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Original Article

Genetic Association Analysis of Dopamine DRD3 Ser9Gly Polymorphism and Schizophrenia in Malay Population

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Abstract

Background: Molecular components of the dopamine receptor (DRD3) play an important role in the pathophysiology of schizophrenia (SCZ). Previous studies have demonstrated an association between the DRD3 Ser9Gly polymorphism and SCZ but the results have been inconclusive.

Method: In this study, we investigated this controversial association between the Ser9Gly (A/G) polymorphism and SCZ using Malay cases-control (261 cases/157 controls) samples. PCR-RFLP was performed to genotype the distribution of the DRD3 Ser9Gly polymorphism.

Results: Both healthy control and SCHZ patient groups were in of Hardy-Weinberg equilibrium for the analyzed genetic variability. There was a significant association between the genotype distribution DRD3 polymorphisms and SCZ (χ^2= 9.359; df = 2; P = 0.009).

Conclusion: We believe that further studies are required to examine the association between others dopamine-related genes and the behavioral phenotypes of SCZ.

Keywords: *Schizophrenia, Dopamine receptor, Single nucleotide polymorphism, Restriction fragment length polymorphism*

Introduction

Schizophrenia (SCZ) is common but complex disease with worldwide lifetime risk of 1% (1). The Burden of Disease Study in 2004 showed that mental disorders contributed 8.6% of total disability-adjusted life year (DALY), ranking as the 4th leading cause of disease burden in Malaysia (2). From 2003 to 2005, a total number of 7351 cases of SCZ have been registered in Malaysia. The registered cases increased about 216 cases, from 2292 cases to 2508 cases between these 3 yrs. With the Malaysian population of 25 million, these numbers represent a very small proportion of the population, indicating that under-reporting cases may occur especially in East Malaysia (2).

Dysregulation in the dopaminergic system has been suggested to play an important role in the pathophysiology of SCZ (3). The dopamine D3 receptor (DRD3) is localized to limbic areas of the brain and involved in the reinforcing effects of emotional, cognitive and endocrine functions. It is thought to be implicated in SCZ and other neuropsychiatric disorders (4). It is well known that DRD3 may mediate the therapeutic actions of antipsychotic drugs (5).

The DRD3 gene maps to chromosome 3q13.3 (6) and there is a polymorphic site in its first exon that leads to a serine to glycine amino acid substitution at position 9 (Ser9Gly) in the extracellular N-terminal domain of the receptor (7). Several case- control studies between the DRD3 Ser9Gly polymorphism and SCZ have been conducted and these studies have yielded variable results. Some studies suggested a significant association between the

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DRD3 Ser9Gly polymorphism and SCZ (8-11), while others did not find any evidence of either excess of homozygosity, allelic or genotypic association (12-14). Even several meta-analyses reported that the DRD3 Ser9 Gly polymorphism might not confer susceptibility to SCZ (3, 15-16). With the increased of variable results obtained from studies in both European and Asian populations, there is a need to examine the association between the DRD3 Ser9Gly polymorphism with SCZ in Malaysia. In the present study, we were interested in the genotyping the Malay population which is the major ethnic in Malaysia.

Materials and Methods

This case-control study involved 261 in-patients with SCZ (137 males; 124 females) recruited from Hospital bahagia, Ulu Kinta, Perak, Malaysia. The patients had a mean age of 46.5 (S.D. 13.6). All patients were evaluated using the Mini International Neuropsychiatric Interview (MINI). Patients with co-morbidity were excluded. A total of 157 volunteer control subjects (86 males; 71 females), with a mean age of 38.4 (S.D. 14.2) were recruited from blood donation centers at Universiti Tunku Abdul Rahman and Kuala Lumpur. All controls were free of any psychiatric illness, drug abuse and family history of psychiatric disorders. All participating subjects were unrelated, born in Malaysia and self-identified as being of Malay descent. This study was approved by the Medical Research Ethics Committee, Ministry of Health, Malaysia.

