}$	218.18	222.57	231.98	241.39	245.79	7.34
$26^{+0} - 26^{+6}$	228.13	232.67	242.40	252.13	256.68	7.59
$27^{+0} - 27^{+6}$	237.66	242.35	252.40	262.45	267.15	7.84
$28^{+0} - 28^{+6}$	246.73	251.58	261.95	272.31	277.16	8.09
$29^{+0} - 29^{+6}$	255.32	260.31	271.00	281.68	286.68	8.34
$30^{+0} - 30^{+6}$	263.37	268.52	279.52	290.53	295.67	8.59
$31^{+0} - 31^{+6}$	270.86	276.16	287.48	298.81	304.10	8.84
$32^{+0} - 32^{+6}$	277.75	283.20	294.84	306.49	311.93	9.09
$33^{+0} - 33^{+6}$	284.01	289.60	301.56	313.53	319.12	9.33
$34^{+0} - 34^{+6}$	289.59	295.33	307.62	319.90	325.64	9.58
$35^{+0} - 35^{+6}$	294.46	300.35	312.96	325.56	331.45	9.83
$36^{+0} - 36^{+6}$	298.59	304.63	317.55	330.47	336.51	10.08
$37^{+0} - 37^{+6}$	301.94	308.13	321.37	334.61	340.80	10.33
$38^{+0} - 38^{+6}$	304.46	310.80	324.36	337.92	344.26	10.58
$39^{+0} - 39^{+6}$	306.14	312.63	326.51	340.38	346.87	10.83

Table 2. Fetal Head Circumference Percentiles by Gestational Age and Gender

Gestational Age (Week)	Male (mm)			Female (mm)		
	3rd Centile	50th Centile	97th Centile	3rd Centile	50th Centile	97th Centile
11+0 – 11+6	55.93	63.07	70.20	54.43	61.56	68.70
12+0 – 12+6	68.03	75.62	83.21	66.39	73.98	81.57
13+0 – 13+6	80.21	88.26	96.30	78.45	86.49	94.53
14+0 – 14+6	92.45	100.95	109.44	90.55	99.05	107.54
15+0 – 15+6	104.70	113.65	122.60	102.67	111.62	120.57
16+0 – 16+6	116.94	126.34	135.74	114.78	124.18	133.58
17+0 – 17+6	129.11	138.97	148.82	126.82	136.67	146.53
18+0 – 18+6	141.19	151.50	161.81	138.77	149.08	159.39
19+0 – 19+6	153.15	163.91	174.67	150.59	161.35	172.12
20+0 – 20+6	164.93	176.15	187.36	162.25	173.46	184.68
21+0 – 21+6	176.52	188.19	199.85	173.70	185.37	197.04
22+0 – 22+6	187.86	199.98	212.11	184.92	197.04	209.16
23+0 – 23+6	198.94	211.51	224.08	195.86	208.43	221.01
24+0 – 24+6	209.70	222.72	235.75	206.49	219.52	232.54
25+0 – 25+6	220.11	233.59	247.07	216.77	230.25	243.73
26+0 – 26+6	230.14	244.07	258.01	226.67	240.61	254.54
27+0 – 27+6	239.75	254.14	268.52	236.15	250.54	264.92
28+0 – 28+6	248.90	263.74	278.58	245.17	260.01	274.85
29+0 – 29+6	257.57	272.86	288.15	253.70	269.00	284.29
30+0 – 30+6	265.70	281.44	297.19	261.71	277.45	293.20
31+0 – 31+6	273.27	289.47	305.66	269.14	285.34	301.54
32+0 – 32+6	280.23	296.88	313.54	275.98	292.63	309.28
33+0 – 33+6	286.56	303.67	320.77	282.18	299.28	316.39
34+0 – 34+6	292.21	309.77	327.33	287.70	305.26	322.82
35+0 – 35+6	297.16	315.17	333.18	292.51	310.52	328.53
36+0 – 36+6	301.36	319.82	338.28	296.58	315.04	333.51
37+0 – 37+6	304.77	323.69	342.61	299.86	318.78	337.70
38+0 – 38+6	307.36	326.74	346.11	302.32	321.70	341.07
39+0 – 39+6	309.10	328.93	348.75	303.93	323.76	343.58

$$SD = 1.023236 + 0.240938 * GA$$

Where sex = 1 for males and sex = 0 for females

Table 2 shows the 3rd, 50th, and 97th percentile values and standard deviations by GA and gender for fetal HC. Figures 2 and 3 show fetal HC charts with mean ± 2SD and ± 3SD for male and female fetuses, respectively.

