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SEROPREVALENCE OF HEPATITIS B MARKERS IN PREGNANT WOMEN IN KENYA
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ABSTRACT

Objective: **To evaluate hepatitis B serological markers in pregnant women from various geographical sites in Kenya.**

Design: **A cross-sectional observational study of women attending antenatal clinics.**

Setting: **The Kenyatta National Hospital and eight hospitals from five provinces in Kenya.**

Subjects: **All women in their third trimester of pregnancy attending the antenatal clinic over the period June 2001 to June 2002.**

Main outcome measures: **For each pregnant woman age and gestation were documented. Hepatitis serological markers were evaluated.**

Results: **A total of 2,241 pregnant women were enrolled. Among them 205 women (9.3%) were positive for HbsAg and from these 18 (8.8%) were found to have HbeAg. Protective antibodies (anti-HbsAg) were detected in 669 (30.2%) of the women. There were notable significant regional differences for HbsAg rates.**

Conclusions: **These results confirm the presence of high disease carrier rate and the corresponding previously reported low level of HbeAg suggesting questionable low rate of perinatal transmission but high rate of horizontal transmission.**

INTRODUCTION

Human infection with Hepatitis B virus (HBV) is a global health problem. More than one third of the world population has been reported to have serological evidence of past or present HBV infection. It is estimated that 350 million people globally are chronic carriers of the hepatitis B virus of who 170 million reside in Africa (1). Areas of particularly high endemicity include sub-Saharan Africa and Asia since the disease is associated with poor socio-economic conditions (2). In high endemic areas infection is usually acquired early in life and the perinatal route of transmission of HBV infection is of great consequence (2, 3).

Chronic HBV infection is known to be the most common cause of hepatocellular carcinoma and liver cirrhosis resulting in a 25% lifetime risk of death from these complications which account for 700,000 and 300,000 deaths respectively each year worldwide (1,4).

It is estimated that 40% of infants born of Hepatitis B surface Antigen (HBsAg) carrier mothers will be infected at birth. This perinatal transmission rate could rise up to 90% when mothers have Hepatitis B e Antigen (HBeAg) as well. In sub-Saharan Africa a high prevalence of HBsAg carriage

and a relatively lower prevalence of HBeAg carriage has been observed among women but few studies have been conducted to evaluate the burden of the perinatal mode of HBV transmission (1, 5).

Hepatitis B disease depletes the limited health resources of developing countries such as Kenya where this disease is highly endemic. Epidemiological studies show that almost half of the Kenyan population will have been infected with hepatitis B by the age of 30 to 40 years (2,3). This would inevitably lead to a large number of adults with cirrhosis, chronic liver diseases and hepatoma.

Cross-sectional studies done in Nairobi and rural Kenya determined the prevalence of HBsAg to range between 3–30% (3, 6-9). At the Kenyan coast it was established that 11.4% of the population was HBsAg positive and 36% of the HBsAg positive women of childbearing age were also positive for HBeAg (6). In a study of a rural community in Kenya a perinatal transmission rate of 40% was observed in children born to mothers who were HBeAg positive (10).

In countries where Hepatitis B is highly endemic children are infected early in life either perinatally or horizontally thereby maintaining the pool of chronic carriers. It is therefore imperative that all children in such countries are immunised against Hepatitis B early in life commencing at an age appropriate for the principle mode of transmission of the virus. Immunisation as a public measure aims at interrupting transmission of infection by protecting the children and the community at large.

In Taiwan a study done on the Universal Hepatitis B vaccination found the incidence of hepatocellular carcinoma in children of six to nine years of age to decline from 0.52 to 0.13 per 100,000 after two years of implementation of universal vaccination (11).

A study conducted in Kenya in 1990 showed that the Hepatitis B vaccine is safe and effective when administered at birth. In this pilot study done in Kiambu district hepatitis B vaccination was given integrated with the Kenya Expanded Programme on Immunisation (KEPI) Diphtheria Pertussis and Tetanus in three doses. The acceptance was good and it improved the overall coverage of KEPI vaccines (12).

