



Acanthosis Nigricans in the Knuckles of Infants: A Novel Clinical Marker of High Metabolic Risk

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ABSTRACT

Introduction: Acanthosis nigricans (AN) is an early clinical sign of insulin resistance (IR) primarily in adults. The prevalence and association of AN and IR in infants, however, remains uncertain. We aimed to describe the prevalence of AN and its association with IR in a group of Latin-American infants.

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Methods: We studied a random sample of 227 healthy infants between 9 and 24 months of age. After a complete clinical history was obtained and a physical examination was performed, fasting plasma glucose and serum insulin were measured. Three blinded evaluators assessed AN in each patient. Infants with AN were categorized as cases. The HOMA-IR index cutoffs of ≥ 90 th and ≥ 95 th percentiles were considered IR.

Results: There were 49 infants with AN (21.6%) (cases) and 178 without AN (78.4%) (controls). Cases had a significantly higher mean serum

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insulin, fasting plasma glucose, and HOMA-IR levels of 3.67 ± 2.56 $\mu\text{U}/\text{ml}$ vs. 2.42 ± 1.45 $\mu\text{U}/\text{ml}$, $P = 0.005$; 84.2 ± 12.6 mg/dL vs. $77 \pm \text{SD}$ 9.9 mg/dL , $P \leq 0.001$; HOMA-IR 0.77 ± 0.54 vs. 0.46 ± 0.28 , $P \leq 0.001$, respectively. More cases than controls presented HOMA-IR levels \geq 95th percentile (cases 18.4%; controls 0.5%, $P \leq 0.001$) and \geq 90th percentile (cases 32.7%; controls 1.6%, $P \leq 0.001$). AN in the knuckles had a high sensitivity and a negative predictive value (NPV) for detecting patients with HOMA-IR levels above the 95th percentile (sensitivity 90%; NPV 99.4%) and above the 90th percentile (sensitivity 84.2%; NPV 98.3%).

Conclusion: AN in the knuckles is a prevalent, non-invasive, costless, and reliable screening clinical tool that can be used for early detection of infants with IR and a high metabolic risk.

Keywords: Acanthosis nigricans; Hyperinsulinemia; Infants; Insulin resistance; Metabolic risk; Obesity

INTRODUCTION

In the last 25 years, pediatric obesity has doubled and is currently affecting more than 107 million children worldwide [1]. This will inevitably translate into an increase of the already unstoppable obesity and type 2 diabetes pandemic. Moreover, it would lead to a rise in chronic diabetes complications, including mortality, while at the same time worsening the high economic burden for the individual patient and any public health system [2–6]. In addition, many of these individuals will also suffer from other pathophysiological associated illnesses such as non-alcoholic fatty liver disease, polycystic ovarian syndrome, and some types of cancer [7–9]. Early detection of individuals at high metabolic risk and opportune lifestyle interventions are the most reliable measures to counter both epidemics [2, 3, 10]. Therefore, identifying clinical markers that could be used to recognize individuals at a high metabolic risk early in life would not only be desirable but also encouraging.

One of the earliest detectable pathophysiological abnormalities in the natural history of

obesity and type 2 diabetes is insulin resistance (IR) [11–15]. The euglycemic insulin clamp is recognized as the gold standard to diagnose IR; however, it is expensive and technically difficult to perform. Several biochemical indexes such as HOMA-IR, QUICKI, and Matsuda have demonstrated a fairly acceptable diagnostic accuracy for IR, yet they are invasive and require fasting and a clinical indication to assess IR. Recently, numerous studies, primarily in adult populations, have demonstrated that acanthosis nigricans (AN) is an early clinical sign of IR [16, 17]. In a young Latin-American cohort the knuckles were the most common site of AN, particularly in non-obese subjects and was positively associated with IR [17]. Identification of individuals with high metabolic risk much earlier in life would be ideal; however, it is not yet possible. There is a paucity of evidence, however, regarding the association of IR and AN in infants and studies have previously assessed AN only in the neck without a correlation with biochemical markers of IR [18–21]. Therefore, we conducted a cross-sectional comparative study in a group of Latin-American infants between 9 and 24 months of age with the aim of describing the prevalence of AN and determining its association with IR. Secondary objectives were to assess AN diagnostic accuracy for IR and to identify the presence of AN in different weight categories and their association with IR.

