Prevalence of iron, folate, and vitamin B12 deficiencies in 20 to 49 years old women: Ensanut 2012

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Abstract

Objective. To describe the prevalence of iron, folate, and B12 deficiencies in Mexican women of reproductive age from the National Health and Nutrition Survey (Ensanut) 2012. **Materials and methods**. Data came from a national probabilistic survey, representative from rural and urban areas, and different age groups. Blood samples were obtained from 4 263, 20 to 49 years old women for serum ferritin, vitamin B12 and serum folate concentrations. The prevalence of deficiencies, was assessed using adjusted logistic regression models. **Results**. The deficiency of folate was 1.9% (95%CI 1.3-2.8), B12 deficiency was 8.5% (95%CI 6.7-10.1) and iron deficiency was 29.4% (95%CI 26.5-32.2). No differences were found when compared with 2006, 24.8% (95%CI 22.3-27.2). **Conclusions**. The vitamin B12 deficiency is still a problem for women of reproductive age and their offspring in Mexico, while folate deficiency disappeared as a problem. Iron deficiency needs prevention and fortification strategies.

Key words: health surveys; iron deficiency; folic acid deficiency; vitamin B12 deficiency; women; Mexico

Resumen

Objetivo. Describir las prevalencias de deficiencias de hierro, folato y B12 en mujeres mexicanas en edad reproductiva de la Encuesta Nacional de Salud y Nutrición (Ensanut) 2012. **Material y métodos**. Datos derivados de una encuesta probabilística nacional, representativa del área rural y urbana por grupos de edad. Se obtuvo una submuestra de sangre en 4 263 mujeres de 20 a 49 años de edad para medir las concentraciones séricas de ferritina, vitamina B12 y folato. Las deficiencias fueron evaluadas mediante regresiones logísticas múltiples ajustadas. **Resultados**. La prevalencia de deficiencias de folatos fue de 1.9% (IC95% 1.3-2.8) y vitamina B12 de 8.5% (IC95% 6.7-10.1). La deficiencia de hierro no fue diferente a la de 2006: 29.4% (IC95% 26.5-32.2) vs 24.8% (IC95% 22.3-27.2). **Conclusiones**. La prevalencia de deficiencias de hierro y vitamina B12 todavía representan un problema en mujeres mexicanas (20-49), mientras que la deficiencia de folato ha dejado de ser un problema de salud pública. Aún se requieren estrategias de prevención en México.

Palabras clave: encuestas de salud; deficiencia de hierro; deficiencia de ácido fólico; deficiencia de vitamina B12; mujeres; México

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Micronutrient deficiencies are a common public health problem in developing countries. These deficiencies are due to inadequate food intake, poor diet quality, low bioavailability and/or gastrointestinal infections, among other factors.[1, 2]

Within the major problems among women of reproductive ages are, on the one hand, iron deficiency, with implications in the development of anemia, low physical capacity, preterm birth, low birth weight, perinatal mortality, immune response, cognitive and emotional development of the offspring, among others.[3,4] The deficiency of iron could have a close association with anemia, especially due to menstrual losses.[5] It has been shown that iron deficiency results in increases in serum and erythrocyte folate, because they are not used for hemoglobin synthesis.[6]

On the other hand, deficiencies of folate and vitamin B12 are associated with megaloblastic anemia, and both vitamins are involved in various common metabolic pathways.[7,8] Folate deficiency is associated with atherosclerosis,[3] stunting,[9] and during pregnancy it increases the risk of low birth weight, and neural tube closure defects, such as spina bifida, anencephaly and encephalocele.[3,10,11] B12 deficiency is also present in memory loss, dementia and depression,[3,12] cardiovascular disease,[13] and cerebrovascular ischemia.[14]

Despite the lack of information from national surveys, it is estimated that there is a high prevalence of deficiencies of folate and B12 in women.[15] In Latin America, about 40% of adults have marginal or deficient levels of B12, whereas low folate concentrations were less common.[16] In the National Nutrition Survey 1999 in Mexico, it was found that 5% of women showed low levels of folate in the blood; however, these data are difficult to interpret because they comes from the microbiological determination of erythrocyte and serum folate.[17] Similarly, in a subsample of this survey, in addition to the low prevalence of folate, prevalences of 25% and 20% of deficiency and marginal concentrations of B12 in plasma were found.[16] Because of these limitations, this analysis involves similar methodologies that give us the advantage of making comparisons with previous published data.