The World Health Organisation (WHO) set 1997 as the target date for the inclusion of hepatitis B vaccination in national immunisation programmes in all countries. To date 129 countries have integrated routine hepatitis B immunisation for infants into their national programmes and in many of them universal immunisation at birth has been shown to significantly reduce the rate of chronic carriage (13). The WHO Expanded Programme on Immunisation recommends that all countries with HBsAg carriage rate of ž2% which includes Kenya should implement universal infant Hepatitis B immunisation. The WHO has proposed two different schedules for hepatitis B immunisation whose choice depends on the main mode of transmission. When horizontal transmission is predominant the first of three doses of Hepatitis B vaccine is given at the age of six weeks and subsequent doses at four weekly intervals. In countries with high rate of perinatal transmission the first dose should be administered at birth and subsequent doses at 6 and 10 weeks of age (14).

Factors that play a major role in determining the rate of vertical transmission include ethnicity and local epidemiology hence the need to investigate many sites (2, 12).

The purpose of this study was to assess the seroprevalence of Hepatitis B markers in pregnant women from various geographical sites in Kenya with the aim of elucidating the significance of the perinatal mode of Hepatitis B as well as confirming the previously reported high HBV endemicity in Kenya.

MATERIALS AND METHODS

Design: This was a multi-centre, hospital based, cross sectional, observational study done between June 2001 and June 2002 under the auspices of Kenya Paediatrics Association.

Study sites: The study geographic regions and sites are shown in Table 1.

Inclusion criteria: All women in their third trimester of pregnancy who gave informed consent to participate in the study and were willing to be bled for determination of hepatitis B markers.

Table 1

Study geographic regions and sites

Study geographic region	Study site code	Study site/City
Nairobi	A	Kenyatta National Hospital
Coast	B	Aga Khan Hospital, Mombasa
	I	Coast General Hospital, Mombasa
Nyanza/ Western	C	New Nyanza Provincial General Hospital, Kisumu
Rift Valley	D	Rift Valley Provincial General Hospital, Nakuru (Central Rift)
	E	Moi Teaching and Referral Hospital, Eldoret (North Rift/Western)
	F	AMREF Lopiding Static Health Facility (North Rift)
Eastern	G	Isiolo District Hospital
Central	H	Nyeri Provincial General Hospital

Data Collection

All women in their third trimester of pregnancy (from 25 weeks of gestation) had their age documented in a prepared questionnaire. The patients were then examined by a doctor in order to determine the gestation of pregnancy after which a blood sample of 10 ml was obtained for determination of hepatitis B markers.

Management of blood samples: A 10 ml sample of venous blood was collected into a vacutainer tube on first contact. The blood sample specimens were centrifuged at 3,000 rpm for 15 minutes then stored in cryotubes at minus 20°C until processed in KEMRI where analysis was done using:

- R-PHA-KEMRI-HEPCELL kit for HBsAg (KEMRI in-house Reverse passive Haemagglutination test)
- MONOLISA® kit (cat no. 72212) for anti-HBeAg (ELISA test)
- MONOLISA® kit (cat no. 72400) for anti-HBsAg antibody (ELISA test).

Statistical Analysis

All statistical analyses were performed under the responsibility of the Statistical Platform of Aventis Pasteur, Lyon-France using the SAS software, version 8.2 for PC (SAS Institute, Cary, NC, SA).

Confidence intervals for the single proportion were calculated using the exact binomial method (Clopper-Person method, quoted by Newcombe) (15), i.e., using the inverse of the beta integral with SAS.

Parameters were described with their 95% confidence interval (CI).

Overall comparison between regions and differences between study sites was done using Chi Squared test (or a Fisher Exact test in case of small numbers).

For significant overall differences paired tests were used to compare regions using two by two tables (Chi Squared or Fisher Exact test) (15).

