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Causas de anemia en adultos mayores: el papel de la hepcidina, vitamina A y D

Tesis para obtener el grado de Doctor en Ciencias en Nutrición Poblacional

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“La vida se ve mejor a los 93 años…compruébenlo”.

Chavela Vargas.

Contenido

Abreviaturas empleadas

AM	Adultos mayores
AI	Anemia por inflamación
AMC	Anemia de múltiples causas
UEA	Anemia sin explicar *(UEA por sus siglas en inglés)*
AGP	Alfa glicoproteína 1 ácida *(AGP por sus siglas en inglés)*
BMP	Proteínas morfogenéticas óseas *(BMP, por sus siglas en inglés)*
CRP	Proteína C reactiva *(CRP por sus siglas en inglés)*
ENSANUT	Encuesta Nacional de Salud y Nutrición
ECNT	Enfermedad crónica no transmisible
ERC	Enfermedad renal crónica
EPO	Eritropoyetina
HAMP	Péptido antimicromibiano hepcidina *(HAMP por sus siglas en inglés)*
IL-6	Interleucina 6
TRF1/2	Receptor de transferrina *(TRF por sus siglas en inglés)*
TNF	Factor de necrosis tumoral *(TNF por sus siglas en inglés)*
VD	Vitamina D
VA	Vitamina A
DVA	Deficiencia de vitamina A
DVD	Deficiencia de vitamina D
1,25(OH)2D	*1*-alfa,*25*-dihidroxicolecalciferol
25(OH)D	25 hidroxicolecalciferol

1. Introducción

La anemia en el adulto mayor (AM) es un problema creciente de salud pública que se ha asociado con una menor calidad de vida y además, es un predictor de la morbimortalidad en el corto plazo (1)(2)(3). En México, de acuerdo a los datos de la Encuesta Nacional de Salud y Nutrición (ENSANUT) de 2015, la anemia afectó a 1 de cada 4 AM; mientras que en el 2012, ésta fue de 1 en cada 6 AM. A pesar de la alta prevalencia de anemia observada en esta población, no ha habido estudios en México enfocados a identificar sus principales causas, siendo las causas nutricionales, las que tienen el mayor potencial de ser modificables. Es posible que la magnitud de las causas de anemia varíen de acuerdo a lo reportado en AM de países desarrollados (4). Esto resulta de particular interés por los siguientes motivos: 1) la prevalencia de anemia en la población mexicana es mayor a lo observado en países vecinos (Chile, Brasil, Ecuador) (5–7); 2) la esperanza de vida de la población mexicana avanza progresivamente: la tasa de crecimiento media anual de la población de 60 años o más es actualmente de 3.4 por ciento, la mayor comparada con el grupo de 0 a 14 años o con el de 15 a 59 años de edad (8,9); considerando que la prevalencia de anemia aumenta conforme avanza la edad, la carga de enfermedad derivada de la anemia impactará substancialmente en los costos del sector salud mexicano.

La anemia en el AM tiene numerosas causas que no necesariamente están asociadas con la progresión de la edad. En ese sentido, la carga de enfermedad crónica en esta población, asociada con la polifarmacia, dificulta la identificación de las causas de anemia a nivel poblacional (10). Considerando lo anterior, son pocos los estudios poblacionales que han documentado las causas de anemia en AM; aceptándose por la comunidad científica que una tercera parte tiene un origen nutricional, otra tercera parte inflamatoria y finalmente, una tercera parte es inexplicable. (11) (Cuadro A)

La anemia de la enfermedad crónica o anemia de la inflamación (AI) es una condición prevalente en pacientes con infección crónica, enfermedades inflamatorias crónicas no infecciosas, enfermedades autoinmunes, cáncer y enfermedad renal crónica (12,13). La AI se caracteriza por ser normocítica e hipocrómica, asociada con pobre pronóstico y baja calidad de vida. El manejo de la AI usando hierro (por vía oral o intravenosa) o agentes estimulantes de la eritropoyesis es inefectivo para algunos pacientes por lo que son necesarias nuevas terapias alternativas para su control y prevención (14). La patogénesis de la AI es multifactorial, involucra daño en la eritropoyesis (causado por un incremento en citosinas proinflamatorias), daño en la movilización del hierro y acortamiento de la sobrevida

de las células rojas (15). La hepcidina, hormona sintetizada por el hígado en respuesta a un exceso de las reservas corporales de hierro o a un proceso inflamatorio (16,17); controla la homeostasis del hierro y es considerada la principal hormona mediadora de la AI. La hepcidina actúa ligándose a su receptor *ferroportina*, la cual es la única exportadora del hierro intracelular en mamíferos. El eje hepcidina-ferroportina induce la endocitosis y degradación lisosomal de ambas moléculas, resultando en disminuida absorción intestinal, disminuida liberación de hierro de los macrófagos y hepatocitos, disminución de hierro plasmático (12) e inhibición de la utilización del hierro por las células blanco y en particular, las células eritroides (16,18). En desórdenes inflamatorios crónicos, los factores humorales y celulares (*ej. TNF e IL-1*), pueden causar supresión de la respuesta de la médula ósea a eritropoyetina (EPO), afectar la producción de EPO y contribuir a la patogénesis de la AI (19)(20).

En esta población de AM, una alta proporción de la anemia (33%) no tiene una causa conocida y se postula que dicha subpoblación de anémicos podría estar siendo caracterizados por un proceso inflamatorio crónico de baja intensidad.(11)(21) Algunas posibles explicaciones para el incremento en la prevalencia de anemia de etiología desconocida es un estado proinflamatorio subclínico crónico,(22) contribuyendo el envejecimiento en este proceso (23); y una resistencia progresiva de la médula ósea de los progenitores eritroides a la eritropoyetina. En este contexto, la hepcidina podría jugar un rol tanto en la inflamación como en la regulación de la disponibilidad del hierro para la eritropoyesis. Sin embargo, de acuerdo a la evidencia disponible, resulta incierto si el estado pro-inflamatorio crónico asociado con el envejecimiento, causa una sobrerregulación de *HAMP* y contribuye al desarrollo de la anemia.

La inadecuada caracterización de las causas de la anemia derivado de los múltiples criterios empleados para su definición (cuadro B) (22,24–31), conlleva a una inconsistencia en la interpretación de resultados sobre la contribución de la inflamación a la anemia, los valores de hepcidina que la caracterizan y su correlación con interleucina 6, el principal inductor de la inflamación. En el cuadro B, se describen las diferentes conceptualizaciones empleadas para distinguir las causas de anemia en los adultos mayores (ver cuadro B).

Ciertas deficiencias nutricionales pueden modular la respuesta inflamatoria hacia una mayor expresión de interleucinas pro-inflamatorias, contribuyendo al desarrollo de la AI. Las deficiencias de retinol (o vitamina A) y de vitamina D (VD) en diferentes grupos poblacionales se han asociado con mayor riesgo de deficiencia de hierro y anemia (32–

37); y aunque los mecanismos fisiopatológicos son inciertos, es posible que ambas vitaminas participen en modular la expresión de la hepcidina (38–41) (33,42).

Diversos estudios epidemiológicos han mostrado consistencia en la relación de la deficiencia de VD (DVD) y mayor riesgo de anemia en adultos. En el cuadro C, se distinguen los estudios que han analizado dicha asociación. (33)(34)(35)(41)
Esta asociación se puede explicar por la acción antiinflamatoria que tiene la VD o por su acción directa en la supresión de la hepcidina. Estudios in vitro, han mostrado que la 1,25D es un potente regulador de la expresión de *HAMP*(40,43) al actuar directamente como un ligando de unión del receptor de VD al elemento de respuesta de VD en la región promotora del gen. (41) Esto se ha mostrado en diversos estudios (piloto) de suplementación con vitamina D en adultos sanos y jóvenes (<60 años) o con algún grado de enfermedad renal crónica, donde las concentraciones de hepcidina y de algunas citosinas proinflamatorias han disminuido significativamente secundario a la suplementación, en comparación con sujetos controles (40,41,44–46) (ver Cuadro D). Dichas asociaciones no han sido documentadas en AM, donde un estado pro inflamatorio crónico coexiste ya sea derivado del proceso mismo de envejecimiento o de las comorbilidades crónicas que presenta.