To better understand the implication of gender-specific nomograms, we compared the numbers of microcephaly (below 2SD of mean) detected by using our revised unisex and gender-specific equations in the test population. Only ultrasound data from 20+0 to 39+6 weeks of gestation was studied. As shown in Table 3, using unisex chart would report significantly fewer cases as microcephaly in male fetuses than male-specific nomogram (P <0.001). Although

not statistically significant, there is a trend that the unisex chart would define more microcephaly cases in females; the percentage of microcephaly in females is 4 times of that in males. Whereas, if using a gender-specific nomogram, the percentage of microcephaly remains similar in both male and female population.

When comparing the differences in HC measurements among the ethnic Chinese, Malay and Indian groups in the sample population, no statistical significances were observed among the 3 races (Chinese versus Malay, P = 0.125; Chinese versus Indian, P = 0.122).

Figure 4 compares our gender-specific HC charts with another commonly used chart – Hadlock et al,[10] and shows different distribution of fetal HC values, particularly at mean and +2SD.
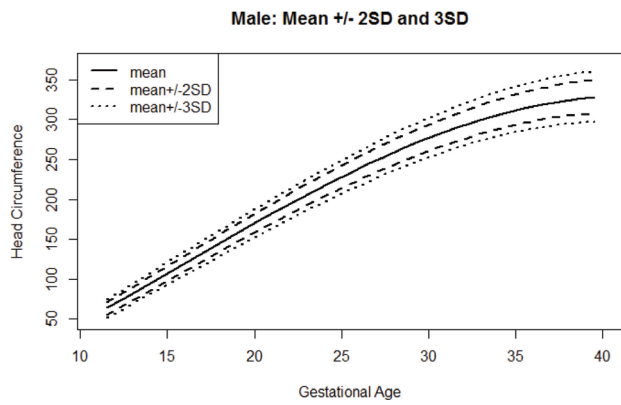
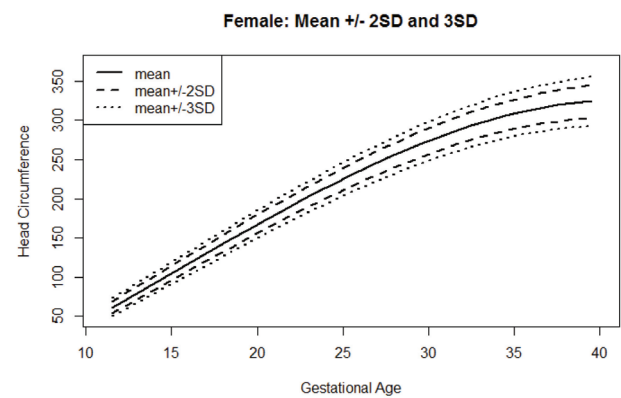Fig. 2. Fetal head circumference (HC) of male fetuses with mean ± 2SD and ± 3SD.

Fig. 3. Fetal head circumference (HC) of female fetuses with mean ± 2SD and ± 3SD.

Potential numbers of fetal microcephaly cases were estimated using Hadlock chart compared to our gender-specific charts. Using Hadlock's chart would report significantly fewer microcephaly cases in male fetuses (*P* <0.01). There is no statistical difference in female fetuses, but Hadlock tends to report more female fetuses as microcephaly (Table 3).

Discussion

Using data from the current sample of 6155 Chinese women locally, we have revised the existing fetal HC nomogram and equation.

A statistically significant and GA-dependent difference of HC was observed between the genders, with male fetal HC larger than females. Gender-related difference in fetal biometry has been previously reported.[11-15] In a large population, a small shift in the GA distribution might significantly affect rates of prematurity, intrauterine growth restriction and postdatism.

As the majority of people infected with Zika have no symptoms, infection during pregnancy may only manifest as fetal abnormalities, notably microcephaly. While universal testing is not recommended, antenatal HC monitoring is potentially an important part of surveillance in pregnancy in a Zika active area.[16] The use of unisex growth charts may make a pathologically small HC less obvious in a male fetus, potentially increasing the rate of false-negative diagnoses; vice versa, this may increase the false-positive of microcephaly in female fetuses, as shown in Table 3. Hence, in line with WHO definition of microcephaly by age and sex,[4] we produce a set of gender-specific HC nomograms with ±2SD and ±3SD for easy reference for prenatal surveillance of microcephaly.