Peripheral blood sample was obtained from each subject. Genomic DNA was prepared using the Promega Wizard DNA Isolation Kit (USA). The distribution of the DRD3 Ser9Gly polymorphism was determined by PCR-RFLP analysis. Amplification was performed with the following primers: 5'-GCTCATCTCCAACTCTCACA-3' and 5'-AAGTCTACTCACCTCGTA-3' (17). The PCR products were then digested with 5 U *MscI*. DNA fragments were visualized by 2.5% agarose gel electrophoresis and stained with ethidium bromide. The fitness of genotype frequency distribution to the Hardy-Weinberg equilibrium (HWE) was tested using Arlequin version 3.11 (http://anthro.unige.ch/arlequin). Allelic and genotype frequency differences between patients and controls were analyzed using the Chi-square (χ^2) test of the Statistical Package for the Social Sciences, version 12.0.

Results

PCR product of DRD3 and *MscI* restriction enzyme-digested PCR products are shown in Fig. 1. The distribution allelic and genotypic frequencies of DRD3 Ser9Gly in patients and controls are summarized in Table 1. The genotype distribution of the polymorphism was in HWE for both patients (P= 0.210) and controls (P= 0.179). There was no significant difference between the two groups regarding the allelic distribution (χ^2= 0.004; df = 1; P= 0.925; OR = 0.982 (95% CI= 0.545–1.769)). A significant genotype distribution between patients and controls was observed (χ^2= 9.359; df= 2; P= 0.009). However, no significant trend for an excess of Gly-containing genotype (Ser/Gly and Gly/Gly) was observed in patients compared with controls (χ^2= 0.325; df= 1; P= 0.569; OR= 0.850 (95% CI = 0.486– 1.487)). Besides, there was no evidence that Ser9Gly homozygosity served as a risk factor for SCZ (χ^2= 1.527; df = 1; P = 0.217; OR = 1.422 (95% CI = 0.813–2.487)).

Table 1: Allelic and genotype distribution of DRD3 Ser9Gly polymorphism in patients and controls

	n	Allele (%)		Genotype		
		Ser	Gly	Ser/Ser	Ser/Gly	Gly/Gly
Schizophrenia	261	347 (66.5)	175 (33.5)	120 (46.0)	107 (41.0)	34 (13.0)
Control	157	210 (66.9)	104 (33.1)	66 (42.0)	78 (49.7)	13 (8.3)

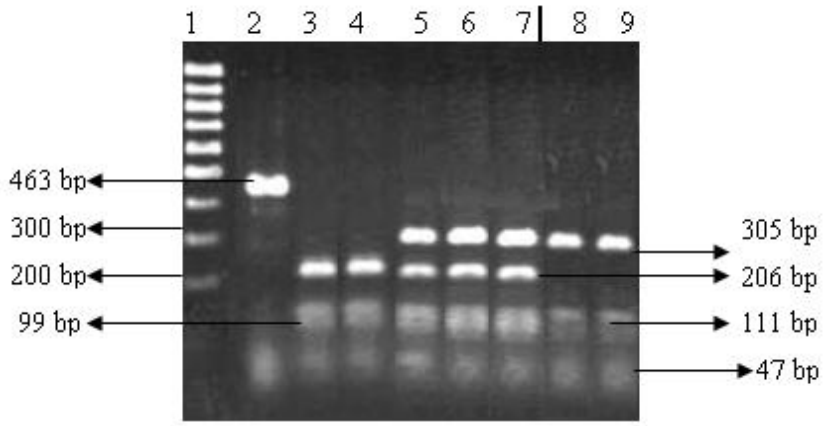

Fig. 1: PCR product of DRD3 and *MscI* restriction enzyme-digested PCR products

Lane 1: Low Range DNA Ladder (MassRuler™), Lane 2: PCR product of DRD3, Lane 3: Gly/Gly genotype (control) Lane 4: Gly/Gly genotype (patient), Lane 5 and 6: Ser/Gly genotype (control), Lane 7: Ser/Gly genotype (patient) Lane 8: Ser/Ser genotype (control), Lane 9: Ser/Ser genotype (patient)

Discussion

The results of the current case-control study suggested a significant difference in genotype distribution between patients and controls. This is in partial accordance with several case-control studies conducted among Asians and Caucasians. Hoogendoorn et al. (18), Chang et al. (19) and Fathalli et al. (14) suggested negative association between allelic and genotype distributions with SCZ, while Utsunomiya et al. (3) found statistically significant evidence of such association.