Ethical considerations: Ethical approval was obtained from the ethical committees of all the hospitals involved. The subjects signed a written informed consent form (available in two languages, Swahili and English) before being enrolled in the trial and after having been informed of the nature of the trial, the potential risks and their obligations. All infants born to the study subjects were given a total of three hepatitis B vaccines free of charge starting at birth.

RESULTS

A total of 2,241 pregnant women were included in this trial between 22 June 2001 and 10 June 2002 in nine study sites in Kenya. The number of pregnant women included per study geographic region and study site is presented in Table 2.

Up to 300 pregnant women were included per study geographic region except in the Rift Valley area (including three study sites: D, E and F) where 741 pregnant women were recruited.

Table 2

Number of pregnant women enrolled per region and site

Study geographic region	Study site code	Study site / City	Subjects enrolled	
			No.	(%)
Total planned			2,239	-
Total included			2,241	100
Nairobi	A	Kenyatta National Hospital, Nairobi	300	13.4
Coast	B and I		300	13.4
	B	Aga Khan Hospital, Mombasa	100	4.5
	I	Coast General Hospital, Mombasa	200	8.9
Nyanza	C	New Nyanza Provincial General Hospital, Kisumu	300	13.4
Rift Valley	D, E and F		741	33.1
	D	Rift Valley Provincial General Hospital, Nakuru (Central Rift)	233	10.4
	E	Moi Teaching and Referral Hospital, Eldoret (North Rift/Western)	208	9.3
	F	AMREF Lopiding Static Health Facility (North Rift)	300	13.4
Eastern	G	Isiolo District Hospital	300	13.4
Central	H	Nyeri Provincial General Hospital	300	13.4

Table 3

Demographics

Study geographic region	Study site code	Age (years)			Previous children			Residents†		
		No.	Mean +/- SD*	[Min-Max]	No.	Mean +/- SD*	[Min-Max]	No.	Mean +/- SD*	[Min-Max]
Total		2223	25.2+/-5.41	12-43	2239	1.2+/-1.43	0-10	2236	3.9+/-3.11	0-100
Nairobi	A	299	27.8+/-5.01	17-43	299	0.9+/-1.01	0-5	299	3.3+/-1.43	1-9
Coast	B and I	294	25.7+/-5.08	12-39	300	0.9+/-1.22	0-6	300	5.9+/-6.92	2-100
	B	94	26.3+/-5.05	12-39	100	1.1+/-1.26	0-6	100	5.2+/-2.35	2-13
	I	200	25.4+/-5.09	14-39	200	0.8+/-1.18	0-6	200	6.2+/-8.29	2-100
Nyanza	C	299	22.7+/-4.98	13-41	300	1+/-1.35	0-6	300	3.8+/-2	0-13
Rift Valley	D, E and F	737	25.2+/-5.1	16-42	741	1.5+/-1.56	0-8	740	3.8+/-1.83	1-12
	D	232	25.2+/-5.07	17-42	233	1.2+/-1.23	0-6	233	3.6+/-1.74	1-12
	E	207	25.4+/-5.23	17-42	208	1.3+/-1.52	0-6	207	4+/-2.03	1-10
	F	298	25.1+/-5.05	16-40	300	1.9+/-1.72	0-8	300	3.9+/-1.74	2-10
Eastern	G	294	24.7+/-5.54	16-41	299	1.5+/-1.71	0-10	298	3.7+/-1.88	1-12
Central	H	300	25.4+/-5.92	14-41	300	1+/-1.15	0-6	299	3.3+/-1.46	1-9

* = Standard deviation, No. = Number of subjects with available data

The ages of pregnant women ranged from 12 to 43 years with a mean of 25.2 ± 5.41 years and most mothers were in their 20s. In each study geographic region the ages of pregnant women were comparable with Coast having the youngest subject (12 years) and Nairobi the oldest (43 years). Parity ranged from 0 to 10 and was similar among the regions. The number of household residents ranged from 0 to 100 with the Coast study geographic region (study site of Mombasa) having the highest number of residents with a mean of 5.9 while Nairobi and Central study regions had the lowest mean of 3.3.