As shown in our previous study,[6] there was no difference in HC measurements among Chinese, Indian and Malay. As Chinese is the largest component of the population in Singapore and allows completion of a large sample collection in a most reasonable time frame, in the current study, data from Chinese population was used to construct the reference chart. Again, comparison was made among the 3 ethnic groups in this study and showed no statistically significant difference in the mean values of fetal HC across all GAs.
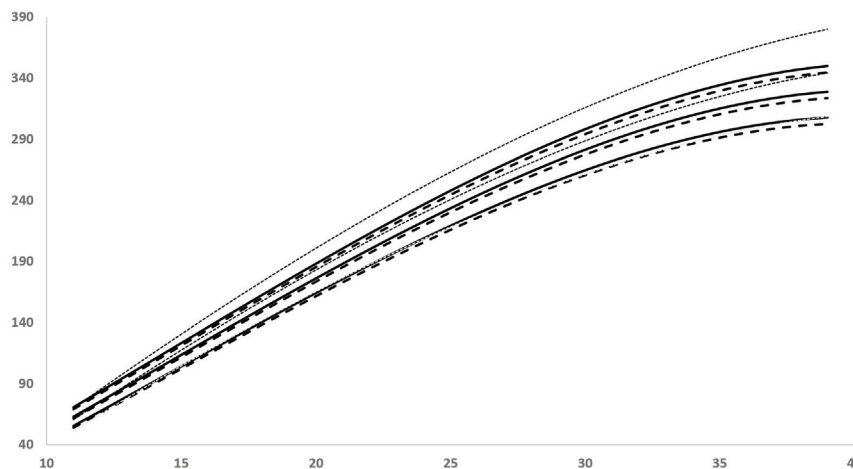

Fig. 4. Comparison of the revised gender-specific fetal head circumference charts (male – solid line, female – broken line) with Hadlock chart (dotted line), mean ± 2SD.

Table 3. Estimation of Microcephaly Using Gender-Specific, Unisex and Hadlock Equations

	Male Population			Female Population		
	Male-Specific Nomogram	Unisex Nomogram	Hadlock	Female-Specific Nomogram	Unisex Nomogram	Hadlock
Total number		1028			927	
Microcephaly	28	11	10	29	41	40
Percentage (%)	2.72	1.07	0.97	3.13	4.42	4.31
P value		0.00969	0.005376		0.1801	0.2199

Thus, the findings support the use of 1 set of gender-specific HC nomograms for all 3 races.

Comparison to Hadlock chart, a widely used formula which is incorporated in many ultrasound machines, was made with our new reference chart. In this study, we found that the present cohort of Singaporean fetuses had different HC measurements compared to the Hadlock's group. The population studied by Hadlock only consisted of Caucasian women from the Houston, Texas area. Geographical, ethnical and socioeconomic diversities may contribute to the difference. Of note, different techniques of HC measurement were used for the 2 studies. In Hadlock's study, HC was measured directly by using a hand-held map measurer or an electronic digitiser;[10] whereas, in the current study, HC were calculated from BPD and OFD. Although these methods have been shown to give equivalent results,[17] to establish a reference standard, a measuring method that is more compatible with current practice should be used. When we tested these 2 equations in the test population, significantly fewer male cases were defined microcephaly using Hadlock chart (P <0.01). This would have an impact on the incidence of microcephaly with the advent of Zika endemic. Therefore, we propose the use of this revised nomogram, which better fits the setting of our local requirement.

A key strength of our analysis was the large sample size which ensured greater precision was achieved when estimating centiles, especially the extreme ends.[8] In addition, meticulous standardisation and ongoing auditing of adherence to ultrasound measurement protocols have been in place since 1994[6,18-20] to ensure consistency and to minimise intra- and inter-observer variability. Our intra- and inter-observer study has shown excellent reliability of sonographers. A limitation of the study is its retrospective design with potential uncontrolled confounders. However, these data have been prospectively collected to build the database over the 10-year study period, and there is consistent use of the same standard of ultrasound practice in the same hospital where these data are acquired under the direction of the same maternal-fetal medicine sonologist.

Conclusion

There are statistical differences in female and male HC measurements throughout all GAs; with the ongoing outbreak of Zika virus infection, we recommend the use of 1 gender-specific nomogram for all ethnic groups in the Singaporean population for antenatal ultrasound surveillance of microcephaly.

