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## ORIGINAL ARTICLE

### DETERMINING SEROPREVALENCE OF HEPATITIS B AND C VIRUS INFECTIONS AND ASSOCIATED RISK FACTORS AMONG APPARENTLY HEALTHY MOTHERS IN ADDIS ABABA, ETHIOPIA

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#### ABSTRACT

**Introduction:** Viral hepatitis is a global public health problem affecting millions of people every year, causing disability and death. Hepatitis B (HBV) and hepatitis C (HCV) viruses spread horizontally, mainly through sexual contact and contaminated needles, and vertically. Both cause considerable morbidity and mortality worldwide. Maternal infection is a risk factor for vertical transmission.

**Objective:** To determine the sero-prevalence of HBsAg and anti-HCV antibody among non-pregnant, apparently healthy mothers and to identify potential risk factors associated with HBV or HCV infection.

**Methods:** A community based cross sectional study was conducted on 454 apparently healthy women, in Addis Ababa, Ethiopia from May 2016 to June 2017. A systematic random sampling method was used to recruit participants.

**Result:** A total of 454 mothers were enrolled. Sero-prevalence of HBsAg and HCV was found to be 3.7% and 2.0%, respectively. HBe antibody was detected in 36.3% of the mothers. None of the participants were co-infected with both viruses. Previous history of liver disease, history of jaundice, HIV infection, and family history of liver disease were significantly associated with HBV infection. Marital status, caring for hepatitis patients, and a history of liver disease were factors significantly associated with HCV infection.

**Conclusion:** Apparently healthy mothers in Addis Ababa had intermediate level of endemicity for hepatitis B and C infections. Routine screening and vaccination of high risk reproductive mothers against HBV is advisable. Emphasis should be given to health education and promotion of infection control practices. Population based studies are strongly recommended to help monitor disease transmission patterns and to design evidence-based interventions against the spread of hepatitis infections in Ethiopia.

**Key words:** Sero-prevalence, risk factors, hepatitis B and C virus, apparently healthy mothers

#### INTRODUCTION

Viral hepatitis is one of the major causes of chronic liver disease and liver failure. In many countries, viral hepatitis is the leading cause of liver transplants. Such end-stage treatments are expensive, easily reaching up to hundreds of thousands of dollars per person (1). In addition, viral hepatitis places a heavy burden on the economy of nations due to loss of productivity. Although the burden of viral hepatitis is very high, it has not received the attention it deserved from the global community (1,2). Various reasons account for this negligence, including the relatively recent discovery of the causative viruses, the mostly silent or benign nature of the disease in its early stages, and the insidious way in which it causes chronic liver disease (1). Among viral hepatitis, hepatitis B and C infections are more often seen in blood, tissue, or organ recipients

and people working or receiving treatment in health care facilities (2). The WHO has estimated that there are more than 2 billion people infected with hepatitis B virus (HBV) and about 378 million HBV carriers worldwide, leading to approximately 620,000 HBV related deaths every year (2,3). Further, it has been reported that 4.5 million new HBV infections occur worldwide each year, of which a quarter progresses to liver disease (2, 3). HBV infection is endemic in Asia and sub-Saharan Africa and thought to be the main etiological factor in over 75% of the chronic liver disease burden (3). In sub-Saharan Africa, the prevalence of HBV surface antigen (HBsAg), which indicates active infection, is 3 - 20% and prevalence of markers for past exposure range from 60 - 99. Since Ethiopia is located in Sub-Saharan Africa, it is considered an area of high endemicity for HBV infection.

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Meanwhile, approximately 170 million individuals are infected with hepatitis C virus worldwide, leading to approximately 250,000 to 350,000 deaths per year (4).

Viral hepatitis is a key public health problem that poses an enormous risk for disease transmission in the general population, especially in children and mothers (2). Reliable epidemiological data are essential for planning health programs and identifying risk groups. For example, the selection of appropriate treatment depends on the genotype of the virus, the presence or absence of cirrhosis, and on the degree of liver fibrosis (2). Therefore, it is important to know the number of people infected with and dying from hepatitis related liver disease, the prevalence of hepatitis related morbidity, and the distribution of genotypes and fibrosis stages (3). Unfortunately, estimates of these key epidemiological parameters are limited by the lack of data from in developing countries like Ethiopia.