Table 4

Study subjects gestation in weeks

Study geographic region	Study site code	Gestation week		
		No.	Mean+/- SD*	[Min-Max]
Total		2240	33+/-4.04	25-43
Nairobi	A	299	33.7+/-3.97	25-42
Coast	B and I	300	33.2+/-3.77	25-40
	B	100	32.6+/-3.71	25-40
	I	200	33.5+/-3.78	25-40
Nyanza / Western	C	300	34.2+/-4.11	25-43
Rift Valley	D, E and F	741	32.4+/-4.16	25-42
	D	233	33.8+/-3.64	25-40
	E	208	33.4+/-3.95	25-41
	F	300	30.7+/-4.08	25-42
Eastern	G	300	31.7+/-3.58	25-39
Central	H	300	33.6+/-3.89	25-42

All the women recruited were in their 25th to 43rd weeks of gestation.

Immunogenicity

A total of 205 mothers (9.3%) were HBsAg carriers and among these subjects 18 (8.8%) were also HBeAg positive. Regarding seroconversion 669 mothers (30.2%) had protective antibodies (anti-HBsAg).

Table 5

Seroprevalence of HBV markers in all subjects

Seroprevalence	n/N	(%)	95% CI
HbsAg	205/2214	9.3	8.1 ; 10.5
HbeAg	18/205	8.8	5.3 ; 13.5
Anti-HBsAg antibody	669/2214	30.2	28.3 ; 32.2

N:Number of subjects with available data
n: Number of positive subjects

Table 6

Seroprevalence of HBV markers by region / investigational site

Study Region	Study site code	HBsA			HBeAg		
		n/N	(%)	95% CI	n/N	(%)	95% CI
Total		205/2214	9.3	8.1 ; 10.5	18/205	8.8	5.3 ; 13.5
Nairobi	A	23/298	7.7	5.0 ; 11.4	1/23	4.3	0.1 ; 21.9
Coast	B and I	15/300	5.0	2.8 ; 8.1	0/15	0.0	0.0 ; 21.8
	B	9/100	9.0	4.2 ; 16.4	0/9	0.0	0.0 ; 33.6
	I	6/200	3.0	1.1 ; 6.4	0/6	0.0	0.0 ; 45.9
Nyanza/Western	C	21/300	7.0	4.4 ; 10.5	3/21	14.3	3.0 ; 36.3
Rift Valley	D, E and F	99/739	13.4	11.0 ; 16.1	8/99	8.1	3.6 ; 15.3
	D	16/231	6.9	4.0 ; 11.0	2/16	12.5	1.6 ; 38.3
	E	37/208	17.8	12.8 ; 23.7	0/37	0.0	0.0 ; 9.5
	F	46/300	15.3	11.4 ; 19.9	6/46	13.0	4.9 ; 26.3
Eastern	G	34/277	12.3	8.7 ; 16.7	4/34	11.8	3.3 ; 27.5
Central	H	13/300	4.3	2.3 ; 7.3	2/13	15.4	1.9 ; 45.4

N: Number of subjects with available data
n: Number of positive subjects

The site with the highest HBV carrier rate was Eldoret with 17.8% while the lowest carriage of 3% was found in Coast general hospital Mombasa. The region with the highest rate was Rift Valley with 13.4% and the site with the lowest rate was Central with 4.3%. In the Coast region the Aga Khan hospital site had three times (9.0%) the chronic carriage rate of HbsAg compared to 3% found at the Coast General hospital. The percentage of mothers who were HBeAg positive among the HBsAg positives ranged between 0% in sites B, I and E and 15.4% in site H. Only 18 subjects were HbeAg positive in this trial and no difference between the different regions was observed (p-value = 0.532).