Based on the insufficient information in Mexico regarding the nutritional status of folate and vitamin B12 in probabilistic surveys, this analysis aims to describe the national prevalences of iron, folate, and B12 deficiencies among Mexican women at reproductive age (20-49 years of age) from the National Health and Nutrition Survey (Ensanut) 2012, and to compare the prevalence of iron deficiency measured by ferritin in the Ensanut 2006 with the Ensanut 2012 results.

Materials and methods

Population and study design

Ensanut had a subsample with blood samples of around one out of three of the participants. We analyzed 4 263 women aged 20 to 49 years with ferritin, vitamin B12, and folate serum determinations, and 900 women 20 to 49 years old with estimated intake of nutrients from the Ensanut 2012. This is a probabilistic survey designed to be representative at national, urban/rural, and four regions of the country levels, visiting about 50 528 households. Further details of the sample design and operation of the survey were published previously.[18]

Collection, preparation and storage of blood samples

Blood samples were withdrawn from the antecubital vein with evacuated tubes.* They were spun down *in situ* at 3 000 rpm on a portable centrifuge.‡ Serum was stored in cryotubes covered with aluminum foil to protect them from light, and stored in liquid nitrogen until delivered to the Nutrition Laboratory of the National Institute of Public Health, Cuernavaca, Mexico.

Laboratory methods

Serum ferritin concentrations, folate and vitamin B12 were measured by immunoassay using chemiluminescent microparticle immunoassay technology and CRP by an immunoturbidimetric method, using latex microparticles at an absorbance of 572 nm (CMIA) using commercial kits§ in a autoanalyzer.#

C-reactive protein (CRP) was used to adjust the values of ferritin, to correct the influence of inflammation by using the equation proposed by Thurnham.[19]

Ferritin, folate and vitamin B12

Iron deficiency was defined when serum ferritin concentrations were below <15 μg/L.[19] Also when the serum concentrations of serum vitamin B12 were lower than 200 pg/ml, they were considered to be deficient,[20] and for folate when lower than 4 ng/ml.[21] Individuals were classified as with inflammation if CRP was >5 mg/L in

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* Vacutainer, Beckton Dickinson Inc (Lakes NJ, USA).
‡ Hettich (Tuttlingen, Germany).
§ ABBOTT Co (Wisbaden, Germany).
# ARCHITECT i 2000 (Wisbaden, Germany).

order to avoid the inflammation effect on ferritin level, as Thurnham and cols. proposed.[22]

Data collection

Information collected includes sociodemographic variables, health and nutrition conditions, anthropometry, diet, and being beneficiary of food assistance programs, like human development program *Oportunidades* (today *Prospera*), milk distribution program *Liconsa* and medical care programs from the Health Sector.

Anthropometry

Measurements of height and weight were obtained by trained and standardized personnel.[23] The height was measured by using a stadiometer* with a precision of 1 mm, and weight was measured with digital scales,* with a 100 g precision. BMI was calculated using the height and weight data (kg/m^2), and women were classified, according to WHO criteria, as[24,25] underweight (<18.5 kg/m^2), normal BMI (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2).

Sociodemographic information

Socioeconomic and family information was obtained through a questionnaire administered to the respondent of the survey. An index of wellbeing (WBI) conditions was calculated using a principal components analysis, which included characteristics of housing construction, housing utilities (water, sewage and electricity), and possession of goods. Based on this information, WBI was the first component.[26] This component accounted for 40.5% of the total variability with a lambda value of 3.24; the WBI was divided into quintiles: extremely low, very low, low, medium, high, and very high. Information on the number of live births from women was gathered, building up four categories: 0 (without children), from 1 to 4, 5 to 9, and more than 10.

Dietary information

In a subsample of the Ensanut 2012, dietary information was obtained using a semi-quantitative food frequency questionnaire of the last seven days (11% of the population). The questionnaire included 102 foods and was administered by trained and standardized staff.[27] Information on food consumption was transformed into energy and nutrient intake, using a food composi-

* SECA brand (Germany).

tion table collected by the National Institute of Public Health (INSP).[28] Further details on the processing of dietary data have been published previously.[29] Finally, the average daily intake of iron, folate, and vitamin B12 was estimated.