METHODS

Study Participants

Approval was obtained from the institutional review board and ethics committee of our university prior to data collection. From April 2016 to April 2017 we enrolled a consecutive and random sample of 227 healthy male and female infants between 9 and 24 months of age born at the “Dr. Jose E. Gonzalez” University Hospital. Parents and/or tutors were contacted via telephone to request their participation in the study. Infants were excluded from the study if they were taking any medication or had any illness that could impair insulin secretion or action, were critically ill, had a congenital

disease, or if their parents or tutor refused to participate in the study. Infants younger than 9 months were arbitrarily excluded as most were not able to withstand the required 4-h fasting as a result of lactation or more frequent eating patterns. All parents or tutors provided informed consent before initiating the evaluation.

Infants who presented AN on the knuckles were categorized as cases. This group was further subdivided into patients with AN only in the knuckles (AN1), patients with AN in the knuckles in addition to other sites (neck, axillae, or elbows) (AN2), and AN in other sites but not on the knuckles (AN3). All participants who did not present AN on the knuckles or any other site were categorized as controls.

Study Protocol

Infants accompanied by their parents or tutors arrived to our institution at about 0800 hours, having at least a 4-h overnight fast. A comprehensive clinical history of each participant was obtained from the parents or tutor. Afterwards, participants underwent a complete physical examination that included anthropometric measures and a standardized close-up photography of the neck, axillae, elbows, and knuckles in order to evaluate and classify AN as previously described [22]. When available, biological mothers of the infants also underwent physical examination, including anthropometric measures and AN evaluation [22]. Afterwards, a single 6-ml blood sample was drawn to obtain plasma glucose and serum insulin levels.

Measurements

Participants' weight and height were determined on a calibrated Seca® 232 and a stadiometer (TAQ Sistemas Médicos, S.A. de C.V., Mexico City, Mexico). Weight and height measurements of the participants' mothers were determined on a calibrated Seca® 700 and a stadiometer (TAQ Sistemas Médicos, S.A. de C.V.). BMI was calculated using anthropometric measurements. Participants were classified as normal, overweight, and obese utilizing the sex-specific World Health Organization BMI for age

birth to 2 years child growth standards [23]. Three experienced, independent, and blinded evaluators assessed AN presence in each patient. Concordance between the observers was evaluated by selecting a random sample of 30 patients. Disagreements between observers were resolved by consensus. Skin phototype was determined as previously reported [24]. Fasting plasma glucose determinations were assessed by the glucose oxidase method (Stat-Fax Spectrophotometer, Awareness Technology, Palm City Fl., intra-assay CV 1.4%, inter-assay CV 0.6%), and for fasting serum insulin, an electrochemiluminescence immunoassay (Hitachi-Cobas e411, Roche, Mannheim, Germany, intra-assay CV $\leq 2\%$) was used. The HOMA-IR index was obtained as previously recommended [25].

Currently, there are no HOMA-IR index cutoff values reported for the age and ethnicity of our study population. Therefore, cutoff values of ≥ 90 th and ≥ 95 th percentiles were considered abnormal.

Statistical Analysis

To determine the sample size needed to measure AN prevalence, a pilot study with 40 infants was conducted in which the presence of AN was documented as 17%. To compare HOMA-IR indexes between cases and controls at least 42 infants per group were required. Categorical variables are reported as percentages and frequencies; continuous variables are reported as means and standard deviations. Normality was determined using the Kolmogorov–Smirnov test. Categorical variables were compared using Pearson's χ^2 test or Fisher's exact test for 2×2 tables. An unpaired Student's *t* test or Mann–Whitney *U* test were used to compare continuous variables according to normality. When more than two groups were compared, one-way ANOVA or the Kruskal–Wallis test was performed according to normality. The Bonferroni correction was used as a post hoc test. Inter-observer reliability coefficients for AN assessment were calculated using Cohen's kappa. $P \leq 0.05$ was considered statistically significant. IBM SPSS, Version 22.0 (IBM Corp., Armonk, NY) was used to perform the statistical analysis.

Statement of Ethics Compliance

Approval was obtained from the institutional review board and ethics committee of our university, Universidad Autonoma de Nuevo Leon. Also, this study complied with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights, and the Springer policy concerning informed consent. All parents or tutors of the infants that participated in the study provided informed consent before enrolling in the study.