The differences in HBsAg carrier rate between the different sites and regions are demonstrated in Tables 7 and 8.

Table 7

HBsAg seroprevalence : Pair wise comparisons between regions

p-value	Coast	Western/Nyanza	Rift Valley	Eastern	Central
Nairobi	0.173	0.737	0.010	0.068	0.082
Coast		0.302	<0.001	0.002	0.699
Nyanza /Western			0.003	0.031	0.158
Rift Valley				0.637	<0.001
Eastern					<0.001

Table 8

HBsAg seroprevalence : Pair wise comparisons between sites

p-value	B	I	C	D	E	F	G	H
A	0.683	0.028	0.737	0.730	<0.001	0.004	0.068	0.082
B		0.025	0.511	0.512	0.043	0.111	0.377	0.076
I			0.053	0.065	<0.001	<0.001	<0.001	0.445
C				0.974	<0.001	0.001	0.031	0.158
D					<0.001	0.003	0.044	0.192
E						0.462	0.089	<0.001
F							0.288	<0.001
G								<0.001

A statistically significant overall difference of HBsAg seroprevalence was observed between some regions as seen between Coast and Rift Valley, Rift Valley and Central and Eastern and Central (Table 7). Similarly statistically differences was demonstrated between some sites like; A and E, I and E, F, and G, C and E, D and E, E and H, F and H, and G and H (Table 8) (p value < 0.001).

Only 18 subjects among the HbsAg carriers were HBeAg positive in this trial and no difference between the different regions was observed (p-value=0.532).

Using the HbsAg and HbeAg data obtained in this study from all the regions and overall the annual incidence rates (IR) of HBV infection was calculated (Table 9).

Table 9

Estimated annual Incidence Rate [IR/100,000] of chronic HbsAg carriage in each region

Region	HBsAg %	HbeAg %*	IR/100,000
Nairobi	7.7	4.3	503
Coast	5.5	0.0	200
Nyanza	7.0	14.3	871
Rift Valley	13.4	8.1	1176
Central	4.3	15.4	563
All regions	9.3	8.8	855

*Percentage of HbeAg women among those who were HbsAg (IR= Number of newborn per 100,000 live births who will become chronic carriers of HBV in the absence of neonatal vaccination).

Calculations are based on Chauvin *et al* (16). The IR of chronic HbsAg carriage in the study regions ranged between 200 in Coast and 1346 in Eastern with an average of 855. Only Coast region had a low IR of 200 while all the other four regions had an IR of more than 500/100,000.

DISCUSSION

In this study the average prevalence of HbsAg carriage was 9.3%, which is within the range found by other authors in Kenya (3,8-11). This suggests that Hepatitis B infections have remained fairly constant over the years and is still high by WHO classification (16). However the prevalence found in Lopiding (15.3%) was about half of that found in the same region (29%) by Bowry *et al* a decade ago (9). There were definite differences in HBsAg carriage rates among the regions. The lowest carriage rate 4.3 % was found in the Central Province region while the highest rate of 13.4% was found in the Rift valley region. This could be due to the better social economic status and sanitation found in the ethnic group in the Central region leading to a lower transmission rate. Although the Rift valley region had the highest HBsAg carriage rate there was a significant difference between the northern site of Eldoret (17.8%) together with Lopiding (15.3%) and the southern site of Nakuru 6.9%. This could be explained by the fact that Female Genital Mutilation (FGM) that is suspected to play a role in hepatitis transmission is practiced in the main ethnic groups in the northern region. The main ethnic group in Nakuru is the Kikuyu among whom FGM is less practiced. In Mombasa the Aga Khan hospital site had three times the chronic carriage rate of HBsAg of that found at the Coast General hospital in the

same region. This difference could be attributed to the socio-cultural practices amongst the two groups attending the hospital who have different social economic status with the former being more affluent.