Geographic and medical services

Localities with 2 500 inhabitants or less were considered as rural areas, and those with >2 500 inhabitants were urban.

According to the sample frame of Ensanut 2012, the Mexican states were grouped into four major geographic regions: Northern, Center, Mexico City and Southern, taking into account the regionalization.[30] A woman was considered beneficiary of a food aid program when stated that any member of the household received benefits from the program (food or micronutrient supplementation) provided by cash transfer program *Oportunidades* (*Prospera*), or the milk program *Liconsa*, wich distributes among the poorest population fortified milk at subsidized prices. Information on the type of medical services that the participants were attending fell into Mexican Institute of Social Security (IMSS), Institute for Security and Social Services of the State (ISSSTE), the low cost medical insurance program (*Seguro Popular*), private services or other institution (*Petróleos Mexicanos* [Pemex], Army or Navy Mexican Forces), or "not affiliated", because for these medical services it is mandatory to provide vitamins and mineral supplementation to women in reproductive age.

Households were classified as indigenous if at least one member of the family spoke an indigenous language.

Ethical issues

The protocol of Ensanut 2012 was approved by the Committees for Research Ethics and Biosafety from the INSP (Cuernavaca, Morelos, Mexico). Written informed consent from women participating in the study was obtained, after careful explanation of the aims and purposes of the survey.

Statistical analyses

The information is described as frequencies with confidence intervals (95%CI). The prevalence of deficiencies of iron, folate and vitamin B12 were estimated and stratified by location, geographic region, socioeconomic status, living in an indigenous household and program beneficiaries. National average distributions of serum micronutrients, and logarithmically modified

dietary intakes were reported. Diet is included in the models as a covariate.

Comparisons of prevalence of ferritin deficiency between 2006 and 2012 were made by means of confidence intervals at 95%.

Logistic regression models were performed to assess the effect of age, BMI category, area, region, wellbeing, ethnicity (indigenous and non-indigenous), and parity, beneficiaries of nutrition programs, and affiliation to a medical service, dietary intake of iron, folate and vitamin B12. Statistical significance was set at α=0.05.

Sampling weights for the micronutrients sample were computed taking into account all the sampling probabilities of: selection of households, selection of individuals and selection of individuals for blood sample. Additionally, adjustment for non-response and a calibration for age and sex was done.

The analysis was performed using Stata software version 12.1; the adjustment for sample design was performed by the module SVY of complex samples, and the SPSS software version 15.0.

Results

Description of the sample

The response rate of blood samples donor was 87%. We did not find differences between participants and non-responders in wellbeing index. Information from 4 137 women with serum ferritin, folate and vitamin B12 concentrations were analyzed. This sample represents 24 584 667 women 20-49 years old of the Mexican population. The description and distribution of variables from this population are presented in table I. Most women lived in urban areas (78.9%), one out of seven was overweight or obese, 40% were affiliated to *Seguro Popular*, 33% attended public health services, and 86% of women did not smoke.

Table II shows the serum concentrations and the average dietary intake of the nutrients of interest. Serum ferritin concentrations decreased in 2012 (22.9 ug/L, 95%CI 21.6-24.3) compared to those of the 2006 survey (29.9 ug/L, 95%CI 27.9-32.0). The mean concentration of serum folate and vitamin B12 were 11.6 ng/mL (95%CI 11.3-11.8) and 366.4 pg/mL (95%CI 366.4-376.3), respectively.

Folate deficiency

Mexican women 20-49 years old presenting folate deficiency were 1.9% (95%CI 1.3-2.8). Rural areas had a higher prevalence compared with urban areas (3.5%,