In Ethiopia, few community-based sero-epidemiology studies have previously been done on the prevalence of HBV and HCV. These studies, which were investigating the prevalence of these viruses among pregnant mothers, blood donors, HIV infected individuals, health care workers, and medical waste handlers, have indicated that hepatitis infections are endemic in Ethiopia, with varying levels of endemicity from region to region (6). However, community-based hepatitis studies focusing on the prevalence of hepatitis B or/and C infection in mothers have not been conducted. Thus, the current study aims to measure the prevalence of hepatitis B and C infection and contributing factors among apparently healthy mothers. This study is important to define the prevalence of these viral infections and associated risks among apparently healthy mothers, in order to adopt effective preventive strategies, guidelines, and educational programmers. Overall, screening asymptomatic people is important for early diagnosis and intervention.

## METHODS AND MATERIALS

**Study area:** This study was conducted in Addis Ababa city, which has a population of about 3.6 million and an average household size of 5.8 persons per household (7). According to the 2007 national census, 98.6% of the housing units in Addis Ababa have access to safe drinking water. In addition, 14.9% have flush toilets, 70.7% have pit toilets, and 14.3% have no toilet facilities (8).

Values for other reported common indicators of the standard of living for Addis Ababa showed that as of 2012 0.1% of the inhabitants fall into the lowest wealth quintile (7). Adult literacy for men and woman is 93.6% and 79.9% respectively the highest in the nation for both sexes. Further, the civic (urban) infant mortality rate is 45 infant deaths per 1,000 live births, which is less than the nationwide average of 77; at least half of these deaths occurred in the infants' first month of life. HIV prevalence in Addis Ababa mothers was (5.2%) (8).

### **Study design, sample size and sampling procedures:**

A community-based cross-sectional study was conducted from June 2016 to May 2017. Administratively, Addis Ababa is divided into 10 Sub-cities and 116 Woredas. According to 2012 CSA, there are about around 700,000 households. Three sub-cities (Gulele, Kirkos, and Lideta) and seven Woredas within these sub-cities were selected randomly. 50-70 households were selected by health extension workers from each Woreda using systematic random sampling technique. The proportional allocation value, which was used for selection, was calculated by dividing the total number of households in the study area to the total number of selected households. In the end, a total of 454 non-pregnant apparently healthy mothers were included in this study. **Data collection and laboratory investigations:** Five milliliter of venous blood was collected and transported to the laboratory, where it was allowed to clot. Then, serum was separated by centrifugation at room temperature at 3000 rpm for four minutes, and then it was stored at -20 °C until further use.

The principal investigator used a structured interview to collect data on sociodemographic variables and associated risk factors from the study participants. The data was collected the by principal investigator. Sandwich Elisa was used to measure serum level HBsAg, Anti-HBc, and anti-HCV in all samples. Bio-Rad ELISA kits were used for this study (9).

**Data processing and analysis:** Data entry and analysis was done using SPSS version 20.0. The Chi-square test was used to determine the association between serological results and different hepatitis infection associated factors. In addition, it was used to compare categorical data, to evaluate the difference in prevalence between groups in the bivariate logistic analysis, and to determine statistical significance.

To determine the association between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using logistic regression analysis. A  $p$ -value  $< 0.05$  was considered as statistically significant.

**Quality assurance:** Standard operating procedures were followed during blood sample collection, processing, and analysis of data. All ELISA experiments were conducted according to the manufacturer's instruction. The performance of Monolisa™ HBsAg ULTRA was determined using test samples. A sensitivity of 100% and a specificity of 99.94% were recorded. The Monolisa™ Anti-HBc PLUS test resulted in 99.53% sensitivity and 99.5% specificity. The Monolisa™ HCV Ag-Ab ULTRA assay had 100% sensitivity and 99.83% specificity. According to the manufacturer's instruction, positive samples were tested in duplicates before final interpretation. In addition, positive and negative controls were run as the test runs.

**Ethical considerations:** The study received ethical approval from Ethical Review Committee and Institutional Review Board (IRB) of Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology and Parasitology and the Armauer Hansen Research Institute (AHRI). A support letter was obtained from the Addis Ababa Health Bureau.