Por otro lado, se conoce que el retinol media la mayoría de las funciones vía interacción con los receptores retinoides, los cuales, actúan como factores de transcripción controlando la expresión de diversos genes blanco (47). La VA modula el estatus inflamatorio, de metabolismo de hierro y eritropoyesis (32). A pesar de que diversos estudios han demostrado una interacción entre estos dos nutrientes, los mecanismos moleculares por los cuales, la deficiencia de vitamina A (DVA) puede afectar el metabolismo y absorción del hierro de forma independiente a la sobrerregulación de la expresión de EPO, aún no son completamente entendidos (32). Consistentemente, la suplementación de VA en población de alto riesgo de anemia (niños, mujeres en edad reproductiva o embarazadas), han mostrado mejoría en las concentraciones de Hb, disminuyendo hasta un 26% el riesgo de anemia, de forma independiente de su estatus de hierro y sin afectar la prevalencia de deficiencia de hierro en población más joven.(48) Algunos autores apoyan la hipótesis de que la VA no afecta la absorción del hierro, sino los mecanismos involucrados en la movilización del hierro. (38,39). Se desconoce si la DVA podría tener un rol independiente en el desarrollo de la anemia en los AM. La DVA no es considerada un problema de salud

pública en países desarrollados por lo que no hay estudios que hayan analizado su asociación con etiologías específicas de la anemia. (Ver mapa conceptual)

No hay estudios que documenten la relación entre las deficiencias de VA y VD, su asociación sobre la hepcidina y respuesta inflamatoria y el mayor riesgo de anemia en la población de AM. Pocos estudios en la literatura han empleado y documentado las concentraciones de hepcidina sérica y biomarcadores de inflamación para la adecuada caracterización de la inflamación como causa de anemia en el AM (22,26,27,49); y muy pocos han considerado el ajuste de distintas covariables que puedan confundir dicha relación (50).

En vista de los cambios demográficos que México está experimentando, la adecuada caracterización de las causas de anemia en los AM, resulta imperativa para el desarrollo de intervenciones que incrementen su calidad y esperanza de vida activa. Identificar cuáles causas de anemia pueden ser prevenibles o tratadas, ayudará a mejorar los esquemas de tratamiento de la anemia en el AM.

Esta disertación expande el conocimiento existente al explorar e identificar las principales causas de anemia así como los factores nutricionales que modulan la respuesta inflamatoria y participan como factores de riesgo, en la población de AM.

Por tal motivo, este estudio tiene los siguientes objetivos:

Objetivo general

Identificar las principales causas de anemia y sus factores asociados en adultos mayores de la zona sur de México

Objetivos específicos

Objetivo 1. Describir la magnitud de las principales causas de anemia y sus factores asociados en la población de AM de la zona sur.

Objetivo 1.1. Identificar si existe una asociación entre los niveles de hepcidina con el riesgo de anemia por inflamación y otras etiologías

Objetivo 1.2. Identificar si existe una asociación entre el estatus de VA y VD y el riesgo de anemia por inflamación y otras etiologías

Objetivo 2. Determinar la asociación de los niveles séricos de retinol y de vitamina D con las concentraciones séricas de hepcidina.

Cuadro A. Magnitud de las causas de anemia documentadas en la literatura científica en población de adultos mayores

Referencia (autor, año)	**Guralnik, 2004**	**Ferrucci, 2010**	**Tettamanti, 2010**	**Price, 2011**	**Artz, 2011**	**Waalen, 2011**	**Den Enzel, 2013**	**Santos, 2013**	**Bach, 2014**	**Fonseca, 2015**	**Contreras, 2015**	**Jamieson, 2016**	**Gowanlock, 2016**	**Ernst, 2017**	**Xu, 2017**
Período de estudio	1991-1994 fase 2	1998	2001-2004	2006 y 2010	2005 y 2009	1998 y 2001	1997 y 1999	2005-2008	2004-2005	2013	2012	2007-2008	UK	Alemania	China
Edad AM	>65 años	>65 años	>65 años	>65 años	>65 años	>=65	>85 años	>60 años	>64 años	adultos y AM>65a	>60	adultos y AM>50a	>60 años	*angiopatía	≥60
Nombre del estudio	NHANES III	InCHIANTY, Italia	"Salute e Anemia", Biella Italia	*STANFORD	*CHICAGO	Scripps/Kaiser, Sn Diego	Leiden 85plus, Holanda	São Paulo Ageing & Health Study,	Innsbruck Austria	EMPIRE study	ENSANUT-12	International Polar Inuit Health Survey	NA	LURIC	China Health and Nutrition Survey
Prevalencias (%)															
Anemia	10.6	14.7	14.2	-	-	6.7	23.3	10	21.1	21.0 (19.1–23.1)	13	-	100	16.7	18.9
Hombres	11	-	15.2	-	-	-	-	-	-	22.2	15	28.6	-	-	-
Mujeres	10.2	-	12.6	-	-	-	-	-	-	19.9	12	23.6	-	-	-
Causas de anemia															
Nutricionales	34.3	-	-	-	-	-	-	-	-	-	-	-	-	20.5	-
Deficiencia de hierro	*16.6*	17.44	16	12	25	24.4	11.4	10.6	14.4	29.9 (5.4% IDA)	15.2 (1.5% IDA)	1.1 M y 3.9% H	10.4	6	10.1
Deficiencia de folato	*6.4*	10.46	10.1	-	-	-	5.2	1.8	6.7	-	-	-	-	14.5	
Deficiencia de B12	*5.9*			-	-	-		15.8	2	-	-	-	-		
No Nutricionales	65.7	-	-	-	-	-	-			-	-	-	-	-	-
Enfermedad renal crónica	*8.2*	10.46	15	4	3.4	9.3	7	62	45.1	-	-	-	4.5	20.2	-
Inflamación	*19.7*	24.4	17.4	6	9.8		20.1	35.1	62.1	-	-	-	5.4	34.1	6.5
Sin explicar	33.6	37.2	26.4	35	44	61.8	25.4	12.3	-	-	-	27.5 H y 19.7 M	20.5	25.2	-
Talasemia	-	-	14.4		4.6	-	-	-	-	-	-	-	-	-	-
Otras	-	-	0.6	6	5.7	-	-	-	-	-	-	-	20.7	-	-
Alcohol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terapia de depr andróg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Radiación	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hematológicas	-	-	-	22	7.5	-	-	3.5	-	-	-	-	31.2	-	-
Disfunción tiroidea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemodiálisis	-	-	-	-	-	4.4		-	-	-	-	-	-	-	-
Múltiples causas	-	-	-	-	-	-	33.7	-	-	-	-	-	3.5	-	-

Cuadro B. Conceptualización de las causas de anemia identificadas en la literatura científica en población de adultos mayores