Table I
SAMPLE DESCRIPTION. MEXICO, ENSANUT 2012

Characteristic	N (Sample)	Weighting factors	
		N (Thousand)	%
Age (years)			
20-29	1 217	8 581	34.9
30-39	1 663	9 482.1	38.6
40-49	1 257	6 521.5	26.5
Area			
Urban	2 646	19 401.2	78.9
Rural	1 491	5 183.5	21.1
Region			
North	926	4 912.6	20.0
Center	1 462	7 218.3	29.4
Mexico City	121	4 829.7	19.6
South	1 628	7 624.1	31.0
Socioeconomic status			
Q1	866	3 412.5	13.9
Q2	925	4 197.4	17.1
Q3	864	4 406.1	17.9
Q4	786	6 626.4	27.0
Q5	696	5 942.3	24.2
Nutritional state (BMI-WHO classification)			
Underweight	53	296.6	1.2
Overweight	1 437	8208	33.9
Obesity	1 518	8 773.7	36.3
Ethnicity			
Indigenous home	540	2 301.9	9.4
Parity (num. children)			
None	39	220.6	1.1
1 to 4	3 038	18 328.1	90.5
5 to 9	443	1 668	8.2
>10	15	38.2	0.2
Suplements consumption			
Yes	90	641.4	7.8
Medical service			
Public	1 167	8 104.6	33.0
Seguro Popular	2 158	9 827.4	40.0
Private or other institution	48	491.3	2.0
Not affiliated	764	6 161.4	25.1
Smoking			
Do not smoke	3 665	21 159.9	86.1
Every day	166	1 834.1	7.5
Weekly	56	463	1.9
Monthly	25	203.8	0.8
Ocasionally	108	849.5	3.5
At least once a year	11	65.7	0.3
Scholarship			
No schooling	209	765.6	3.1
Primary education	1 380	6 802.2	27.7
Jr High school	1 498	8 640.4	35.1
High school education	650	4 762.1	19.4
University	400	3 614.4	14.7
Programs beneficiaries			
Homes with *Oportunidades*	1 157	3 880.3	16.7
Homes with *Liconsa*	315	2 401.8	10.3

Table II

MEAN SERUM CONCENTRATIONS OF FERRITIN, FOLIC ACID, B12 VITAMIN AND THEIR DIETARY INTAKES. MEXICO, ENSANUT 2012

		Weighting factors		
	n	N (thousand)	Mean	95%CI
Serum concentrations				
Ferritin 2006 (µg/L)	3 524	35 219.6	29.9	(27.9-32.0)
Ferritin 2012 (µg/L)	4 136	24 582.9	22.9	(21.6-24.3)
Folate (ng/mL)	4 029	24 522	11.6	(11.3-11.8)
B12 vitamin (pg/mL)	3 884	23 069	366.4	(356.7-276.3)
Dietary intake/day				
Heme iron (mg)	902	8 186	3.9	(3.8-4.0)
Non heme iron (mg)	902	8 186	5.8	(5.7-6.0)
Total folate (ug)	902	8 186	221.4	(211.4-231.8)
B12 vitamin (ug)	856	7 546	1.9	(1.7-2.2)
C vitamin (mg)	1 000	8 186	78.4	(73.2-83.7)
Total fiber (g)	902	8 186	15.2	(14.2-16.2)

95%CI 2.2-5.7 *vs* 1.5%, 95%CI 0.9-2.5). The Southern with the highest prevalence (4.6%, 95%CI 3.1-6.8) and Central (1%, 95%CI 0.4-2.6) regions showed higher prevalences than the Northern region (0.1%, 95%CI 0-0.6). Quintile 1 of wellbeing had a higher prevalence than quintile 4 (3.6%, 95%CI 2.2-5.9 *vs* 0.4%, 95%CI 0.1-1.6). Indigenous women had a prevalence of 7.2% (95%CI 4.3-11.6), higher compared with non-indigenous (1.4%, 95%CI 0.8-2.3). No differences in the prevalence of folate deficiency by beneficiaries of social or medical care programs were found (table III).

In a multiple logistic model the Southern women were 30.1 fold more likely to be folate deficient than women from the Northern (95%CI 5.01-180.74); the same happened with indigenous *vs* non indigenous women (OR= 3.08; 95%CI 1.31-7.24). By contrast, the beneficiaries of health care systems such as Pemex's, or the Army and Navy Forces's (OR= 0.08; 95%CI 0.01-0.98); as well as those not affiliated's (OR= 0.19, 95%CI 0.05-0.69), had lower prevalence of folate deficiency (table IV).