## RESULTS

**Socio-demographic characteristics:** A total of 454 mothers were involved in this study and the mean age of the study subjects was  $32.75 \pm 5.79$  (SD) years, (range: 20 to 57 years). The majority of the mothers belonged to the 30-34 year old age group (33.5%), followed by 25-29 (25.1%) and 35-39 (25.1%) age groups. In addition, most (81.7%) of the study participants were married.

**Prevalence of HBV and HCV infection:** Overall, 42.3% (95% CI: 39.5-46.1%) of apparently healthy mothers were positive for HBsAg, HBcAb or HCV antibody. The seroprevalence for HBsAg was 17/454 (3.7%), while it was 165/454 (36.3%) for HBcAb and 9/454 (2.0%) for anti-HCV. 17/454 (3.7%) of the study participants were positive for both HBsAg and HBcAb. The prevalence of HBV and HCV infection segregated by age can be found in Table 2.

**Risk factors associated with HBV and HCV infections:** A positive association was observed between HBsAg seropositivity and participants' number of

Among participants with history of liver disease ( $n=31$ ), history of jaundice ( $n=9$ ), and family history of hepatitis ( $n=38$ ), the prevalence of HBsAg was 13.9%, 30.8% and 10.5% respectively. On the other hand, age, level of education, marital status, occupation, alcohol consumption habits, caring for hepatitis patients, history of operation or cesarean section, history of female genital mutilation, history of sharp injury, history of blood transfusion, history of ear-piercing, dental procedure, having multiple sexual partner, and history of abortion, did not have significant association with having hepatitis B virus infection.

In addition, binary and multivariate logistic regressions were used to determine the association between the HBV associated risk factors with HBV infection. Few predictor variables showed statistically significant association with HBV infections. Mothers with a history of hepatitis were 5.5 times more likely to be HBV positive than mothers who had no such history (AOR = 5.5; CI (1.8-16.5);  $p=0.003$ ). Mothers who had a history of jaundice (AOR=17.8 CI; (4.0-75.5);  $p=0.03$ ) were 17.8 times more likely to be HBV positive than their counterparts. A statistically significant association was observed between having contact with HBV infected household members and HBV infection ( $P = 0.05$ ). Mothers who had a previous history of household contact were 3.2 times more likely to have infection with HBV than those a without previous history of household contact (AOR= 3.2; CI (1.0-10.4);  $P=0.05$ ) (Table 3).

Participants' age group ( $p=0.012$ ), marital status ( $p=0.05$ ), caring for hepatitis patient ( $p=0.01$ ), blood transfusion history ( $p=0.03$ ), history of jaundice ( $p=0.00$ ), and family history of liver disease ( $p=0.012$ ) were significantly associated with HCV infection (Table 4). However, previous history of dental procedure, body tattooing, having multiple sexual partner, body piercing with sharp objects, and history of surgical procedure showed no significant association with having hepatitis C virus infection. In addition, HCV associated factors and their association with HCV infection was determined using binary and multivariate logistic regression. History of jaundice (AOR=19.2; CI (3.5-104.9);  $p=0.02$ ) and alcohol consumption habit (AOR=6.9; CI (1.3-37.0);  $p=0.02$ ) were significantly associated with HCV positivity at 95% confidence interval. In the multivariate analysis, after adjustment for all other confounding variables, age group, marital status, history of blood transfusion and family history of liver disease had no impact on the acquisition of HCV infection as shown in Table 4.

Table 1: Sociodemographic characteristics of study participants.

Sociodemographic characteristics		Numbers	Percentage (%)
<b>Age group</b>	20-24	21	4.6
	25-29	114	25.1
	30-34	152	33.5
	35-39	114	25.1
	40-44	34	7.5
	45-49	14	3.1
	50-54	3	0.7
<b>Education</b>	55-59	2	0.4
	Illiterate	52	11.5
	1-8	260	57.3
	9-12	106	23.4
<b>Marital status</b>	≥College	36	7.9
	Married	371	81.7
	Unmarried	9	2.0
	Divorced	67	14.8
<b>Occupation</b>	widowed	7	1.5
	Housewife	249	54.9
	Employed	57	12.5
	Daily laborer	55	12.1
<b>Family size</b>	Private	93	20.8
	1-4	194	42.5
	5-6	210	46.2
	≥7	50	11.0
<b>Total</b>		454	100

Table 2: The prevalence of HBsAg, HBcAb and Anti-HCV antibodies segregated by sociodemographic characteristics.