Referencia (autor, año)	Guralnik, 2004	Ferrucci, 2010	Tettamanti, 2010	Price, 2011	Artz, 2011	Waalen, 2011	Den Enzel, 2013	Santos, 2013	Bach, 2014	Fonseca, 2015	Contreras, 2015	Jamieson, 2016	Gowanlock; 2016	Ernst, 2017	Xu; 2017
Nombre del estudio	NHANES III	InCHIANTY	"Salute e Anemia"	Veteranos de Stanford	AM clínica hematol	Scripps/Kaiser study	Leiden 85plus Study	Paulo Ageing & Health S	AM, clínicas de Austria	Portugal	Mexico	Canadá	UK	Alemania	China
Causas de anemia															
Nutricionales															
Deficiencia de hierro	Dos de los siguientes criterios: saturación de transferrina <15%, ferritina <12 ng/mL, y protoporfirina eritrocitaria > 1.24 µM	Razón TfR/log(ferritin)>1.5 o ferritina<12ng/mL	Fe< 50 µg/dL en mujeres y <60 µg/dL en hombres, baja ferritina (<15ng/mL), saturación de transferrina<16%, o incremento en la capacidad total de ligación de hierro (>450g/dL)	1 de los siguientes criterios: Hierro sérico<60 mcg/dl, saturación de transferrina <15% y ferritina <30ng/ml o incremento en 1g/dl posterior al tx con hierro.	Ferritina<50ng/mL	Ferritina<20ng/mL y saturación de transferrina<20%	Ferritina <20 mg/L en hombres y <15 mg/L en mujeres, hierro sérico <10mmol/L, transferrina >3.7 g/L, o % saturación de transferrina<20%	Ferritina sérica<12 mg/L	Ferritina <30 ng/mL	Ferritina <15 ng/mL	Ferritina <15 ng/mL	Ferritina <15 ng/mL	Ferritina <50 ng/mL o ausencia de hierro en médula ósea	Dos de los siguientes criterios: saturación de transferrina <15%, ferritina<12 ng/mL, y MCV<80 um3	Ferritina <15 ng/ml y CRP<10 mg/l
Deficiencia de folato	Folato eritrocitario< 232.49 nM (102.6 ng/mL) ó Y folato sérico<5.89 nM (2.6 ng/mL) en visitas a domicilio	Folato sérico<2.2 ng/mL	Folato sérico<3ng/mL	Por debajo de los límites de laboratorio o respuesta a suplementación con folato.	Folato sérico<4.0 bg/mL o RBC<316ng/mL		Folato plasmático<7.0 nmol/L	Folato sérico<2.6 ng/mL)	Folato sérico <3.8 ng/mL				Folato eritrocitario <340 nmol/L	Folato sérico <2.6 ug/L	
Deficiencia de B12	B_{12}< 147.56 pM (200 pg/mL).	Vitamina B12 <147.56pM (200 pg/mL)	Vitamina B12 <200pg/ML y VCM >95fL	Por debajo de los límites de laboratorio o respuesta a la suplementación con cobalamina.	Vitamina B12 <200pg/mL ó <300pg/mL con AMM>0.4mmol/L		Vitamina B12 <150 pmol/L	Vitamina B12 <200 pg/mL)	Vitamina B12 <141 pmol/L				Vitamina B12 <148 pmol/L	Vitamina B12 <200 ng/L	
No Nutricionales	Sin evidencia de deficiencia de hierro, folato o B12, los sujetos con anemia fueron evaluados para otras causas de anemia.														
Enfermedad renal crón	Depuración de creatinina <30 mL/min.	Depuración de creatinina<30 mL/min	Afectados por insuficiencia renal	eGFR (ecuación MDRD)<30ml/min/1.73ml	eGFR<30mL/min/1.73m^2 fórmula MDRD	Creatinina>1.16 en mujeres y >2 en hombres SIN deficiencia de hierro	Depuración de creatinina<30mil/min		Tasa de filtración glomerular<60 mL/min/1.73 m^2				CKD-EPI fórmula <30 mL/min/1.73 m2	CKD; eGFR < 60 ml/min	
Inflamación	Bajo hierro sérico <10.74 µM [< 60 µg/dL]) sin evidencia de deficiencia de hierro	Hierro circulante < 10.74 M ó [<60 g/dL] sin evidencia de deficiencia de hierro	Bajo hierro circulante en presencia de incrementadas reservas (ferritina>100ng/mL, saturación de transferrina>25% y menor a 50% y reducida capacidad de ligación del hierro (<250g/dL)	Enfermedad inflamatoria aguda activa, incluye infección, enfermedad autoinmune o malignidad	Presencia de alguna enfermedad crónica o subaguda diagnosticada sin ser las condiciones previamente descritas.		CRP>5 ó CRP>5 + ferritina>300µg/L ó Hierro sérico <20µmol/L ó saturación de trasnferrina<20%	Fe sérico <50 mg/dL sin evidencia de anemia por deficiencia de hierro	CRP >0.7 mg/dL				Dx de desórdenes inflamatorios crónicos: vasculitis, enfermedad del tejido conectivo, enfermedades autoinmunes, artritis reumatoide, polymialgia reumática, arteritis de céls gigantes y EII.	CRP > 1 mg/dL ó hierro sérico <60 ug/L	CRP ≥10 mg/l
Sin explicar	Si no cumple las condiciones anteriores	Si no cumple las condiciones anteriores	Si no cumple las condiciones anteriores	Si no cumple las condiciones anteriores	Si no cumple las condiciones anteriores.	Si no cumple las condiciones anteriores	Estatus normal de hierro, normal B12, folato, función renal y valores normales de CRP	Sin definir				Anemia y ferritina sérica >=15	Si no cumple los criterios anteriores	Si no cumple las condiciones anteriores	
Talasemia			Bajo o muy bajo VCM y Hb corpuscular media, incrementada cuenta eritrocitaria, normal o incrementado hierro circulante en presencia de normal o incrementadas reservas de hierro		VCM<82fL con cuenta normal eritrocitaria, sin deficiencia de hierro, no enfermedad inflamatoria y apropiado grupo étnico, confirmado por HbAc2										
Otras			Otros tipos		Condición clínica con alta probabilidad de desarrollar anemia								Etiología de anemia identificada que no se clasifica dentro de los criterios antes señalados		

Continúación Cuadro B.

Referencia (autor, año)	Guralnik, 2004	Ferrucci, 2010	Tettamanti, 2010	Price, 2011	Artz, 2011	Waalen, 2011	Den Enzel, 2013	Santos, 2013	Bach, 2014	Fonseca, 2015	Contreras, 2015	Jamieson, 2016	Gowanlock; 2016	Ernst, 2017	Xu; 2017
Nombre del estudio	NHANES III	InCHIANTY	"Salute e Anemia"	Veteranos de Stanford	AM clínica hematol	Scripps/Kaiser study	Leiden 85plus Study	Paulo Ageing & Health S	AM, clínicas de Austria	Portugal	Mexico	Canadá	UK	Alemania	China
Causas de anemia															
Alcohol				Consumo de alcohol >=80g/d											
Terapia de deprivación de andrógeno				Si terapia hormonal fue previo a los 12 meses											
Radiación				Si había una historia de terapia de radición por cáncer de próstaa											
Hematológicas				Síndrome mielodisplásico de acuerdo a la OMS (sin ref)	Requiere confirmación por aspirado de médula ósea definido por criterios OMS			Sin definir					Sx mielodisplásico confirmado por aspirado de médula ósea o sospecha por parámetros clínicos		
Disfunción tiroidea					Tirotropina<0.1 mcU/mL o >10mcU/mL										
Hemodiálisis						Códigos de procedimientos de ICD9 en pacientes con hx de hemodiálisis									
Múltiples causas							Combinación de uno o más anormalidades de laboratorio (con o sin deficiencia de hierro)						Criterio de múltiples etiolgías sin claridad sobre la principal		

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Cuadro C. Evidencia de la asociación de las concentraciones de Vitamina D con la etiología de anemia en población adulta mayor