Our inability to detect association between homozygosity with SCZ, as demonstrated in more homogenous Caucasian populations (8, 15) may due to genetic heterogeneity of the Malay population. Fathalli et al. (14) reported a lack of association between homozygosity with SCZ in Caucasians recruited from different countries. The issue of population stratification cannot be excluded even in the Japanese, which is considered a more homogenous ethnic (3). In Malaysia, ethnicity measures identity rather than origin and ancestry. Immigrants from Indonesian Archipelago have been absorbed into the Malay community (20). Thus, the measurement by self-identification suggested the possibility of population which may contribute to false positive and false negative (21). Despite of the limitation of ethnicity, this has merits in terms of clinical diagnosis. We can rule out other confounding factors, such as different diagnostic tools, or the use of inpatients or outpatients. All of the schizophrenic subjects were inpatients from one psychiatric hospital (*Hospital Bahagia Ulu Kinta*) and clinical records were complete.

Although our results are inconclusive to associate the DRD3 Ser9Gly polymorphism with SCZ, this gene cannot be excluded as an implication in SCZ. Talkowski et al. (16) reported that the Ser9 Gly polymorphism was associated with SCZ only when it was present in a common haplotype spanning intron 1 to the 3' region of DRD3 gene but not when it was present on other haplotypes. Thus, further studies with linked variants for this region may contribute to SCZ susceptibility.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

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Original Article

Breastfeeding Practices in Infants in the West Region of Cameroon

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Abstract

Background: The study was conducted to evaluate the knowledge, attitudes and practices of breast-feeding in the West region of Cameroon.

Methods: A cross sectional descriptive study was conducted in two health facilities on 195 mother-infant pairs, seen at the out patient and vaccination units of the Bafoussam Regional Hospital over a period of one month from 1st to 30th September 2008. The socio-demographic characteristics of mothers, knowledge on breastfeeding and the practice of breastfeeding were studied. Data was analyzed using the SPSS software. The chi square and student *t*- test were used for comparison and results considered significant for $P< 0.05$.

Results: Breastfeeding was practised by 99.48% of the mothers. Only 33.8% of the mothers knew that they had to exclusively breastfeed up to 6 months, and 20% effectively breastfed up to 6 months. The mean duration of breastfeeding was 5.06 months and negatively correlated with the number of children and the profession of the mother. In 69.74% of the women, nothing was given to the baby before the first breastfeed. Discontinuation of breastfeeding was done averagely around 15.24 months and earlier in married women and in those with a higher educational level.

Conclusion: Although the majority of parents practised breast feeding, only a minority understood its benefits, so more should be done to educate the community on the benefits of exclusive breast-feeding for up to six months.

Keywords: *Breastfeeding practices, Knowledge, Attitudes, Cameroon*

Introduction

Optimal exclusive breastfeeding up to 6 mo of age is recommended by United Nation's Children's Fund (UNICEF) as having the single greatest potential impact on child survival of all preventive interventions, with the potential to prevent 1.4 million deaths in children under five in the developing world. A further 6% or close to 600,000 under five deaths can be prevented by ensuring optimal complimentary feeding (1). Breastfed children have at least six times greater chances of survival in the early months than non-breastfed children, because breast milk drastically reduces deaths from acute respiratory tract infections and diarrhoea, two major child killers, as well as from other infectious diseases (2). Breastfeeding rates have been on the increase worldwide in the last decade but only 38% of children less than 6 mo of age are exclusively breastfed and 39% benefit from breastfeeding up to 2 yr of age in developing countries (1).

Despite the known advantages of breastfeeding we observe a decline in the practice especially in the urban areas in developing countries. In Côte-d'Ivoire, studies carried out in certain neighbourhoods in Abidjan, revealed that 90% of mothers give artificial milk to babies less than 4 mo in addi-

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tion to breast milk (3). In Cameroon and according to the 2004 Demographic Health Survey (DHS), although 99% of babies are breastfed at birth, the mean duration of breastfeeding is 17.4 mo, and only 24% are exclusively breastfed up to 6 mo (4). The causes of the low rate of exclusive breastfeeding during the first 6 mo are multifactorial and comprise taboos in the community and insufficient knowledge (of the mothers and the health personnel) of the national recommendations on breastfeeding. We decided to study the feeding patterns of infants by assessing the knowledge and practices of mothers, the beliefs and taboos in this part of Cameroon, which could help ameliorate the rate of breastfeeding in Cameroon as a whole.