Sociodemographic characteristics		Numbers	HBsAg Positive (%)	HBcAb Positive (%)	HCV Positive (%)
<b>Age group</b>	20-24	21	0(0.0)	6(28.6)	0(0.0)
	25-29	114	8(7.0)	34(29.9)	1(0.9)
	30-34	152	7(4.6)	55(36.2)	4(2.6)
	35-39	114	2(1.8)	40(35.1)	3(2.6)
	40-44	34	0(0.0)	19(55.9)	0(0.0)
	45-49	14	0(0.0)	8(57.1)	0(0.0)
	50-54	3	0(0.0)	2(66.7)	1(33.3)
	55-59	2	0(0.0)	1(50.0)	0(0.0)
<b>Education</b>	Illiterate	52	0(0.0)	11(21.2)	1(2.0)
	1-8	260	12(4.6)	97(37.3)	4(1.5)
	9-12	106	3(2.8)	43(41.0)	3(2.8)
	≥College	36	2(5.6)	14(38.9)	1(2.8)
<b>Marital status</b>	Married	371	16(4.3)	137(37.2)	4(1.1)
	Unmarried	9	1(11.1)	2(22.2)	0(0.0)
	Divorced	67	0(0.0)	24(35.8)	4(6.0)
	widowed	7	0(0.0)	1(28.6)	1(14.3)
<b>Occupation</b>	Housewife	249	11(4.5)	92(36.9)	4(1.6)
	Employed	57	0(0.0)	23(40.4)	1(1.8)
	Daily laborer	55	4(7.3)	16(29.1)	2(3.6)
	Private	93	2(2.2)	34(36.6)	2(2.2)
<b>Family size</b>	1-4	194	3(1.5)	66(34.0)	2(1.0)
	5-6	210	9(4.3)	77(36.7)	7(3.3)
	≥7	50	5(10.0)	22(44.0)	0(0.0)
<b>Total</b>		454	17(3.7)	165(36.3)	9(2.0)

Table 3: Association between different risk factors and HBV infection.

s.no	Variables	HBsAg test result		COR (CI) 95%	AOR (CI) 95%	P-value
		Positive %	Negative			
1.	Diagnosis history of hepatitis					
	Yes	5(13.9)	31	5.5(1.8-16)	5.4(1.8-16.5)	0.03
No	12(2.9)	406	1.0(reference)			
2.	Caring of hepatitis patients					
	Yes	2(4.8)	40	1.3(0.3-6.0)	1.0(reference)	0.72
No	15(3.6)	397	1.0(reference)			
3.	Operation					
	Yes	4(2.5)	153	1.7(0.6-5.0)	1.0(reference)	0.34
No	13(4.4)	284	1.0(reference)			
4.	Sharp injury					
	Yes	10(4.2)	227	1.3(0.5-3.6)	1.0(reference)	0.57
No	7(3.2)	210	1.0(reference)			
5.	Blood transfusion					
	Yes	2(2.7)	25	2.3(0.5-10.6)	1.0(reference)	0.29
No	15(3.5)	412	1.0(reference)			
6.	History of jaundice					
	Yes	4(30.8)	9	14.6(4.0-54)	17.8(4-75.8)	0.03
No	13(3.0)	428	1.0(reference)			
7.	Tattoo					
	Yes	3(3.4)	86	1.2(0.33-4.13)	1.0(reference)	0.82
No	14(3.8)	351	1.0(reference)			
8.	Ear-piercing					
	Yes	16(3.6)	425	1.2(0.06-3.7)	1.0(reference)	0.46
No	1(7.7)	12	1.0(reference)			
9.	Dental procedure					
	Yes	5(3.2)	152	1.2(0.27-2.3)	1.0(reference)	0.66
No	12(4.3)	285	1.0(reference)			
10.	Multiple sexual partner					
	Yes	4(5.8)	65	1.3(0.25-7.1)	1.0(reference)	0.75
No	13(3.4)	374	1.0(reference)			
11.	Family history of hepatitis					
	Yes	4(9.5)	38	3.2(1.0-10.4)	4.3(1.5-11)	0.05
No	13(3.2)	399	1.0(reference)			
12.	History of abortion					
	Yes	3(2.2)	136	2.0(0.2-3.4)	1.0(reference)	0.25
No	14(4.4)	301	1.0(reference)			
13.	Vaccine					
	Yes	0	4	0.0	1.0(reference)	0.99
No	17	433	1.0(reference)			

Table 4: Association between risk factors and HCV infection.