RESULTS

Allocation Analysis and AN Prevalence

A total of 227 infants were included in the study. Concordance coefficients between observers for the classification of AN in the neck, axillae, elbows, and knuckles are shown in Table 1. In all cases, Cohen's kappa (inter-observer reliability) was rated high. There were 49 infants with AN (21.6%) and 178 controls (78.4%). Among cases, 44 (19.3%) were categorized as AN1, 5 (2.2%) as AN2, and none as AN3 (Table 2).

Study Population

Anthropometric and Demographic Characteristics

Infants' mean age was 16.4 ± 4.7 months and 47.1% were female. More than 80% were classified as skin phototype 2 and 3. Overweight and obesity were seen in 14.1% and 5.7%, respectively. Overall, 57.3% of the study

population had a family history of diabetes. There were no significant differences in the anthropometric, demographic, or perinatal characteristics between groups with the exception of a higher BMI in cases (17.4 ± 3.42) compared to controls (16.47 ± 2.28 ; $P \leq 0.001$) (Table 2).

Biological Mothers' Characteristics

Only 212/227 (93.4%) biological mothers were able to attend the study consult and underwent physical exploration and AN assessment. Their mean age was 25.5 ± 5.8 years with a mean BMI of 27.35 ± 6.52 kg/m². More than two-thirds of the biological mothers presented AN on any site (66%). There was a major proportion of mothers with AN on any site in the cases group compared to controls, although it was not statistically significant (77.3% vs. 63.1%, respectively; $P = 0.054$). There was an overall prevalence of 4.8% of gestational diabetes mellitus (GDM) of the study participants' mothers. There were no differences in any of the mothers' characteristics between groups (Table 2).

HOMA-IR Index Comparison

Mean fasting insulin, glucose, and HOMA-IR values are shown in Table 3. Cases had a significant higher mean serum insulin level (3.67 ± 2.56 μU/ml) when compared to controls (2.42 ± 1.45 μU/ml, $P = 0.005$). This same trend was seen for fasting plasma glucose (cases 84.2 ± 12.6 mg/dL; controls 77 ± 9.9 mg/dL, $P \leq 0.001$) and HOMA-IR (cases 0.77 ± 0.54 ; controls 0.46 ± 0.28 , $P \leq 0.001$). A major proportion of cases were above the 95th insulin percentile (cases 14.3%; controls 2.2%, P

Table 1 Concordance of acanthosis nigricans classification by clinical assessment

N = 30	Observer 1 vs. 2		Observer 1 vs. 3		Observer 2 vs. 3	
	Agreeing exactly (%)	Kappa	Agreeing exactly (%)	Kappa	Agreeing exactly (%)	Kappa
Neck	90	0.78	96.6	0.93	93.3	0.86
Axillae	83.3	0.66	90.0	0.80	80	0.6
Elbows	93.3	0.86	93.3	0.86	96.6	0.93
Knuckles	90.0	0.77	93.3	0.85	90.0	0.77

Table 2 Demographic and anthropometric characteristics of the study population

Characteristic	Acanthosis nigricans in the knuckles			P value
	All patients (n = 227)	Controls 178 (78.4)	Cases 49 (21.6)	
Patients' characteristics at time of visit				
Mean age (rectified) (SD), months	16.4 (± 4.7)	16.4 (± 4.8)	16.1 (± 4.6)	0.71
Female sex	107 (47.1)	78 (43.8)	29 (59.2)	0.05
Mean weight (SD), kg	10.5 (± 1.63)	10.38 (± 1.56)	10.93 (± 1.82)	0.05
Mean height (SD), m	0.79 (± 0.06)	0.79 (± 0.05)	0.79 (± 0.07)	0.69
Mean BMI (SD), kg/m ²	16.47 (± 2.24)	16.22 (± 1.74)	17.38 (± 3.39)	< 0.001
BMI-for-age percentiles				
≤ 3	13 (5.7)	13 (7.3)	0 (0)	0.09
3 to ≤ 15	28 (12.3)	23 (12.9)	5 (10.2)	
15 to ≤ 50	66 (29.1)	53 (29.8)	13 (26.5)	
50 to ≤ 85	73 (32.2)	55 (30.9)	18 (36.7)	
85 to ≤ 97	34 (15)	27 (15.1)	7 (14.3)	
≥ 97	13 (5.7)	7 (3.9)	6 (12.2)	
Fasting hours, mean (SD)	6.23 (± 3)	6.3 (± 3.06)	5.97 (± 2.81)	0.46
Family history of diabetes	130 (57.3)	105 (59)	25 (51)	0.31
BMI				
Normal	182 (80.2)	145 (81.5)	37 (75.5)	0.08
Overweight	32 (14.1)	26 (14.6)	6 (12.2)	
Obese	13 (5.7)	7 (3.9)	6 (12.2)	
Phototype				
2	81 (35.7)	66 (37.1)	15 (30.6)	0.10
3	110 (48.5)	88 (49.4)	22 (44.9)	
4	35 (15.4)	24 (13.5)	11 (22.4)	
5	1 (0.4)	0 (0)	1 (2)	
AN1			44 (89.8)	
AN2			5 (10.2)	
Perinatal characteristics				
Born to women with GDM	11 (4.8)	10 (5.6)	1 (2)	0.30
Delivery				
Vaginal	125 (57.9)	98 (58.3)	27 (56.3)	0.86
Cesarean section	91 (42.1)	70 (41.7)	21 (43.8)	