Table III

PREVALENCE OF FOLIC ACID, B12 VITAMIN AND IRON DEFICIENCIES. MEXICO, ENSANUT 2012

	Folate (2012)				B12 vitamin (2012)				Ferritin (2006)				Ferritin (2012)			
		Weighting factors				Weighting factors				Weighting factors				Weighting factors		
	n	N (Thousands)	%	(95%CI)	n	N (Thousands)	%	(95%CI)	n	N (Thousands)	%	(95%CI)	n	N (Thousands)	%	(95%CI)
National	4 132	24 522	1.9	(1.3-2.8)	3 884	23 069	8.5	(6.7-10.7)	3 511	35 091.0	24.8	(22.3-27.2)	4 136	24 582.9	29.4	(26.5-32.2)
Area																
Urban‡	2 561	19 339	1.5	(0.9-2.5)	2 414	18 189	7.2	(5.1-9.9)	2 334	25 869.4	25.1	(22.2-28.0)	2 645	19 399.5	28.5	(25.0-32.0)
Rural	1 468	5 183	3.5*	(2.2-5.7)	1 372	4 880	13.4*	(10.2-17.4)	1 177	9 221.6	23.8	(19.3-28.2)	1 491	5 183.5	32.5	(28.9-36.1)
Region																
North ‡	879	4 908	0.1	(0-0.6)	825	4 647	1.9	(1.2-3.2)	580	5 689.8	20.3	(16.1-24.5)	925	4 910.9	22.8	(18.7-26.9)
Center	1 416	7 209	1.0*	(0.4-2.6)	1 351	6 922	7.2*	(5-10.2)	1 487	10 866.5	24.9	(20.3-29.4)	1 462	7 218.3	30.5*	(26.8-34.2)
Mexico City	110	4 781	0.9	(0.2-4.6)	102	4 344	10.9*	(4.7-23.3)	126	5 923.3	17.3	(10.4-24.3)	121	4 829.7	30.2	(18.4-42.1)
South	1 624	7 624	4.6*	(3.1-6.8)	1 508	7 155	12.6*	(10-15.7)	1 318	12 611.4	30.2*	(26.7-33.7)	1 628	7 624.1	31.9*	(28.7-35.1)
Socioeconomic status (quintiles)																
Q1‡	850	3 412	3.6	(2.2-5.9)	804	3 269	16.7	(12.6-21.8)	937	7 681.9	28.4	(23.8-33.0)	866	3 412.5	30.5	(25.6-35.4)
Q2	908	4 149	3.1	(1.6-6.0)	844	3 712	12.0*	(8.6-16.4)	891	8 079.3	25.6	(20.5-30.7)	925	4 197.4	35.9	(30.1-41.6)
Q3	849	4 402	2.0	(0.8-4.9)	799	4 139	8.4*	(4.5-15.1)	755	6 989.6	28.6	(23.3-33.8)	863	4 404.3	33.6	(26.4-40.9)
Q4	761	6 626	0.4*	(0.1-1.6)	720	6 293	6.9*	(3.1-14.7)	562	6 807.7	21.7	(15.9-27.4)	786	6 626.4	28.4	(20.9-35.9)
Q5	661	5 933	1.8	(0.6-5.0)	619	5 655	3.3*	(2-5.4)	353	5 385.7	17.8*	(12.5-23.2)	696	5 942.3	21.9*	(17.1-26.8)
Medical service																
Public‡	1 118	8 047	1.4	(0.6-3)	1 058	7 564	6.0	(4.1-8.7)	-	-	-	-	1 167	8 104.6	25.2	(21.0-29.4)
Seguro Popular	2 125	9 823	3.2	(2-5)	1 995	9 320	10.3*	(8.1-12.8)	-	-	-	-	2 158	9 827.4	33.6*	(29.2-38.0)
Private or other institution	46	491	0.2	(0-1.8)	44	486	22.5	(4-66.7)	-	-	-	-	48	491.3	33.8	(2.6-65.0)
Not affiliated	740	6 161	0.8	(0.3-2.5)	689	5 699	7.8	(3.7-15.6)	-	-	-	-	763	6 159.7	27.7	(21.2-34.2)
Ethnicity																
Indigenous‡	535	2 302	7.2	(4.3-11.6)	498	2 184	18.6	(13-25.9)	387	3 135.3	24.3	(17.5-31.1)	540	2 301.9	31.6	(24.4-38.9)
Non indigenous	3 494	22 220	1.4*	(0.8-2.3)	3 288	20 885	7.4*	(5.6-9.9)	3 123	31 942.6	24.8	(22.2-27.4)	3 596	22 281.0	29.1	(26.1-32.2)

* Statistically different *p*<0.05, complex samples logistic regression
‡ Reference