REFERENCES

1. Costello A, Dua T, Duran P, Gülmezoglu M, Oladapo OT, Perea W, et al. Defining the syndrome associated with congenital Zika virus infection. Bulletin of the World Health Organization 2016;94:406-406A. Available at: http://dx.doi.org/10.2471/BLT.16.176990. Accessed on 13 November 2016.

2. Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al. Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. Lancet 2016;387: 2125-32.

3. Johansson MA, Mier-Y-Teran-Romero L, Reefhuis J, Gilboa SM, Hills SL. Zika and the risk of microcephaly. N Engl J Med 2016;375:1-4.

4. WHO. Fact sheet – Microcephaly (updated 2 March 2016). Available at: http://www.who.int/mediacentre/factsheets/microcephaly/en/. Accessed on 13 November 2016.

5. Lai FM, Yeo GS. Reference charts of fetal biometry in Asians. SMJ 1995;36:628-36.

6. Yeo GS, Chan WB, Lun KC, Lai FM. Racial differences in fetal morphometry in Singapore. Ann Acad Med Singapore 1994;23:371-6.

7. Papageorghiou AT, Thilaganathan B, Bilardo CM, Ngu A, Malinger G, Herrera M, et al. ISUOG Interim Guidance on ultrasound for Zika virus infection in pregnancy: information for healthcare professionals. Ultrasound Obstet Gynecol 2016;47:530-2.

8. Napolitano R, Donadono V, Ohuma EO, Knight CL, Wanyonyi SZ, Kemp B, et al. Scientific basis for the standardization of fetal head measurements by ultrasound: a reproducibility study. Ultrasound Obstet Gynecol 2016;48:80-5.

9. Altman DG, Chitty LS. Charts of fetal size: 1. Methodology. Br J Obstet Gynaecol 1994;101:29-34.

10. Hadlock FP, Deter RL, Harrist RB, Park SK. Estimating fetal age: computer-assisted analysis of multiple fetal growth parameters. Radiology 1984;152:497-501.

11. Wald N, Cuckle H, Nanchahal K, Turnbull AC. Sex differences in fetal size early in pregnancy. Br Med J (Clin Res Ed) 1986;292:137.

12. Parker AJ, Davies P, Mayho AM, Newton JR. The ultrasound estimation of sex-related variations of intrauterine growth. Am J Obstet Gynecol 1984;149:665-9.

13. Bromley B, Frigoletto FD Jr, Harlow BL, Evans JK, Benacerraf BR. Biometric measurements in fetuses of different race and gender. Ultrasound Obstet Gynecol 1993;3:395-402.

14. Schwarzler P, Bland JM, Holden D, Campbell S, Ville Y. Sex-specific antenatal reference growth charts for uncomplicated singleton pregnancies at 15-40 weeks of gestation. Ultrasound Obstet Gynecol 2004;23:23-9.

15. Melamed N, Meizner I, Mashiach R, Wiznitzer A, Glezerman M, Yogev Y. Fetal sex and intrauterine growth patterns. J Ultrasound Med 2013;32:35-43.

16. World Health Organisation, Interim Guidance update. Pregnancy management in the context of Zika virus infection. Accessed on 13 May 2016. Available at: http://apps.who.int/iris/bitstream/10665/204520/1/WHO_ZIKV_MOC_16.2_eng.p df?ua=1. Accessed on 13 November 2016.

17. Hadlock FP, Kent WR, Loyd JL, Harrist RB, Deter RL, Park SK. An evaluation of two methods for measuring fetal head and body circumferences. J Ultrasound Med 1982;1:359-60.

18. Yeo GS, Lai FM, Wei X, Lata P, Tan DT, Yong MH, et al. Validation of first trimester screening for trisomy 21 in Singapore with reference to performance of nasal bone. Fetal Diagn Ther 2012;32:166-70.

19. Lai FM, Yeo GS. Down syndrome screening in Singapore--the effectiveness of a second trimester serum screening policy modelled on 29,360 pregnancies in KK Women's and Children's Hospital. Singapore Med J 1998;39:69-75.

20. Thia EW, Wei X, Tan DT, Lai XH, Zhang XJ, Oo SY, et al. Evaluation of an objective method of image assessment for first-trimester nasal bone. Ultrasound Obstet Gynecol 2011;38:533-7.