Table 2 continued

Characteristic	Acanthosis nigricans in the knuckles			P value
	All patients (n = 227)	Controls 178 (78.4)	Cases 49 (21.6)	
Preterm birth	29 (12.8)	23 (12.9)	6 (12.2)	0.9
Mean gestational age (SD), weeks	38.4 (± 2.9)	38.2 (± 3)	38.9 (± 2.4)	0.16
Mean birth weight (SD), g	3026 (± 626.9)	3020.2 (± 636)	3047.7 (± 597.5)	0.83
Mean height, mean (SD), cm	49.3 (± 4.2)	49.4 (± 4.3)	48.8 (± 3.9)	0.38
Breastfed	204 (90.2)	158 (89.3)	46 (93.9)	0.54
Mean duration of breastfeed (SD), months	7.60 (± 6.16)	7.35 (± 6.2)	8.5 (± 5.99)	0.23
Infant formula	185 (81.9)	146 (82.5)	39 (79.6)	0.84
Mean duration using milk formula (SD), months	9.7 (± 7.2)	9.8 (± 7.2)	9.1 (± 7.4)	0.46
Biological mothers' characteristics				
Mean age (SD), year	25.5 (± 5.8)	25.7 (± 6)	24.8 (± 5.2)	0.34
AN presence, any site	140 (66)	106 (63.1)	34 (77.3)	0.05
GDM presence, any pregnancy	16 (7)	15 (8.4)	1 (2)	0.12
Mean BMI (SD), kg/m ²	27.35 (± 6.52)	27.08 (± 6.78)	28.4 (± 5.36)	0.28
Metformin use during pregnancy	5 (2.2)	5 (2.8)	0 (0)	0.23
Previous use of corticosteroids	15 (6.6)	12 (6.7)	3 (6.1)	0.87

Data are presented as numbers (percentages) of patients unless otherwise indicated

BMI body mass index, AN1 acanthosis nigricans in the knuckle only, AN2 acanthosis nigricans in the knuckles and other site, GDM gestational diabetes mellitus

≤ 0.001) and above the 90th insulin percentile (cases 30.6%; controls 4.4%, $P \leq 0.001$). Similar results were observed when both groups were compared by the glucose 95th percentile cutoff (cases 14.3%; controls 6.1%, $P \leq 0.001$) and 90th percentile cutoff (cases 22.4%; controls 8.4%, $P \leq 0.001$). Finally, more cases than controls presented HOMA-IR levels ≥ 95th percentile (cases 18.4%; controls 0.5%, $P \leq 0.001$) and ≥ 90th percentile (cases 32.7%; controls 1.6%, $P \leq 0.001$).

Table 4 compares the HOMA-IR between cases and controls allocated by BMI. The majority of cases and controls had a normal BMI (cases 75.5%; controls 81.5%) compared to the overweight (cases 12.2%; controls 14.6%) and obese categories (cases 12.2%; controls 3.9%, $P = 0.084$) (Table 2). Cases with a normal BMI

presented higher HOMA-IR levels than controls (cases 0.76 ± 0.54 ; controls 0.45 ± 0.29 , $P = 0.003$). Although not statistically significant, this trend was also observed in overweight (cases 1.04 ± 0.64 ; controls 0.53 ± 0.32 , $P = 0.053$) and obese patients (cases 0.59 ± 0.48 ; controls 0.54 ± 0.30 , $P = 0.945$). A minor proportion of cases and controls with normal BMI presented HOMA-IR levels above percentile 95 (cases 19.9%; controls 1.4%, $P \leq 0.001$) and 90 (cases 29.8%; controls 2.4%, $P \leq 0.001$). This trend continued when overweight cases and controls were compared for HOMA-IR ≥ 95th percentile (cases 33%; controls 0%, $P = 0.002$) and ≥ 90th percentile (cases 7.7%; controls 50%, $P = 0.01$). However, in the obese group there were no differences in insulin or HOMA-IR in both the ≥ 95th and ≥ 90th percentiles.