Table IV

Odds ratios for folate, B12 vitamin and iron deficiencies in women from 12 to 49 years old. Mexico, Ensanut 2012

	Model 1a 2012 Folate (>4 ng/mL)		Model 2a 2012 B12 Vitamin (<200 pg/mL)		Model 2a 2012 Ferritin (< 15µg/ L)		Model 3a 2012 vs. 2006 Ferritin (< 15µg/ L)	
	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Survey 2012	-						1.16	(0.93-1.43)
Age (years)	0.99	(0.95-1.03)	0.99	(0.95-1.04)	1.02	(0.99-1.04)	0.98	(0.97-0.99) ‡
BMI classification								
<18.5	1.00		1.00		1.0		1.00	
18.5-24.9	0.78	(0.09-6.50)	2.14	(0.34-13.28)	0.43	(0.14-1.32)	1.39	(0.63-3.09)
25-29.9	0.37	(0.04-3.13)	2.69	(0.44-16.54)	0.28	(0.09-0.84)*	1.00	(0.46-2.20)
>30	0.51	(0.06-4.71)	4.96	(0.75-32.61)	0.18	(0.06-0.55)*	1.14	(0.52-2.52)
Area								
Urban	1.00		1.00		1.00		1.00	
Rural	1.13	(0.57-2.24)	1.25	(0.74-2.13)	1.02	(0.74-1.40)	0.79	(0.63-0.99)*
Region								
North	1.00		1.00		1.00		1.00	
Center	4.82	(0.67-34.73)	3.71	(1.84-7.45) ‡	1.44	(1.01-2.05)*	1.37	(1.05-1.78)*
Mexico City	11.02	(0.92-131.38)	7.03	(3.09-15.97) ‡	1.73	(0.87-3.45)	1.20	(0.77-1.87)
South	30.10	(5.01-180.74) ‡	4.32	(2.27-8.19) ‡	1.55	(1.09-2.20)*	1.65	(1.29-2.06) ‡
Socioeconomic status								
Q1	1.00		1.00		1.00		1.00	
Q2	2.46	(0.86-7.04)	0.83	(0.48-1.42)	1.62	(1.11-2.37)*	0.98	(0.76-1.28)
Q3	1.30	(0.28-5.92)	0.50	(0.24-1.06)	1.46	(0.95-2.25)	1.08	(0.82-1.42)
Q4	0.47	(0.08-2.75)	0.44	(0.15-1.27)	1.02	(0.60-1.76)	0.80	(0.58-1.10)
Q5	4.27	(0.78-23.36)	0.15	(0.06-0.40) ‡	0.93	(0.56-1.55)	0.58	(0.41-0.81)*
Ethnicity								
Indígenous	1.00		1.00		1.00		1.00	
Non indígenous	3.08	(1.31-7.24)*	1.58	(0.91-2.74)	0.77	(0.51-1.15)	0.87	(0.65-1.16)
Parity								
0	1.00		1.00		1.00		-	
1-4	0.33	(0.03-3.60)	1.97	(0.18-21.90)	0.76	(0.20-2.88)	-	
5-9	0.78	(0.06-10.69)	2.50	(0.20-31.17)	0.84	(0.21-3.41)	-	
>10	12.81	(0.47-349.72)	5.69	(0.23-140.52)	1.72	(0.21-13.98)	-	
Medical service								
Public	1.00		1.00		1.00			
Seguro Popular	1.14	(0.31-4.16)	0.60	(0.33-1.11)	1.41	(0.93-2.13)	-	
Private or other institution	0.08	(0.01-0.98)*	3.43	(0.66-17.85)	1.25	(0.39-4.02)	-	
Not affiliated	0.19	(0.05-0.69)*	0.72	(0.24-2.19)	1.33	(0.79-2.24)	-	
Programs beneficiaries								
Liconsa	0.48	(0.07-3.46)	1.88	(0.63-5.58)	0.69	(0.39-1.23)	0.81	(0.55-1.19)
Oportunidades	0.78	(0.31-1.94)	1.52	(0.89-2.61)	0.80	(0.56-1.13)	0.99	(0.79-1.24)
Micronutrient deficiencies								
Folate	-		4.07	(1.69-9.82) ‡	1.16	(0.50-2.70)	-	
B12 vitamin	-		NA		2.08	(1.29-3.36)*	-	
Ferritin	1.27	(0.55-2.95)	1.98	(1.25-3.13) ‡	NA			
Constant	0.01	(0.00-0.32)	0.01	(0.00-0.18)	0.61	(0.10-3.89)	0.61	(0.25-1.50)
Dietary intakes/day	*Model 1b*		*Model 2b*				*Model 3b*	
Survey 2012	-		-				1.19	(0.47-3.03)
Heme iron	2.64	(0.40-17.22)	0.29	(0.06-1.41)	0.73	(0.17-3.18)	0.99	(0.78-1.25)
Non heme iron	0.30	(0.12-0.72)*	2.73	(1.00-7.44)*	1.02	(0.47-3.22)	0.98	(0.93-1.03)
Total folate	1.00	(0.99-1.01)	1.00	(1.00-1.01)	1.00	(1.00-1.01)	1.00	(1.00-1.00)
B12 vitamin	2.09	(0.77-5.66)	0.80	(0.53-1.23)	0.61	(0.42-0.88)*	0.98	(0.87-1.10)
Total fiber	0.99	(0.90-1.08)	0.98	(0.91-1.06)	1.02	(0.98-1.06)	1.00	(0.98-1.01)
Constant	0.03	(0.0-2.65)	0.07	(0.01-0.94)	0.56	(0.17-1.86)	0.33	(0.19-0.59)