Materials and Methods

The survey was done in two health facilities (the Regional Hospital and the Integrated Health Center) in Bafoussam town, on 195 mother-child pairs in the external consultations and vaccination units over a one month period (1st to 30th of September 2008). These two health facilities were selected because they are attended by all social groups of the population, and are not Baby Friendly health institutions.

The procedure consisted of consecutively interviewing the mothers on their knowledge and attitudes on their breastfeeding practices and recorded in a pre-established form. The sample size was a convenient sample as we enrolled the women consecutively as they came to the hospital.

For the mother we noted her age, ethnic group, parity, matrimonial status, religion, profession, level of education, and knowledge on breastfeeding (the time breastfeeding was initiated, duration of exclusive breastfeeding, advantages of breast milk, their attitudes towards breastfeeding, and the type of feeding given to their babies after birth).

Data collected was analysed with the statistical package of social sciences (SPSS) version 10.1. The Chi square test was used to compare percentages and the student *t*-test to compare averages. The results were significant for a *P* value <0.05.

Results

During our study, 195 mother-child pairs were surveyed.

Socio-economic characteristics of the mothers

Most of the women (142, 73%) had completed secondary education. The most frequent age group of the mothers was 20-29 yr with about half (108, 55%) consisting of stay at home moms, and the majority (173, 89%) married. The average number of children per mother was 2.7 (range: 1-8 children) and the majority, 135 (69.2%) of women were multiparous.

Maternal knowledge on breastfeeding

Only a third of mothers (34%) who participated in the study knew that exclusively breastfeeding up to 6 months was better, and even fewer knew the advantages of breast milk (15.6%). The minority (7.8%) knew that breastfeeding the child had to be done frequently day and night (33%) (Table 1).

Attitudes of the mothers towards breastfeeding after delivery

The minority of mothers (7.8%) initiated breastfeeding within the first 30 min following delivery and one mother did not breastfeed her baby because she was HIV positive. This was the only HIV positive woman in our study population and she declared that she had opted for feeding with breast milk substitutes following nutritional counselling during her prenatal visits. Fifty-four (27.8%) women breastfed their babies between the first and the second hour following delivery while more than 60% breastfed three or more hours later (Table 2). Some of the deliveries were done in the hospital and some in peripheral health centres, and most of the mothers request discharge from the hospital within 24 h without receiving nutritional counselling for their babies from the nurses or doctors. In 136 (69.74%) mothers, nothing was given to their babies before the first breastfeeding. In 28 mothers (14.36%), water was given, and in 22 (11.30%) sugared water was given.

Out of the 195 mothers who participated in the study 59 gave pap before the first breastfeeding for

several reasons amongst which was the absence of breast milk flow in 44 (74.60%) of them (Table 3).

Mode of feeding

Globally, breastfeeding had been done by 194 (99.48%) of the mothers surveyed, and one who was seropositive fed her child with breast milk substitutes. Out of the 194 who had breastfed only 39(20%) had exclusively breastfed up to 6 mo and 155(79.9) had introduced other liquids and foodstuffs in addition to breast milk as water, breast milk substitutes, honey, pap and "fufu". The mean duration of exclusive breastfeeding was 151.82 d (5 mo) and significantly declined with parity and the profession of the mother ($P< 0.05$). There was no statistical correlation between the duration of exclusive breastfeeding up to 6 mo and the age of the mother, matrimonial status and the level of education ($P> 0.05$) (Table 4). Main reasons for early weaning before 6 mo were "insufficient" milk in 27 (52.94%) mothers, and the child "eating much" in 16 (31.38%) mothers.

The mean age of breastfeeding cessation was 457.31 d (15.24 mo) with extremes of 4 mo to 24 mo. The matrimonial status and the level of education of the mothers negatively influenced the duration of breastfeeding in general ($P> 0.05$).

The mean age of introduction of mixed feeding was 52.67 d (1.75 mo) with extremes of 1 d to 180 d.

Table 1: Knowledge of mothers on breastfeeding

	n	%
Exclusive breastfeeding for 6 months	66	33.8
Placing the child on the breast within 30 minutes following delivery	15	7.8
Giving breast milk to the child on demand, day and night	64	32.82
Advantages of breast milk*	30	15.6
Total	175	

The mothers were asked whether they knew any benefits breast milk can have on the babies.
And in those who said they knew they were asked to enumerate at least one.