Variables	HCV test result		COR(CI)95%	AOR(CI) 95%	P value
	Positive (%)	Negative (%)			
Marital status					0.01
Married	4(1.1)	367(98.9)	1.0		
Unmarried	0(0.0)	9(100)	NA		-
Divorced	4(6.0)	63(94.0)	5.8(1.4-23.8)	9.3(1.5-58.8)	0.01
Widowed	1(14.3)	6(85.7)	1.5(1.48-15.4)	34.5(1.2-100.0)	0.004
Care of hepatitis patient					
Yes					
No	3(7.1)	39(92.9)	5.2(1.25-21.6)		0.59
Blood transfusion					
Yes	2(7.7)	24(92.3)	5.0(1.0-25.4)		0.21
No	7(1.7)	421(98.3)	1.0		
History of jaundice					
Yes	2(8.3)	11(91.7)	11.3(2.1-60.6)	19.2(3.5-104.9)	0.02
No	7(1.6)	434(98.4)	1.0	1.0	
Family history of liver hepatitis					
Yes	3(7.1)	39(92.9)	5.2(1.2-21.6)		0.72
No	6(1.5)	406(98.5)	1.0		
Alcohol consumption					
Yes			5.6(1.5-21.3)	6.9(1.3-37.0)	0.03
No	4(6.7)	56(93.3)	1.0	1.0	
Total %	5(1.3)	389(98.7)			
	9(2.0)	445(98.0)			454

## DISCUSSION

HBV and HCV infections are significant health problems around the globe. Both infections are associated with a broad range of clinical presentations ranging from clinically asymptomatic, acute hepatitis to chronic hepatitis and liver cirrhosis (5). Population based serological studies conducted on viral hepatitis have demonstrated the diversity of epidemiological patterns with regard to the risk of acquiring infection related to personal attributes, place and risk distribution (10). Screening asymptomatic people is important for early diagnosis and intervention, which may improve health outcomes and enhance our understanding of diseases transmission pattern (10).

The results from this study revealed 3.7% seroprevalence of HBsAg, which lies within the established standard intermediate endemicity of hepatitis B prevalence (11). Even though this finding in agreement with the WHO intermediate level of endemicity, it may not represent the whole community. Because this study mainly focused on apparently healthy mothers in Addis Ababa, who represent only female populations more inclusive sample from both sexes are needed to justify the WHO established endemicity classifications.

This result compares favorably with the results of a study conducted in Jimma on 493 pregnant women, where the seroprevalence was 3.7% (12), and a study done in Addis Ababa on delivering women, which had seroprevalence of 3.0 % (13), and studies done in Woldia and south Gondar on diabetes and non-diabetic patients, where the seroprevalence for both was 3.7% (14). The reported HBsAg prevalence of this study was higher than what was reported in studies conducted in Dessie on healthy female blood donors (1.5%) (15), in Addis Ababa on public health center cleaners (3.57%) (6), and in Jimma on blood donors, 2.1% (16).

In contrast, it was lower than that reported on studies conducted in Dessie on pregnant women (4.9%) (17), in Gondar, Bahir Dar, Dessie and Mekelle on blood donors (6.2%) (18), Shashemene (5.7%) (19), Ghana (10.6%) (20), and in Addis Ababa within the community (6.2%) (21). The observed discrepancies in HBV distribution across different geographical locations might be attributed by variation in socio-demographic characteristics of the study population, such as socio-cultural environment, traditional prac-

Moreover, the variation could also be due to circulating genotypes, which is responsible for disease severity as well as treatment responses, methodological difference (test method, study design etc.), the level of awareness, and behavioral differences for the potential risk factors of HBV infection.