The overall prevalence of HbeAg in HbsAg carriers was 8.8% which is slightly lower than the reported levels of 12 to 17% found in a previous local study in Nairobi (9). However the number of subjects in this study was small and so were not analysed further according to the regions (for comparison) as this would have given a relatively high prevalence.

Determination of HBV DNA in HbsAg positive but HbeAg negative pregnant mothers though an expensive analysis may be considered useful in order to estimate the total proportion (including those who are HbeAg+) who are highly infectious and are likely to transmit the infection to their babies. This would complement HbeAg analysis that alone may not be an entire measure of infectivity.

At the Kenyan coast it has been established that 11.4% of the population was HBsAg positive and 36% of the HBsAg positive women of childbearing age were also positive for HbeAg (8). In yet another study of a rural community in Kenya a perinatal transmission rate of 40% was observed in children born to mothers who were HBeAg positive (10).

This study was designed to evaluate the Hepatitis B serological markers in pregnant women from various geographical sites in Kenya. In countries where Hepatitis B is highly endemic children are infected early in life and so it was important to determine seroprevalence in pregnant women to evaluate the risk of perinatal transmission of this infectious but vaccine preventable disease in Kenya (2).

A total of 2,241 pregnant women (in their 25th to 43rd weeks of gestation) were included in this trial in nine study sites in Kenya. A total of 300 pregnant women were included per study geographical region except in the Rift Valley (including three study sites; D, E, and F) where 741 pregnant women were recruited. The age of the pregnant women ranged from 12 to 43 years with a mean of 25.2 ± 5.41 years. In each geographical region study the age of pregnant women and the number of previous children were comparable. The average annual Incidence Rate of Hepatitis B chronic carriage for Kenya due to vertical transmission in the absence of neonatal vaccination (IR/100,000) in this study estimated at 855 (range 200 to 1346) (Table 8) was high since values more than 500 are considered high. This implies that the perinatal transmission of Hepatitis B may be higher than previously thought despite the relatively low HbeAg antigenaemia and without universal prevention of vertical transmission thousands of neonates will be infected and become chronic carriers (14). The estimated annual incidence of HBsAg chronic carriage due to perinatal transmission in the absence of neonatal vaccination would be 9,234 cases per year [using IR=855/100,000 and 2001 birth cohort of 1,080,000 (WHO data)] with some areas like Eldoret having an IR as high as 12,701 cases (16). This figure refers only to the perinatal transmission excluding early horizontal transmission that contributes more significantly to transmission. The burden of perinatal HBV infection transmission is therefore large and will increase with time leading to many cases of chronic liver diseases and hepatocellular carcinoma. Prevention of perinatal transmission and subsequent horizontal transmission would be achieved by vaccination of all neonates at birth with hepatitis B vaccine. The alternative would be to screen all pregnant women for HBsAg and if positive then to vaccinate their newborns from birth. This is a very expensive exercise and not affordable in a poor setting such as ours (17). The current immunization schedule for Kenya starts at six weeks exposing a large number of newborns to perinatal transmission. A decision needs to be made for Kenya on when to implement the birth dose of hepatitis B vaccination for all newborns in order to alleviate the burden of HBV infection.

In conclusion;

(i) The prevalence of HbsAg in our study population was high at 9.3%. Although the prevalence of HbeAg among the carriers was relatively low at 8%, the burden of the disease is high with an IR of 855 cases on average.
(ii) Although it was not in the objectives of this study a future one should be designed to include follow up of infants born to HbsAg positive mothers to estimate the magnitude of infections caused by horizontal transmission of HBV in the newborn period.
(iii) The seroconversion rate for hepatitis B in our population was 30.2%.

Following the findings of this study The Kenya Paediatrics Association would like to make a

recommendation to the Ministry of Health that Hepatitis B vaccination be given to all newborns at birth during the BCG and oral polio immunisation and the two other subsequent doses with DPT at 10 and 14 weeks respectively.

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