Autor	Estudio	Población	Metabolito VD	Resultados	
Perlstein TS; 2011	NHANES III	AM≥60	25(OH)D	La DVD = 33.3% en la población no anémica. 56% en Anemia por inflamación (p=0.008) y 33% en la anemia sin explicar (p=0.55) DVD y anemia OR=1.47, IC95% 1.06-2.05)	
Hirani V; 2015	CHAMP	AM≥60	25(OH)D	20% de prevalencia de anemia en decil más bajo	
			1,25(OH)D	25% de prevalencia de anemia en decil más bajo. A 2-5 años de seguimiento, los niveles basales de 1,25D (no 25D) se asociaron con un incremento en los niveles de Hb (β value 0.001; p = 0.001)	
Ernst; 2017	LURIC	3,299 pacientes con angiografía coronaria	(25OHD)		**DVD (%)**
				Riesgo de anemia: 25OHD <30 nmol/l: 1.52 (1.15–2.02)	30.1 NoA
				Nutricional: 2.99 (95 % CI 2.04–4.38);	56.3
				CKD: 3.27 (95 % CI 2.23–4.79)	58.6
				AI: 2.16 (95 % CI 1.61–2.91);	48.1
				UEA: 1.84 (95 % CI 1.30–2.60).	44.2
			1,25-dihydroxyvitamin D [1,25(OH)2D]	Riesgo de anemia: 1,25(OH)2D <40 pmol/l: 3.59 (2.33–5.52) // 40–70 pmol/l: 1.68 (1.32–2.15)	3.7 NoA
				Nutricional: 5.35 (95 % CI 3.14–9.11);	17
				CKD: 13.27 (95 % CI 8.57–20.57);	33.6
				AI: 2.26 (95 % CI 1.29–3.97);	7.9
				UEA: 0.99 (95 % CI 0.40–2.46).	3.6

Perlstein TS; et al. Prevalence of 25-hydroxyvitamin D deficiency in subgroups of elderly persons with anemia: Association with anemia of inflammation. Blood. 2011;117(10):2800–6.
Hirani V; et al. Cross-sectional and longitudinal associations between the active vitamin D metabolite (1,25 dihydroxyvitamin D) and haemoglobin levels in older Australian men: the Concord Health and Ageing in Men Project. Age (Omaha). 2015;37(1):1–13.
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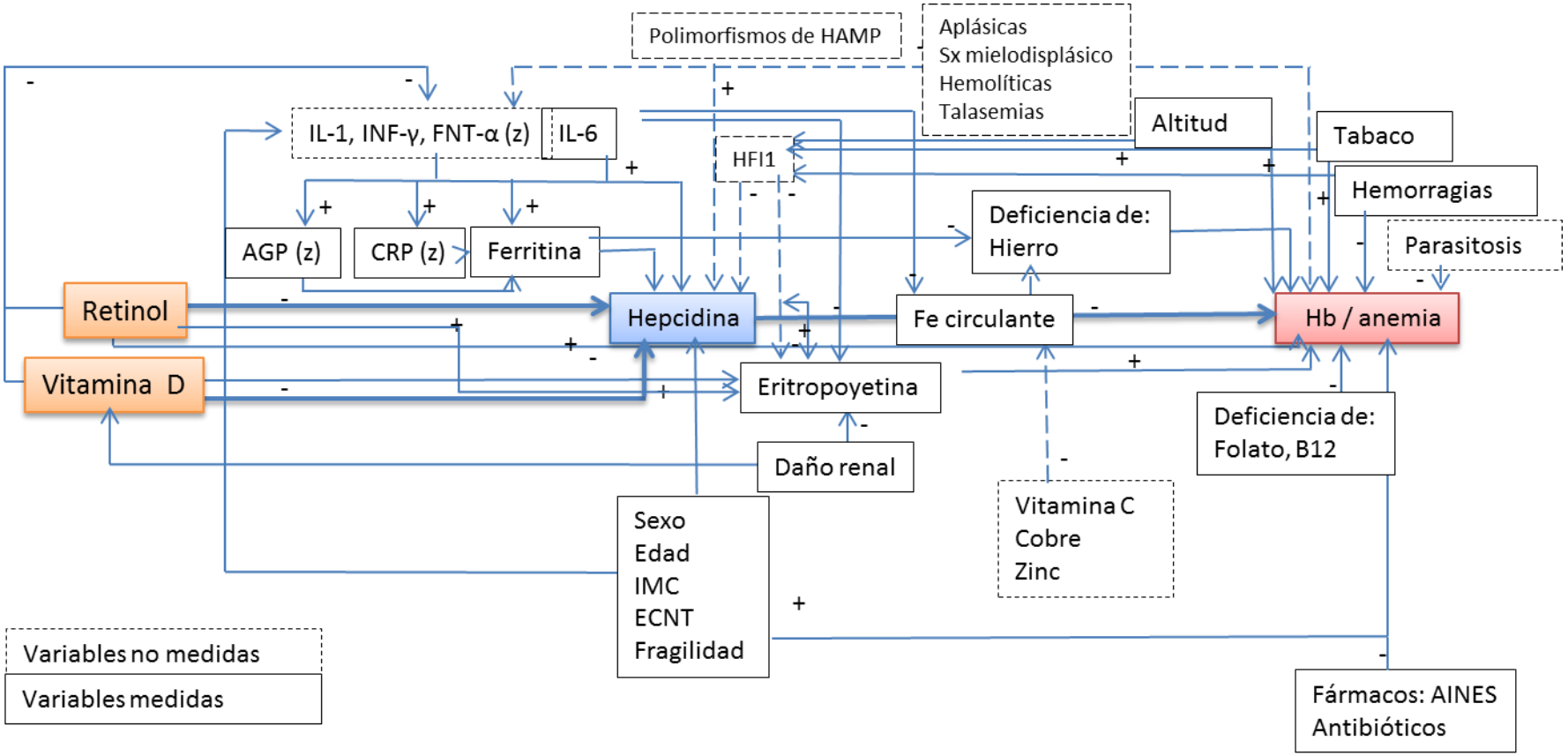
Cuadro D. Evidencia de la asociación de las concentraciones de Vitamina D con hepcidina

Autor	Diseño de estudio	Población	Intervención	Resultados
Bacheta; 2010	Pre-Post	7 voluntarios adultos sanos	Dosis única de vitamina D2 (ergocalciferol, 100,000 IU)	Los niveles circulantes de hepcidina disminuyeron 34% 24h post suplementación y 33% 72h (p<0.05) post suplementación (P<0.01)
Zughaier; 2014	Docle ciego, aleatorizado y controlado por placebo	19 adultos en etapa 2/3 de CKD. CKD (eGFR) 60-89 ml/min/1.73 m2 y 30-59 ml/min/1.73 m 2 para etapas 2 y 3, respectivamente, usando la fórmula MDRDS	Vitamina D3 cholecalciferol, 50,000 IU semanal por 12 semanas, seguido de 50,000 IU cada semana (por 40 semanas) o placebo por un año.	El porcentaje de cambio del basal a los 3 meses en 25(OH)D fue inversamente asociado con el porcentaje de cambio en hepcidina (Spearman rho= -0.38, P=0.02)
Smith; 2016	Docle ciego, aleatorizado y controlado por placebo	28 adultos sanos	Dosis única de 250,000 IU de vitamin D3 o placebo por 1 semana	Las concentraciones de hepcidina plasmática disminuyeron 73% de la medición basal en el grupo suplementado [GMR] = -0.27 (95% CI: -0.11,-0.62); P = 0.005, sin cambios en el grupo placebo (GMR = 0.73 (95% CI: 0.49-1.09); P = 0.11). Las citokinas y ferritina no cambiaron significativamente entre los grupos.
Panwar; 2018	Docle ciego, aleatorizado y controlado por placebo	40 participantes con CKD en etapa 3 o 4 (eGFR 15–60 ml/min/1.73m2)	Calcitriol oral 0.5 mcg diario o idéntico match placebo por 6 semanas	No hubo cambios significativos en hepcidina serica, parámetros de hierro o hemoglobina entre los dos grupos. El caclcitriol no reduce las concentraciones de hepcidina entre sujetos con leve a moderada CKD
Syed; 2017	Transversal	69 pacientes con EII de 5 a19 años	N/A. Insuficiencia de VD (25(OH)D <30 ng/mL)	Insuficiencia de VD (77%) se asoció con mayores niveles de hepcidina (b [SE] = 0.6 [0.2], P = 0.01) y reducida hemoglobina (b [SE] = -0.9 [0.5], P = 0.046)