The overall seroprevalence of HCV (2.0%) in this study is similar to a study conducted in Gabon (2.1%) (22), a study conducted in Sudan (1.9%) (23), seroprevalence of the general population of Ethiopia (2.0%) (24), a study conducted in Poland (1.9%) (25) and a study conducted in a northern Ethiopia volunteer testing and counseling center (2.0 %) (26). This finding was also comparable with other reports found in health center cleaners in Addis Ababa (1.6%)(6) and in blood banks at Gondar, Bahir Dar, Dessie and Mekele (1.7 %) (15).

In contrast, this finding was higher in a report from Dessie blood bank 0.39% (15), Jimma adult blood donors 0.2 % (17), Debretabor hospital among HIV patients 1.3% (25), Arba Minch blood bank, in southern Ethiopia 0.0% (24), in Dessie among pregnant mothers 0.8 % (28), 0.19% in Indian women (29), and in Nigerian pregnant women 1.4% (30). Moreover, our finding was lower than what was reported in studies from Gondar (5 %) (31), Hawassa (6%), Kigali (5.2%), Rwanda (32), and Ghana (7.7%) (33). These differences may be attributed to difference in sensitivity and specificity of methods used, e.g. ELISA vs. Rapid test. Another reason for these differences could be using different study designs. Commonly, facility based study designs may lead to overestimates because subjects may be experiencing symptoms. In addition, convenient sampling techniques may be subject to bias, and population variation, types of risk exposure and sample size may impact results in different studies.

In this study, most of sociodemographic variables were not associated with HCV infection. However, caring for hepatitis patients and history of jaundice were significantly associated with the occurrence of HCV infection. These findings were supported by a study conducted in Ethiopian public hospitals (31). In the present study, no statistical significant differences were observed for HBV and HCV infections in terms of age, sex, occupation and educational status. This study was supported by a study reported in Amhara regional state general populations (34) and a study done in Felege Hiwot Referral Hospital, northwest Ethiopia (35).

The highest prevalence of HBsAg was detected among apparently healthy mothers, who were 25-29 or 30-34 years old. This was in agreement with studies conducted in Shashemene General Hospital, southern Ethiopia; Shenyang, China; Debretabor hospital, South Gondar, Northwest Ethiopia; Addis Ababa, Ethiopia (5, 31, 36, 37), and Nigeria (30). The observed high prevalence of HBV positivity among the younger age group could be due to high risk health behaviors.

The present study investigated the association between HBV infection and level of education. Higher prevalence was observed among those with a primary level of education. This finding was in agreement with a previous study conducted in Ethiopia among pregnant women (37). History of liver disease, history of jaundice, and family history of liver disease were significantly associated with HBV infection and were important predictors of HBV infection. These findings were supported by a study conducted in Bahirdar (27), in Debretabor hospital (28), Gondar hospital, (30), and in Karachi, Pakistan (38).

## Conclusion

The present study showed 3.7% and 2.0% for chronic HBV and HCV infection prevalence, respectively. This indicated an intermediate prevalence of HBV and HCV infection among apparently healthy mothers. This was in line with World Health Organization's regional HBV and HCV infection burden estimates (4, 5). There is a need for timely intervention strategies to alleviate the burden of HBV and HCV infection in this community. This prevalence rate also calls for additional efforts regarding active screening and vaccination for young adults and public health education campaigns in the media to promote better awareness of viral hepatitis risk factors.

In this study, mothers within the ages of 24-29 and 30-34 had the two highest prevalence of chronic HBV infection, 7.0% and 4.6%, respectively. These age groups may be at high risk, and should require routine screening and vaccine schedules (for HBV). Moreover these mothers of reproductive age are at risk of mother-to-infant transmission in the study areas.

Among the assessed variables and clinical presentations, previous history of liver disease, history of jaundice, and family history of liver disease were significantly associated with HBV infections.

On the other hand, marital status, consumption of alcohol, and history of jaundice were significantly associated with the occurrence of HCV infection.

Scaling up of screening of pregnant and non-pregnant mothers for HBV and HCV infections and provision of health education about the risk factors, the mode of transmissions, and prevention are recommended. Population based studies with additional serological markers and molecular techniques are required to design a working strategy for evidence-based intervention. Therefore, screening apparently healthy mothers is an important tool in early diagnosis and intervention.

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### *Competing interests*

There was no conflict of interest between the authors and other parties.

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