Table 2: Time interval between delivery and first breastfeeding*

	n	%
≤ 30 min	15	7.8
1 h – 2 h	54	27.8
3 h – 4 h	41	21.2
5 h – 23 h	42	21.6
≥ 24 h	42	21.6
Total	194	100.0

*One HIV seropositive mother did not breastfeed her child.

Table 3: Reasons given for introduction of other food substances before the first breastfeeding (N = 59)*

Reason	n	%
No milk flow	44	74.60
Delivery by cesarean section	6	10.16
Sick child	5	8.47
Recommended by a health personnel	2	3.40
Evacuation of meconium	1	1.68
Local "Foulbe" tradition	1	1.68
Total	59	100.0

*Of the 195 mothers in this survey 59 gave other foods to the newborn before initiation of breastfeeding.

Table 4: Correlation of socio-economic characteristics of mothers and age of weaning

Socio-demographic characteristics of mothers	Weaning age group (month)				
	1 – 2.5	3 – 4.5	5 – 6.5	7 – 8.5	*P*
Matrimonial status					
Married	5	26	50	9	
Single	0	1	6	0	
Concubine	0	0	2	0	*P* = 0.971
Total	**5**	**27**	**58**	**9**	
Level of Education					
None	1	0	0	0	
Primary	1	7	12	4	
Secondary	3	19	44	5	*P* = 0.153
University	0	1	2	0	
Total	**5**	**27**	**58**	**9**	
Profession					
Student	0	2	1	0	
Housewife	3	16	34	5	
Liberal profession	2	7	19	3	
Civil servants	0	2	4	1	*P* = 0.005
Total	**5**	**27**	**58**	**9**	
Maternal age					
< 20	0	0	2	1	
20 - 29	3	16	40	5	
30 - 39	2	9	16	3	
40 - 45	0	2	0	0	*P* = 0.513
Total	**5**	**27**	**58**	**9**	
Parity					
Primiparous	1	3	16	4	
Multiparous	4	21	41	5	
Grand multiparous	0	3	1	0	*P* = 0.000
Total	**5**	**27**	**58**	**9**	

Discussion

This study led us to assess breastfeeding practices in an urban zone of Bafoussam in the West region of Cameroon. The mothers surveyed were relatively young, and most were multiparous and housewives. Mothers who had secondary education practiced breastfeeding most. This data is in concordance with that reported by Sepou et al (5) in Central Africa and Siyou in Cameroon (6). Of the 195 mothers who participated in the study, 66 (33.8%) knew they had to exclusively breastfeed up to 6 mo. Only 15 (7.8%) knew that breastfeeding had to be initiated within 30 min of delivery. Sixty mothers (32.82%) knew the child had to be breastfed frequently day and night. Only 30 mothers (15.6%) knew the advantages of breast milk (this is not astonishing as in most local African communities breast milk is just accepted as an

important and appropriate nutriment for the new born and most mothers would breastfeed without actually knowing the advantages of breastfeeding to them or their babies). Conversely, Ngofika (7) noted that 69.7% of the mothers had been informed of breastfeeding initiation within 30 min following delivery and 67.3% knew they had to breastfeed as frequently as possible.

Other foods were given to the baby at birth in 30.36% of the mothers surveyed. Reasons for this practice included amongst others, 'insufficient' breast milk flow in 74.6% of the mothers, advice from a health personnel in 3.4% of the mothers, and in accordance with ethnic group practices in 3.36% of the mothers (like in the Foulbé tribe to evacuate the meconium).

In 69.79% of the women, nothing was given to the newborn before initiation of breastfeeding. Contrarily, the 2004 Demographic Health Survey (DHS) in Cameroon (4) noted that 62% of the mothers gave their babies other liquids before initiating breastfeeding. We also noted that only 7.8% of the mothers breastfed their newborns within 30 min following delivery. In Côte-d'Ivoire, in some localities in the capital city Abidjan, 90% of mothers practiced mixed feeding in babies less than 4 mo (3). In India, Dash et al. (8) noted that 48% of babies were breastfed for the first time within six hours following birth. But the World Health Organisation (WHO) and the Cameroon Ministry of Public Health recommend that all babies be breastfed within 30 min following delivery (2). Promotion of early initiation of breastfeeding has the potential to improving child survival. It has been shown that 16% of neonatal deaths could be saved if all infants were breastfed from day one and 22%, if breastfeeding started within the first hour (9).