Bacchetta J; et al. Suppression of iron-regulatory hepcidin by vitamin D. J Am Soc Nephrol. 2014;25(3):564–72.
Zughaier SM; et al. The role of vitamin D in regulating the iron-hepcidin-ferroportin axis in monocytes. J Clin Transl Endocrinol. 2014 1(1):19–25.
Smith; et al. High-dose vitamin D3 reduces circulating hepcidin concentrations: A pilot, randomized, double-blind, placebo-controlled trial in healthy Adults. Clin Nutr. 2017 Aug;36(4):980-985
Panwar B; et al. Effect of calcitriol on serum hepcidin in individuals with chronic kidney disease: A randomized controlled trial. BMC Nephrol. 2018;19(1):1–8.
Syed S; et al. Vitamin D Status Is Associated with Hepcidin and Hemoglobin Concentrations in Children with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2017;23(9):1650–8

Cuadro E. Mapa conceptual



AINES, antiinflamatorios no esteroideos; AGP, Alfa glicoproteína 1 ácida; CRP, proteína C reactiva; ECNT, Enfermedades crónicas no transmisibles; Hb, hemoglobina; HF1, factor inducible de hipoxia 1; FNT-a, factor de necrosis tumoral alfa; IMC, Índice de masa corporal; IL-1, interleucina 1; INF-g, Interferón gamma.

2. Resumen

El presente trabajo explora las principales causas de la anemia y los factores de riesgo asociados en los adultos mayores (AM) de 4 municipios de los estados de Campeche y Yucatán, cuyas prevalencias reportadas en las encuestas pasadas nacionales de salud (ENSANUT 2012 y SAGE-Mex) señalaban a la anemia como un problema de salud pública. Actualmente, no se ha documentado en población latina ni mexicana, cuáles son las principales causas de anemia en este grupo de población. No obstante, la anemia por inflamación (AI) es una causa frecuente en AM de otras poblaciones, por lo que cuantificar su magnitud e identificar si las vitaminas que tienen un rol inmunomodulador (como la vitamina A y vitamina D) pueden estar asociadas con la hepcidina, es de interés para identificar posibles acciones para su prevención y control. El diseño de estudio es transversal cuya recolección de información se realizó en el verano de 2015. Se entrevistaron a 829 AM de la zona urbana de los municipios de Champotón, Campeche, Mérida y Valladolid. Una muestra de suero en estado de ayuno se obtuvo en 803 participantes. Se recolectó información sobre salud física, mental, nutrición, dieta, antropometría y pruebas de funcionamiento físico por personal capacitado, previo consentimiento informado por escrito. Los metabolitos analizados fueron: hepcidina, IL-6, CRP, AGP, hierro sérico, sTfR, ferritina, creatinina, vitamina B12, homocisteína, folato, retinol y 25(OH)D. La anemia estuvo presente en el 35% de la población, sin diferencias por sexo. La principal causa de anemia de etiología conocida fue la enfermedad renal crónica, siendo la diabetes mellitus el más fuerte predictor. La anemia por inflamación, fue la segunda causa más frecuente y ambas causas (ERC y AI) estuvieron caracterizadas por elevada hepcidina e IL-6. Una gran proporción de los AM se clasificó como anemia por causa inexplicable, asociándose en este grupo, los niveles de IL-6, el consumo de antiinflamatorios no esteroideos y la condición de pre-fragilidad. Las causas nutricionales tuvieron una baja contribución a la etiología de la anemia, siendo explicada principalmente por la deficiencia de B12. Las deficiencias de vitamina A y vitamina D se asociaron con anemia de distinta etiología: la DVA se asoció con AI y la DVD con las causas nutricionales y las causas múltiples. La deficiencia de vitamina A pero no de vitamina D, se asoció con mayores niveles de hepcidina. En conclusión, una gran proporción de la anemia en los AM de este estudio tiene un componente inflamatorio, que se asocia con hepcidina y ésta a su vez, con deficiencia de vitamina A.

3. **Artículo 1.** Causes of Anemia in Older Mexican Adults: association between hepcidin concentrations, vitamin A and vitamin D status.

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Journal of Nutrition

Causes of Anemia in Older Mexican Adults: association between hepcidin concentrations, vitamin A and vitamin D status[1-3]

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[3]Abbreviations used: OA, Older Adults; VAD, Vitamin A deficiency; VDD, Vitamin D deficiency; ID, Iron Deficiency; AI, Anemia of Inflammation; ND, Nutritional Deficiencies; CKD, Chronic Kidney Disease; UEA, Unexplained Anemia; IL-6 Interleukin 6; AMC, Anemia of Multiple Causes; HAMP, Hepcidin Antibacterial Protein.

Abstract

Background. Anemia in elderly is a growing public health issue in Mexico, however, its etiology in older Mexican population remains unknow. Whether hepcidin, Vitamin A deficiency (VAD) and D (VDD) might play a role in the etiology of anemia, need to be explored. The aim of this study was to describe the causes of anemia, and the association of hepcidin, VAD and VDD with anemia.

Methods. Cross-sectional study of 803 fasting older adults (OA) from 4 urban localities from Campeche and Yucatán in summer 2015. Anemia etiologies were defined as chronic kidney disease (CKD), nutritional deficiencies (ND) considering iron deficiency (ID) and B12 deficiency, anemia of inflammation (AI), multiple causes (AMC) and unexplained anemia (UEA) along with serum biomarkers. Multinomial regression models were fit to associate the variables by etiology of anemia adjusting by confounders.

Findings. Anemia affected 35% of OA. CKD was present in 29.3% and ND in 7% of anemics. 45% of OA anemics had UEA. Hepcidin and Log-IL-6 were associated with AI (OR=1.01and OR=1.91, $p<0.05$) and CKD (OR=1.01, $p<0.001$; OR=1.29, $p<0.001$, respectively). VAD was associated with AI (OR=3.2, $p<0.001$). VDD was associated with ND and AMC (OR=1.8, OR=5.76, $p<0.05$, respectively). Log-IL6 was associated with UEA (OR=1.08, $p<0.001$).

Interpretation. CKD along inflammation accounted for the main causes of anemia of a known etiology. UEA was frequent and characterized by an inflammatory component. Hepcidin and IL-6 levels were associated to AI and CKD. VAD was associated to AI and VDD was associated with ND and AMC.

Keywords: anemia, inflammation, vitamin A, vitamin D, CKD, hepcidin, unexplained anemia, older adults

Introduction

Anemia in Mexican older adults (OA) is a serious health problem affecting their quality of life and predicting a short-term mortality.(1,2) Data from the National Health and Nutrition Survey (ENSANUT) MC-2016 showed that anemia affected 24.0% of OA (CI95% 20.7-27.6) while Mexico's SAGE data shows that anemia affected 27% OA in 2009 (unpublished data). Even though in these national surveys a high prevalence of anemia in OA stands out, no study has characterized the magnitude of its main causes in the Mexican population.