Table 3 Mean fasting serum insulin, glucose, and HOMA-IR values

Measurements characteristics	Acanthosis nigricans in the knuckles		<i>P</i> value
	Controls 178 (78.4)	Cases 49 (21.6)	
Insulin			
Mean insulin (SD), μ U/ml	2.42 (\pm 1.45)	3.67 (\pm 2.56)	0.005
Insulin percentile 95			
\leq 6.95	174 (97.7)	42 (85.7)	0.001
\geq 6.95	4 (2.2)	7 (14.3)	
Insulin percentile 90			
\leq 5.34	170 (95.5)	34 (69.4)	< 0.001
\geq 5.34	8 (4.4)	15 (30.6)	
Glucose			
Mean glucose (SD), mg/dl	77 (\pm 9.9)	84.2 (\pm 12.6)	< 0.001
Glucose mg/dl			
\leq 100	175 (98.3)	43 (87.8)	< 0.001
\geq 100	3 (1.6)	6 (12.2)	
Glucose percentile 95			
\leq 97	167 (93.8)	42 (85.7)	0.004
\geq 97	11 (6.17)	7 (14.3)	
Glucose percentile 90			
\leq 95	163 (91.5)	38 (77.6)	< 0.001
\geq 95	15 (8.4)	11 (22.4)	
HOMA-IR			
Mean HOMA-IR (SD)	0.46 (\pm 0.28)	0.77 (\pm 0.54)	0.001
HOMA-IR percentile 95			
\leq 1.27	177 (99.4)	40 (81.6)	< 0.001
\geq 1.27	1 (0.5)	9 (18.4)	
HOMA-IR percentile 90			
\leq 1.04	175 (98.3)	33 (67.3)	< 0.001
\geq 1.04	3 (1.6)	16 (32.7)	

Data are presented as numbers (percentages) of patients unless otherwise indicated

Diagnostic Accuracy of AN for IR

The sensitivity and specificity of using AN in the knuckles for detecting patients above the 95th

and 90th percentile of HOMA-IR are shown in Table 5. The presence of AN in the knuckles had a high sensitivity and negative predictive value (NPV) for detecting patients with HOMA-IR

Table 4 Mean fasting Insulin, Glucose and HOMA-IR values analyzed by BMI group

BMI	n	HOMA-IR							
		Mean HOMA-IR (SD)		HOMA-IR ≥ 90th percentile			HOMA-IR ≥ 95th percentile		
		P value		< 1.04	≥ 1.04	P value	< 1.270	≥ 1.270	P value
Normal									
Controls	145	0.45 (± 0.29)	0.003	141 (97.2)	4 (2.8)	< 0.001	143 (98.6)	2 (1.4)	< 0.001
Cases	37	0.76 (± 0.54)		26 (70.2)	11 (29.8)		30 (81.1)	7 (19.9)	
Overweight									
Controls	26	0.53 (± 0.32)	0.05	24 (92.3)	2 (7.7)	0.01	26 (100)	0 (0)	0.002
Cases	6	1.04 (± 0.64)		3 (50)	3 (50)		4 (66.7)	2 (33.3)	
Obese									
Controls	7	0.54 (± 0.30)	0.94	7 (100)	0 (0)	0.09	7 (100)	0 (0)	N/A
Cases	6	0.59 (± 0.48)		4 (66.7)	2 (33.3)		6 (100)	0 (0)	
Insulin									
	n	Insulin							
		Mean insulin (SD), μU/ml		Insulin ≥ 90th percentile			Insulin ≥ 95th percentile		
		P value		≤ 5.34 μU/ml	≥ 5.34 μU/ml	P value	≤ 6.95 μU/ml	≥ 6.95 μU/ml	P value
Normal									
Controls	145	2.37 (± 1.55)	0.009	140 (96.6)	5 (3.4)	< 0.001	143 (98.6)	2 (1.4)	0.001
Cases	37	3.69 (± 2.61)		25 (67.6)	12 (32.4)		32 (86.5)	5 (13.5)	
Overweight									
Controls	26	2.7 (± 1.59)	0.05	25 (96.2)	1 (3.8)	0.002	26 (100)	0 (0)	0.002
Cases	6	5.01 (± 2.62)		3 (50)	3 (50)		4 (66.7)	2 (33.3)	
Obese									
Controls	7	2.84 (± 1.37)	0.62	7 (100)	0 (0)	N/A	7 (100)	0 (0)	N/A
Cases	6	2.26 (± 1.59)		6 (100)	0 (0)		6 (100)	0 (0)	
Glucose									
	n	Glucose							
		Mean glucose (SD), mg/dl		Glucose ≥ 90th percentile			Glucose ≥ 95th percentile		
		P value		≤ 94 mg/dl	≥ 94 mg/dl	P value	≤ 97 mg/dl	≥ 97 mg/dl	P value
Normal									
Controls	145	76.5 (± 9.9)	0.005	136 (93.7)	9 (6.3)	0.04	140 (96.6)	5 (3.4)	0.58
Cases	37	82.1 (± 10.3)		31 (83.8)	6 (16.2)		35 (94.6)	2 (5.4)	
Overweight									
Controls	26	79.5 (± 9.4)	0.57	23 (88.5)	3 (11.5)	0.18	23 (88.5)	3 (11.5)	0.18