Despite the relatively satisfactory rate of breastfeeding in the mothers surveyed, this study noted a low rate (20%) of exclusive breastfeeding up to 6 mo as recommended by WHO and UNICEF (2). The mean duration of exclusive breastfeeding was 5.06 mo. The overall rate of breastfeeding in our study is high (99.48%). Nlend et al (10) reported a slightly lower rate of 86% in Yaounde the capital city of Cameroon in 1997. Other studies in Africa noted rates of 96.5% in Central Africa (5), 83.7% in Agadir in Morocco (11), and 88.6% in Burkina Faso (12). In the North of Brazil, Neusa et al. (13), had a 99% breast feeding rate which is similar to ours. Guerrero et al (14) in Mexico found a slightly lower rate of 91% of mothers who breastfed their babies, whereas in China, Liqian et al (15) found a rate of 62. 8% in urban and 83.4% in rural areas.

Concerning exclusive breastfeeding up to 6 mo, several studies in Africa show low rates. In our study, we found an exclusive breastfeeding rate of 20% at 6 mo, which is greater than the 17.3% observed by Nlend et al (10) in Yaounde. Tietche et al (16) still in Yaounde had the lowest rate of 3% whereas Siyou (6) had 10.7%. In Senegal, Wade reported in his study that only 5% of neonates less than 5 mo were exclusively breastfed and 61% in the same age group received in addition water and other foodstuffs (17). In Cameroon according to the 2004 DHS only 24% newborns are exclusively breastfed up to 6 mo (2). These disparities in the proportion of women who breastfeed exclusively in these studies may be probably due to differences in study design and cultural practices. The prevalence of HIV infection in the adult population in Cameroon is 5.5% and 7.4% in pregnant women (4). Transmission rates during pregnancy, delivery and breastfeeding are not well documented. Njom Nlend et al (18) in 2004, in a cohort of 111 pregnant women in a prevention of mother to child transmission of HIV (PMTCT) programme, and who had taken Zidovudine during pregnancy and Nevirapine during labour, noted that the transmission rate at 15 mo postnatal was 8.8% in neonates who had been on breast milk substitutes and 14.3% in those who had been breast fed.

The average age of introduction of complementary foods was 5.06 mo, and is similar to the 5.03 mo observed by Traoré et al (12) in Burkina Faso. At the national level other studies showed early ages of 3.25 mo for Tietche et al (16) and 3.4 mo for Siyou (6). Reasons for early weaning before 6 mo in our study were: "insufficient" breast milk in 52.94% of the mothers, child "over eating"

in 31.38%, and the profession of the mother in 1.96% of the mothers. Siyou noted "insufficient" milk in 29.8% and maternal illness as causes of early weaning. In Mexico, Guerrero et al (14) found from their study that breastfeeding was interrupted or stopped for the following reasons: advised by a medical personnel (68%), mother suffering from what is popularly known as "coraje or anger" (52%), or «susto or fright» (54%), mother having "insufficient milk" (62%), or milk of "bad quality" (56%) and because either the mother (56%) or the child (43%) is sick. The duration of exclusive breastfeeding within 6 mo of birth decreases significantly with parity and profession of the mother ($P< 0.05$). However Nlend et al. (10) noted that the duration of exclusive breastfeeding increases with the number of children the mother has.

The mean age of breast feeding cessation was 15.24 months which is lower than that obtained by Siyou (6) who noted 9.2 mo. In Brazil, Neusa et al (13) observed that the mean duration of breastfeeding was 65 d for mothers who started other milks within 1 mo and 165 d for the other mothers. The WHO and the Cameroon's Ministry of Public Health recommend that children be exclusively breastfed up to 6 mo and supplemented with other appropriate foods up to 2 yr and even beyond (1).

Breastfeeding cessation is done earlier in married women and in women who have a higher level of education ($P< 0.05$). However Nlend et al (10) observed that the age of the mother negatively influenced the duration of breast-feeding in general.

From this work, it can be concluded that breastfeeding is the most frequent mode of infant feeding of neonates in this region of Cameroon and needs to be maintained and encouraged. However, the rate of exclusive breastfeeding at 6 mo was low and should be improved upon.

We thus recommend that emphasis be placed on targeted information, education and communication of mothers during vaccination, prenatal and pediatric consultations on the appropriate and recommended infant feeding practices.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

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