Few studies have documented the causes of anemia in OA and significant variations in the magnitude of each cause is found between them due to differences in criteria, methods and populations used. (3–11) Despite such differences, one third is due to chronic inflammation and renal impairment and a similar proportion remains unknown (≈30%).(12)

Chronic comorbidities become frequent at older ages and may represent a leading risk factor for anemia in the aging population, as they cause chronic immune activation. Anemia of chronic disease, or anemia of inflammation (AI) is the term referred to immunologically based anemia mediated by inflammatory cytokines that control hepcidin expression, blocking the iron export from hepatocytes, macrophages and enterocytes resulting in poor iron available to cells.(13) Depending on the initial body iron status, as well as the time of exposure to chronic inflammation, AI can also coexist with iron deficiency (ID).(14)

To identify immunomodulatory metabolites that might be implicated in the development of AI can be useful for prevention and treatment. Vitamin A (VA) and Vitamin D (VD) have a crucial role modulating the immune response, and working in interaction both regulate a broader range of gene expression.(15) Both vitamins have been implicated in the development of anemia, suggesting that they play a role in the iron mobilization metabolic pathway.(16)(17) Few studies have explored the association of VD with AI.(18)(19)(20) Such association could happen through direct suppression

of hepcidin expression, since hepcidin antibacterial protein (HAMP) gene has a vitamin D receptor element (VDRE) in their promoter region.(16) This is supported by evidence of VD supplementation causing some reduction in hepcidin concentrations.(21) However other studies do not support this.(22) Evidence that VA is associated with anemia etiologies is also scarce. Supplementation of VA have consistently shown to improve Hb levels in children and pregnancy women at risk of anemia, independently of their body iron status.(17) Some studies in rodents have suggested a possible link between VA status and hepcidin, the mediator of AI. In addition, all those studies have not been focused on OA population in whom the systemic chronic inflammation may be present either by aging or chronic diseases.(23–25)

Contributions of causes of anemia and associated risk factors might be different across populations -specifically from developing countries. The evidence for VA and VD status as causes of anemia in OA is scarce. Therefore, the aim of this study is to analyze the possible causes of anemia and anemia-related factors in older Mexican adults. First, we explored the magnitude of the contribution of each cause of anemia. Secondly, we explored whether hepcidin levels were higher in AI and lower in IDA than non-anemic. Finally, we examined whether VA and VD status are independently associated to AI.

Methods

Study population and data collection

A cross-sectional study in OA aged 60 years and older from four localities of Southern Mexico: Champotón, Campeche, Mérida and Valladolid was carried out between August-September 2015. The states of Campeche and Yucatán were selected because they showed the highest prevalence of OA anemia (20-30%) according to ENSANUT-2012.(26,27)

For the sampling procedure, we used a stratified multistage cluster sample design. We used data from 803 individuals aged 60 years and older out of sample of 829 originally interviewed, who had complete information. Demographic, socioeconomic, health status, and nutritional information were collected using *ad hoc* questionnaires.

Participants were interviewed at home and gave written informed consent letters. The study was approved by the Research, Ethics, and Biosecurity Committees of INSP.

Laboratory analysis

Fasting venous blood samples were drawn and centrifuged *in situ*. Serum was separated and stored in coded cryovials and preserved in liquid nitrogen until delivery to a central laboratory in Cuernavaca, Mexico. Individuals were instructed to refrain from eating any solid or liquid food 8 h before the appointment. The time of the last meal was registered.

Capillary hemoglobin was measured using a portable photometer (Hemocue). C reactive protein (CRP mg/dL), homocysteine (umol/L), B12 (pg/ml), ferritin (range, 0-1000 ng/ml), VitD-25OH (nmol/L) and folate (ng/ml) were measured by immunochemiluminescence method, and creatinine (mg/dl) by a colorimetric method, using commercials Kits (Abbott Diagnostics) in ArchitectCI8200 autoanalyzer. Serum Retinol was determined in an HPLC HP1110 LCDAD (Agilent Technology Waldbronn, Germany), using NovaPack columns C18 4 um 3.9x150 mm with a flux of 1.5 mL/min of the mobile phase methanol, after extraction with 99% ethanol. Serum iron was measured by atomic absorption analyzer (Beckton Dickinson). Soluble transferrin receptor (sTfR) was measured with a commercial immunoassay kit using recombinant human sTfR as standard (Quantikine IVD sTfR ELISA kit; R&D Systems Inc, Minneapolis, MN). According to the manufacturer, the central 5th and 95th percentile of the reference distribution of sTfR concentration is 8.7 to 28.1 nmol/L. Hepcidin was measured using a commercial immunoassay (My Biosource ELISA) kit with a

detection range between 4.69 ng/ml-300 ng/ml. Alpha glycoprotein 1-acid (AGP), erythropoietin (EPO) and interleukin 6 (IL-6) were measured by immunoassay ELISA, using commercials kits (R&D Systems Inc, Minneapolis, MN).

Biochemical analyses were performed at the Centro Médico Nacional Siglo XXI and at the Nutrition Laboratory in the INSP, Cuernavaca; Mexico.

Definition of variables

Ethnicity was considered if an indigenous language was spoken at the household. Household economic status was based on asset ownership and dwelling characteristics. An asset index was considered using a principal component analysis, the first component gave a total variance of 33%. This was divided into tertiles with the uppermost tertile indicating the highest socioeconomic status (SES). Anthropometric information (weight and height) was collected using validated and standardized methods. Body mass index (BMI) was estimated and grouped in two categories: normal weight (18.5–24.9 kg/m^2), and overweight/obese ($\geq 25 kg/m^2$).(28) Chronic comorbidities (hypertension, diabetes, dyslipidemia, myocardial infarction, angina pectoris, heart disease, cirrhosis, arthritis, stroke, chronic lung disease, osteoporosis, and cancer) were considered by self-report if previously diagnosed by a physician. Functional status was based on Katz's index for activities of daily living (ADL) and Lawton scales for those instrumental (IADLs).(29)(30) Drugs consumption was registered and classified as non-steroid anti-inflammatory drugs (NSAID) and steroid anti-inflammatory drugs (SAID). Frailty phenotype was determined according to a slightly modified version of Fried proposal (31) and sarcopenia was defined according to the European Working Group on Sarcopenia in Older People Criteria.(32) Vitamin D deficiency (VDD) was defined at 25(OH)D<50 nmol/L,(33) and vitamin A deficiency (VAD) if serum retinol <20 ug/dL.(34)

Etiology of anemia

Anemia was defined according to WHO criteria: Hb <12 g/dL in women and <13 g/dL in men. (35) Subjects were classified as having anemia related to chronic kidney disease (CKD) if the estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2,(36) or if kidney disease had been previously diagnosed by a physician. ID was considered if serum ferritin concentration <15 ng/mL after correcting for inflammation according Turnham,(37) or if sTfR>28 nmol/L. If no evidence of CKD or ID was detected anemia of inflammation (38) was defined as the presence of cirrhosis, cancer or low serum iron (<60 ug/dL) in the absence of ID, or CRP>5 mg/dL and AGP>1 g/L, or s-ferritin >350 ng/mL. Since ID could coexist with chronic inflammation, renal dysfunction or malignancy, OA with ID and those pathological conditions, anemia was reclassified either as having CKD or AI etiology. Vitamin B12 deficiency (B12D) was defined as <-0.5 SD, considering homocysteine and folate concentration.(39) Folate deficiency (FD) was defined as s-folate <4 ng/mL.(40) Anemia of multiple causes (AMC) was considered if nutritional deficiency (ND) coexists with AI and CKD. If subjects with anemia could not be classified into any of these categories, they were considered to have unexplained anemia (UEA).

Statistical Analysis

Characteristics of the sample are described as proportions, mean and SD, and for variables with biased distributions are described as medians, and interquartile ranges. Bivariate associations for categorical variables between each cause of anemia vs non-anemic group were tested using a Pearson chi-square analysis, adjusting for multiple comparison, for continuous variables, we used a quantile regression model. In both cases, an adjustment by Bonferroni's test was made.