Table 4 continued

		Glucose							
		Mean glucose (SD), mg/dl	Glucose \geq 90th percentile			Glucose \geq 95th percentile			
		<i>P</i> value	\leq 94 mg/dl	\geq 94 mg/dl	<i>P</i> value	\leq 97 mg/dl	\geq 97 mg/dl	<i>P</i> value	
Cases	6	83.2 (\pm 15.4)	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)		
Obese									
Controls	7	76.6 (\pm 8)	0.008	7 (100)	0 (0)	0.03	7 (100)	0 (0)	0.03
Cases	6	98.5 (\pm 15.3)		3 (50)	3 (50)		3 (50)	3 (50)	

Data are presented as numbers (percentages) of patients unless otherwise indicated
BMI body mass index, *N/A* not applicable

Table 5 Sensitivity and specificity of acanthosis nigricans as a marker for HOMA-IR detection

	Overall		Overall	
	HOMA IR > 95th percentile	HOMA IR < 95th percentile	HOMA IR > 90th percentile	HOMA IR < 90th percentile
Cases	9	40	16	33
Controls	1	177	3	175
Sensitivity	90		84.2	
Specificity		81.5		84.2
Positive predictive value	18.4		32.6	
Negative predictive value	99.4		98.3	

levels above the 95th percentile (sensitivity 90%; NPV 99.4%) and 90th percentile (sensitivity 84.2%; NPV 98.3%). The positive predictive value and specificity of AN was higher when detecting HOMA-IR values \geq 90th percentile (32.6% and 84.2%, respectively) than \geq 95th percentile (18.4% and 81.5%, respectively).

DISCUSSION

In this large observational study of infants between 9 and 24 months old, we found that one out of every five infants has AN.

Interestingly, AN in the knuckles was positive in all cases and only a minority of patients (2.2%) had AN in other more classical sites (i.e., neck, axillae). Infants with AN were significantly associated with a higher HOMA-IR, fasting plasma glucose, and serum insulin levels; and to our knowledge, for the first time in the literature, we reliably report a \geq 90th (\geq 1.04) and \geq 95th (\geq 1.27) percentile cutoff for HOMA-IR in infants. AN in the knuckles had a high sensitivity (84–90%) and negative predictive value (98–99%) for both \geq 90th and \geq 95th HOMA-IR percentiles.

Previous studies have assessed AN in children at age 2 years and older and have reported a

range of 4.7–19% with a higher prevalence in overweight and obese children (up to 55%) [20, 26–28]. Nevertheless, these studies often limit their assessment of AN to the neck, with a lack of a high inter-observer reliability. Moreover, most of these did not evaluate its association with biochemical markers of IR. The prevalence of AN in our study population was high (21.6%) with the knuckles being the main contributor to this result. When the knuckles and other sites were considered, AN prevalence was only 2.2%. This finding resonates with previous literature in which, in a healthy population of young Latino adults (18–23 years old), the knuckles were the most prevalent site of AN; hence, this suggests that AN in the knuckles might be one of the earliest clinical signs of IR (however, often ignored because of the false notion that the neck or axillae are more common sites) and of paramount importance to direct efforts to the most needed (i.e., individuals at high metabolic risk) in whom early implementation of lifestyle changes could have a profound impact [15–17]. This finding is also supported by the fact that AN seems to progress with time as the prevalence found at ages 18–23 years was around 50% (compared to 21% in our study) [16]. Hence, exclusion of the knuckles may delay the clinical diagnosis of IR until its appearance in classical and well-known locations of AN (i.e., the neck or axillae), thereby losing invaluable time to start management of IR.