* $p<0.05$
‡ $p<0.001$, complex samples logistic regression

NA: not applicable

Vitamin B12 deficiency

The overall national prevalence of vitamin B12 deficiency for women 20-49 years old (table III) was 8.5% (95%CI 6.7-10.1). In rural areas was higher than in urban (13.4%, 95%CI 10.2-17.4 *vs* 7.2%, 95%CI 5.1-9.9). The lowest prevalence was found in the Northern region (1.9%, 95%CI 1.2-3.2), while the highest prevalence was in the Southern region: 12.6% (95%CI 10.2- 17.4). The women in the lowest quintile of the WBI had 16.7% (95%CI 12.6-21.8) of vitamin B12 deficiency, higher than in the other quintiles.

The *Seguro Popular* beneficiaries had vitamin B12 deficiency in 10.3% (95%CI 8.1-12.8), higher than in beneficiaries of other public health systems (6.0%, 95%CI 4.1-8.7). Indigenous women had 18.6% (95%CI 13.0-25.9), greater than for non-indigenous women 7.4% (95%CI 5.6-9.9).

Table IV shows the odd ratios from the logistic model. The Central (OR= 3.71, 95%CI 1.84-7.45), Mexico City (OR= 7.03, 95%CI 3.09-15.97) and Southern (OR= 4.32, 95%CI 2.27- 8.19) regions had a higher risk related to the Northern region. Also, serum folate (OR= 4.07, 95%CI 1.69-9.82) and ferritin deficiencies (OR= 1.98, 95%CI 1.25-3.13) were a risk factor of vitamin B12 deficiency. The quintile 5 of WBI was protective for vitamin B12 deficiency, compared with the lower BWI quintile (OR= 0.15, 95%CI 0.06-0.40). Dietary iron was not a risk factor for vitamin B12 deficiency.

Iron deficiency

The national prevalence of iron deficiency (ID) in women 20-49 years old was 29.4% (95%CI 26.6-32.3). The Southern (31.9%, 95%CI 28.8-35.2), and Central (30.5%, 95%CI 26.9-34.3) regions had higher prevalence compared to the Northern region (22.8%, 95%CI 18.9-27.2). The prevalence found in quintile 1 of WBI (30.5%, 95%CI: 25.8-35.7) was higher than in quintile 5 (21.9% 95%CI 17.5-21.7). The women affiliated with *Seguro Popular* showed a higher prevalence compared with beneficiaries of other Public Health Systems (33.6%, 95%CI 29.4-38.1 *vs* 25.2%, 95%CI 21.2-29.6, respectively).

The prevalence of iron deficiency found in the 2006 survey (24.8%, 95%CI 22.3-27.2), was no different than that of the 2012 survey (29.4%, 95%CI 26.6-32.3). In a logistic model a protective effect (*p*<0.05) of age was observed in the groups 25-29 and >30 y of age. A higher risk showed in the Center and Southern regions: 1.37 (95%CI 1.05-1.78), 1.65 (95%CI 1.29-2.06), respectively, compared with the Northern region (table IV), and having a vitamin B12 deficiency (OR 2.08, 95%CI 1.29-3.36) was a risk for ID.