For all comparisons, the non-anemic group was the reference category. Pearson's correlation between hepcidin and biomarkers of inflammation was done using log-transformed variables.

Taking into consideration the standard errors that account for multiple groups comparisons and considering potential confounders, a multinomial logistic regression model clustered by state was used to identify associations between hepcidin concentrations, VAD, VDD and other factors of each cause of anemia. Model 1 explored hepcidin concentrations adjusting by sex, age, ethnicity, and socioeconomic status. Model 2 adjusted by model 1 plus VA, VD status and IL-6 levels. Model 3, adjusted by model 2 plus frailty, BMI, NSAID drugs and type 2 diabetes.

Statistical significance was set at $\alpha=0.05$. All analyses were done in STATA SE V15 (College Station, USA).

Results

Descriptive characteristics of OA are shown in Table 1. Briefly, 60% were women, 33% indigenous, 15% were sarcopenic; 30% had T2-diabetes, 50% hypertension and 13% were taking NSAID. The 10.9% of the whole sample had VB12D, 5% ID, 9% VDD, and 2.5% VAD. Anemia affected 35.3% of OA regardless of sex differences (p=0.957). Anemics were older and with higher frequency of functional disability, inflammation, frailty, diabetes, arthritis, cancer, B12D, VAD and VDD than non-anemics (Table 1).

Table 2 shows distribution of types and causes of anemia. Mild anemia was prevalent (85.9%). IDA accounted for 1.1% of OA with anemia. In this respect, only 2 out of 6 OA with IDA, were classified by ferritin <15 ng/ul criteria, while others were classified with sTfR >28 nmol criteria. Nutritional deficiencies, accounted for 7% of total anemia, due mainly to B12D. No folate deficiency was found in this population. CKD accounted for 29.3% of all causes of anemia. AI was present in 14.6% of anemic OA, and 45% of anemia was unexplained.

The characteristics of participants according to presence and type of anemia are reported in Table 3. In the non-anemic group, participants were significantly younger than those with any etiology of

anemia. Prevalence of VAD and CRP>3 mg/dL was significantly higher in the AI compared with non-anemics ($p<0.05$). Levels of inflammatory markers (hepcidin, CRP, AGP and IL-6) were significantly higher in AI, IL-6 in renal disease; IL-6 and CRP were also significantly higher in AMC compared to non-anemics ($p<0.05$). CRP, hepcidin and AGP levels in the UEA, showed no differences with non-anemics ($p>0.05$). Prevalence of functional disability was significantly higher in AI and renal disease, and frailty in AI, compared with non-anemics ($p<0.05$). No significant differences were found by sex, NSAID nor other drugs consumption, and VDD between groups.

In the univariate analysis, Hb was negatively and significantly correlated with the log-normalized hepcidin level in the whole sample ($r=-0.09$, $p=0.0083$); anemics ($r= -0.18$, $p=0.0019$) and non-anemics (-0.07, $p=0.07$); and was positively correlated with log-normalized ferritin ($r=0.10$, $p=0.0022$). A negative correlation between Log EPO and Hb was found in UEA ($rho= -0.25$, $p=0.0029$).

In the multinomial logistic regression models, the factors associated to higher odds of anemia varied by cause in comparison with non-anemics group (Table 5). In model 1, adjusting for confounders, hepcidin levels was associated to CKD, Nutritional, AI and AMC compared to non anemics ($p<0.05$). Model 2, VAD was associated to CKD, AI; while VDD was associated to CKD, ND, MC and UEA ($p<0.05$). In model 3, hepcidin, Log of IL-6, age, NSAID and diabetes were associated to CKD anemia ($p<0.05$). OA with VDD were associated to ND anemia and AMC ($p<0.05$). Hepcidin, VAD, IL-6, age, indigenous, NSAID consumption were associated to higher odds of AI ($p<0.05$). VDD, IL-6, frail condition were associated to AMC ($p<0.05$). IL-6, prefrail, age, female, indigenous and NSAID consumption were associated to UEA ($p<0.05$) (Table 5).

Discussion

The prevalence of anemia in OA from this four localities in Southern Mexico is a serious health problem, affecting 1 out of 3 OA; a much higher prevalence than reported at national level (22%, unpublished data), other Latin American countries (Brazil and Chile) and in the Mexican-American OA population.(41–43) In an attempt for better describing the underlying etiology, a deeper review of literature regarding the causes of anemia in OA was carried out.(3–11) Nevertheless, a high proportion of anemia could not be classified within any of the criteria used. Among the etiologies studied, renal disease was the most predominant etiology; nutritional anemias, particularly IDA had the lowest contribution.

Chronic inflammation in our study had an important role as etiology of anemia and hepcidin seems to be an important mediator. As hypothesized, higher hepcidin levels were found in subjects with AI and associated to CKD. AI has not a clear definition by clinical parameters, and usually is associated to some underling disease (malignancy, infections, renal disease and autoimmune disease). (44)

In this study, among the etiologies of known cause, CKD contributed the most to anemia compared with other studies where the contribution of CKD to anemia has been lower.(45) Differences could be explained by the criteria used to define CKD, the cut offs used and the population at risk. At this respect, diabetes in our study was a stronger predictor of CKD anemia. Diabetes in Mexican population is a serious health problem affecting to 27.4 % of OA population.(46) In 2013, diabetes along with CKD were in the top three causes of disability-adjusted life years in Mexico.(47) Moreover in 2015, diabetes was the second leading cause of death in OA, accounting for 15.9% of all deaths.(48) Strategies to address diabetes prevention will reduce the burden of associated comorbidities, including renal damage and hence anemia of CKD.

The high levels of hepcidin in CKD may be due partly to inflammatory processes characteristic of the disease or to a decrease in renal clearance [of hepcidin] function, or by the uremic toxins

produced by the damaged nephron cells that stimulate hepcidin synthesis. *In vitro*, indoxyl sulfate has shown to induce hepcidin expression in CKD.(49)

Even when AI and CKD categories showed a pattern of higher hepcidin and IL-6 levels than non-anemics, a lack of correlation between hepcidin and biomarkers of inflammation (CRP, AGP and IL-6) was observed. This lack of correlation could be due to the unknown trajectories of hepcidin expression regarding IL-6 and other inflammatory biomarkers in both, healthy OA and in the presence of chronic comorbidities.(50)

Our results are consistent with other authors, where hepcidin levels have been shown to be higher in inflammation as well as in CKD (6)(7) compared with non-anemics. Nevertheless, in those studies hepcidin have not shown a correlation with IL-6 (3)(51).

A surprising finding was that nutritional deficiencies and particularly ID, had a lower contribution as causal of anemia in this population, similar results were found in other studies.(52)(18) Contrary to our expectations, hepcidin levels were not different among the IDA OA vs the OA non-anemic group and no correlation of ferritin and hepcidin concentrations was observed in this IDA group. Ferritin was one of the criteria used to define ID, but as its concentration increases with age and inflammatory conditions distinguishing IDA from AI is challenging due to the variability of s-ferritin through lower values (<30ng/mL) that can be present in both conditions contributing to false-negative results, despite the correction for inflammation.(53)(37) Our results contrast with previous works (6)(7)(3) where hepcidin levels were significantly lower among IDA elderly individuals using lower ferritin levels as diagnosed criteria.