To date, the association between AN presence and IR in children has been scarcely studied. Kobaissi et al. [29] aimed to describe the clinical value of AN as a predictor of insulin sensitivity in overweight Hispanic children (8–13 years), concluding that BMI variation was the main predictor of insulin sensitivity, whereas the presence of AN explained only 4% of the estimated variation. Yet children were reported (by a single observer) with a high prevalence of AN (73.3%). The exclusion of patients with normal BMI limits the applicability of AN to a very specific population [29]. In contrast, our study evaluated with high inter-observer reliability the value of AN as a marker of IR at age 2 or younger, independently of their BMI, ensuring its applicability in a boarder

range of individuals. Furthermore, we found higher HOMA-IR index values and basal serum insulin levels in participants with AN in the knuckles compared to controls. This suggests that AN is an early and reliable clinical sign to detect patients at high metabolic risk prior to any other usual clinical manifestations (e.g., weight gain, hyperglycemia, hypertension).

Our reported high sensitivity (84–90%) and negative predictive value (> 98%) of AN on the knuckles set this clinical sign as an accurate, non-invasive, and straightforward marker of IR early in life. In perspective, if AN on the knuckles is not detected in a child, he or she would have a greater than 98% probability of not having IR using both HOMA-IR 90th and 95th percentiles cutoff values. On the other hand, according to our AN prevalence (21.6%) and with our reported diagnostic performance, around 80% of the total population at this early stage of life could reliably and easily be recognized as not having IR.

Until now, few studies have described the HOMA-IR index in children and reliable cutoff values have not been widely accepted. Borgoño et al. [18] compared the HOMA-IR index of 1-year-old children born from mothers with and without GDM and concluded that for infants born from women with GDM, weight gain was positively associated with IR. In contrast to the results of Borgoño et al., the HOMA-IR index values reported in our study were higher (0.21 vs. 0.53, respectively). However, their population consisted mostly of Caucasian patients (75%) and non-specified race/ethnicity (25%) [18]. Besides, the different insulin and glucose laboratory techniques could explain this variation. In fact, compared to Caucasians, higher HOMA-IR index cutoff values have been suggested for Latin-American population [30]. Therefore, as a result of the paucity of evidence, we opted to define the HOMA-IR index cutoff value by percentile and reliably describe the 90th and 95th percentiles.

Our study has several limitations. First, as a result of the study's design, we cannot accurately predict how higher HOMA-IR or high insulin values will impact infants later in life. However, even though this was not the aim of this study, in an ongoing study, we will follow

the participants of this study and assess their complete evolution through time. In fact, the body of evidence is strong regarding the association between metabolic risk and higher HOMA-IR, insulin, and glucose values. Second, we used HOMA-IR to define IR; however, as a result of our large study sample, performing a euglycemic insulin clamp would not have been feasible because of its technical complexity. Moreover, the euglycemic insulin clamp has not been completely validated in this population. At the same time, we followed a strict protocol and, to our knowledge, this is the first study in a large number of infants with a high inter-observer reliability to evaluate AN prevalence, its association with IR, and AN diagnostic accuracy for IR.

CONCLUSION

More than one in five infants aged 9–24 months have AN in the knuckles and its presence is positively associated with IR. Owing to its high sensitivity and negative predictive value, AN in the knuckles is a non-invasive, easy-to-perform, costless, accurate, and reliable screening clinical tool that can be used for early detection of infants with IR and at high metabolic risk. This could translate into a timely, effective, and feasible way to direct preventive strategies to young individuals at high metabolic risk.

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Compliance with Ethics Guidelines. Approval was obtained from the institutional review board and ethics committee of our university, Universidad Autonoma de Nuevo Leon. Also, this study complied with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights, and the Springer policy concerning informed consent. All parents or tutors of the infants that participated in the study provided informed consent before enrolling in the study.

Data Availability. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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