The quintile 5 WBI (OR= 0.58; 95%CI 0.41-0.81) was protective for ID.

Discussion

The prevalence of vitamin B12 deficiency in women of reproductive age is a problem in Mexico, primarily in rural areas; however, folate deficiency is no longer a problem. In the case of iron deficiency, it rose four percentage points (pp) nationwide between 2006 to 2012.

Based on our knowledge, the information listed in the literature in relation to nutritional status of folate and vitamin B12 in women of reproductive age is limited, making it difficult to compare our results. However, the prevalence of folate and vitamin B12 are very similar to that reported by Allen in 2004 for Mexican women (vitamin B12, 40%).[16]

For the first time, national data on vitamin B12 deficiency in women of reproductive age is presented, showing that the rural population has a higher prevalence of vitamin B12 deficiency. This can be explained because the rural areas in Mexico live on very little vitamin B12 source foods, mainly of animal origin. This is to be considered given the role that this vitamin plays in the generation of chronic diseases,[7,8,13,14,31-33] and because its neurological effects.[34,35]

The serum ferritin levels in women showed an increase of 0.67 pp / year, resulting in an increment of four pp in the six years from the 2006 and the 2012 surveys.

Although this was not significant, there is a greater risk in women belonging to the Central and Southern part of the country. Belonging to the rural area results in a protective effect, perhaps associated with the support from nutrimental supplements provided by food aid programs, even though programs like *Oportunidades* (*Prospera*) and *Liconsa,* that represent around 90% of food aid programs, showed no significant differences. We found no correlation between serum deficiencies and dietary intake of folate, vitamin B12 and iron. Among other subsidies that could contribute to reduce iron deficiency by *Prospera,* it is the cash transfer which probably allows for greater food availability with high nutritional content in *Prospera* homes.[36,37]

Nevertheless, according to Ramírez-Silva and cols., the effects on dietary intake in children were caused by the food supplement and did not coome from enhancements in the home diet.[38] So, in order to avoid maternal iron deficiency and its consequences, like low birth weight, premature delivery, and a host of perinatal complications, especially hemorrhage, it is necessary to monitor the prevalence in the future.[39] Similarly, food consumption must be rich in meat (heme iron) and low in tannins and phytates.[40]

One of the most relevant strengths of this manuscript is its probabilistic design, that provides a sampling that furnishes a national sample, with representativeness of the rural and urban dwelling and geographic regions.

One of the limitations of our analysis is that we do not have information on vitamin B12 in women from previous surveys (Ensanut 2006 and National Nutrition Survey [ENN] 1999) which does not allow us to analyze the trend; however, this information is valuable to the country because it serves as a basis for future comparisons. On the other hand, food consumption was estimated by food frequency questionnaire that do not reflect actual intakes neither correlate well with serum nutrients. But we considered that this method provides a reasonable estimate of micronutrients with the aim of showing the magnitude of the problem.

In relation to folate, comparisons of folic acid deficiency of ENN 1999 with Ensanut 2012 could not be made, since those from 1999 are difficult to interpret because they were erythrocyte folate and serum folate performed with the microbiological assay.[17] Both serum folate and erythrocyte folate are important, as the first is directly related to intake, and even more when we refer to population intake. We hypothesized that the folic acid fortification and supplementation and the high intake of corn tortillas and bread, despite it contains a low amount of folate per 100 g, in Mexico have contributed to the very low prevalence of folate deficiency.

On the other hand, serum folate is an indicator of recent folate intake, and food supplement was no considered in the estimation of total intake of folate. So in order to know women long-term folate status, it is necessary to use in the future red blood cell folate concentrations.

In summary, we present here a moderate deficiency of vitamin B12, almost a null deficiency of folates but a still high prevalence of iron deficiency, that have increased in the last six years in almost four pp, despite the efforts of the federal government to reduce micronutrient deficiencies through two main social programs, first *Prospera,* a cash transfer program that includes a fortified beverage and a tablet containing iron and zinc and one RDA of deficient micronutrients for pregnant and lactating women, and second the program *Liconsa,* that distributes fortified milk with the same micronutrient composition to pregnant and lactating women. This information is relevant for the Mexican population as well as for other developing countries in the Americas region.

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