IDA results found in our study are consistent with ENSANUT 2012 report, where IDA was present in 1.5% of OA population (54), despite the fact that the national survey has the limitation of not considering other causes of anemia that may coexist with ID, and used ferritin as the only biomarker. The contribution of ND to anemia in other OA populations account for ≈1/3 of the

whole OA population, being ID the most important nutritional deficiency.(4)(45) Some differences arise when comparing those data with our findings: 1) the criteria to define ID. Most relevant biomarkers are influenced by inflammation (s-ferritin, transferrin saturation), lack of appropriated adjustment by any inflammation biomarker that could affect the ID prevalence reported(37,55); 2) Temporality of measurement: in the case of the national representative data from NHANES, data were collected in 2002 (4), while our data came from 2015. During that time several events have occurred that may account for the low current prevalence of ID observed in our population: some socials programs that distributed fortified foods, variations in food culture and eating habits, and the contributions of larger life expectancy and chronic diseases epidemiology that brought significant increments in chronic non-transmissible diseases.(47) ID in OA at national level is low in the southern region in comparison with other regions; nevertheless, anemia in the southern region is higher than in other geographical regions of the country, with low contribution of ID to anemia.(54) It is also possible that ID could be underestimated by an inflammatory process and the residual confounding related to the adjustment by inflammation could be higher in the population of this region. Adjustment by inflammation was carried out in this analysis and had a marginal impact on the prevalence of ID (delta 0.25 pp).

None OA in this study had folate deficiency. Folate status was measured in serum that may not reflect the long-term folate status as red blood cells do; however, since the introduction of food fortification with folic acid, FD is no longer considered a public health issue.(56) Vitamin B12 was the main nutritional deficiency in this study, a finding similar to other studies.(45)

A high proportion of anemia was not explained by any of the above criteria. UEA prevalence was similar to that reported in other studies, and being this an exclusion group, the heterogeneity of etiologies not measured is high (rare hematopoiesis disorders, myelodysplastic syndromes, erythropoietin blunted response, among others). (11)(57) In the adjusted model, the Log of IL-6

234 was a predictor of UEA. This finding contrast with previous studies were hepcidin has been
235 measured in serum, plasma and urine (3,6,7)(51) probably because the lack of adjustment for
236 confounders. Artz et cols(58) found a significant correlation of neopterin and Hb levels (r=-0.459,
237 p=0.048), suggesting that a low grade proinflammatory profile underlies the pathophysiology of
238 UEA in OA.(58) Frailty condition has been characterized by an inflammatory profile in OA driven
239 mainly by IL-6.(59) In our study, prefrail condition was associated to UEA after considering
240 confounders. This finding may suggest a potential pro-inflammatory role in the development of
241 anemia of unknown cause. In this regard, a proinflammatory pathway independent of hepcidin may
242 induce anemia by suppressing the erythroid colony formation due to local exposition of such
243 cytokines in the bone marrow affecting erythropoiesis in an independent way to that of impairment
244 in iron mobilization.

245 The NSAID consumption associated to UEA may reflect an underlying disease that merit its
246 consumption or an occult gastrointestinal hematic loss caused by its consumption(60). However, for
247 such association the NSAID consumption must be chronic and data for the whole period of
248 consumption was not collected.

249 *Role of VAD*

250 Studies associating VAD and anemia in OA are scarce because VAD is no longer considered a
251 health priority in developed countries.(61)(62) In our study, VAD prevalence was higher in anemics
252 than non-anemic OA and associated to AI and marginally with CKD. VA has an important role in
253 hematopoiesis and iron metabolism (63), favoring the differentiation and proliferation of red blood
254 cells, in modulating EPO expression (64) and by strengthening the immunity for infections and
255 hence, AI.(15)(65)

256 Some experimental studies in rodents have highlighted a possible relation between VA and hepcidin
257 by modulating the enhanced expression of HAMP in VA deficiency (66,67) while others authors

have proposed an ineffective erythropoiesis by downregulating EPO expression.(68,69) Analyzing the association of VA and hepcidin, VAD status was positively associated by two fold to higher hepcidin concentrations in this sample of OA *[submitted manuscript]*; suggesting that association between VAD and anemia could occur through an inflammatory pathways. Residual confounding due to inflammation can explain the association of VAD to AI, because serum retinol concentrations diminished in the presence of infection and inflammation.(70)

Role of VDD

The exact mechanism by which VD may play a role in the development of anemia is unknown; but some studies have proposed that VD stimulates erythroid precursors and downregulate the pro-inflammatory cytokines expression (thereby reducing the stimulus leading to AI and the proinflammatory milieu in bone marrow).(71)(24,72) Previous studies have suggested that VD is associated with anemia by its direct suppression of HAMP mRNA transcription in AI.(24)(73)(74)(21) However, we did not find an association of VDD and hepcidin *[submitted manuscript]*. Some authors suggest that 1,25(OH)2D rather than 25(OH)D is a more important predictor of anemia risk.(75) Ernst et al (18), previously documented that 1,25(OH)2D and 25(OH)D were both independently associated to different anemia etiologies in adults with coronary heart disease, and the association of anemia was stronger with 1,25(OH)2D concentrations than 25(OH)D levels. In our study, 25(OH) was associated to anemia, but contrary to our expectation, VDD was not associated to AI nor to CKD; it was associated to nutritional deficiencies and to AMC. Whether the active form of VD 1,25(OH)2D may be a better predictor needs to be explored in further studies. VDD and frailty condition were both associated to AMC. Reverse causality may explain it, since anemia may result as a consequence rather than an etiology of multiple comorbidities; OA course with higher frailty and poor health status, which allows for little outdoor exposure and hence lower sun exposure, resulting in higher VDD prevalence.

Strengths and limitations

Anemia is a multifactorial condition. Defining a unique etiology as a cause of anemia in cross-sectional studies is challenging since multiple conditions are present in the same person, so reverse causality may be an alternative explanation in the association observed. Indeed, we carefully selected those criteria following the most recognized and frequently diagnostic parameters used in epidemiological studies to define the etiology of anemia;(4,7,10)(39) nevertheless, comparability of results with other populations deserve caution, not only due to different criteria used to define each etiology but also due to the population at risk. The prevalence of inflammation in this sample which was higher than in other OA studies in México, such as SABE [personal communication] and to other studies that have explored causes of anemia from other OA populations. Since chronic inflammation may confound many of the associations observed, in a *separate analysis* we excluded all subjects with cancer, arthritis and cirrhosis; nevertheless interpretation of results did not change (data not shown).

In addition, thresholds and ranges for most biomarker studied here (i.e. ferritin, eGFR, Hb and hepcidin) need to be defined and validated for OA.(14)

Association of variables was adjusted for multiple comparison and potential confounders in the regression model. However, the limited sample size in the ID, nutritional deficiencies and AMC categories aware us to improve comparability, affecting the power of estimation.

Information regarding the causes of anemia in OA Latin American populations have not been completely documented.(76) This study is the first approach in identifying the different causes of anemia in OA from Mexico where prevalence of anemia remains high. Longitudinal studies focusing on causes of anemia exploring others underlying etiologies as well as their outcomes are necessary in order to identify opportunities for intervention by treating or preventing anemia of a known etiology.

306 In summary, CKD and inflammation accounted for the main causes of anemia of know etiology,
307 both characterized by higher IL-6 and hepcidin concentrations. An important proportion of OA had
308 UEA. ID had the lowest contribution as a cause of anemia. Prefrail condition and Log of IL-6 were
309 associated with UEA. VAD was associated with CKD anemia. VDD was associated with nutritional
310 deficiencies and AMC. Further longitudinal studies are needed to understand the role of VA and
311 VD status on inflammatory biomarkers and in the development of anemia in OA.

Acknowledgments

VDG and ASR designed the study; VDG conducted the research; VDG and ASR analyzed data; VDG wrote the paper; VDG, ASR, SV and MFA collaborated in writing the discussion; VDG had primary responsibility of the final content. All authors read and approved the final manuscript.

Table1. Descriptive characteristics of older adult population by anemia condition

Variable		Total	Non anemic	Anemic	P value*
n sample		*829*	*533*	*291*	
Sex (women)		60.9	61	61.2	0.5
Age group (years)					
	60-69	49.1	54.6	39.5	
	70-79	34.7	33.8	36.1	<0.001
	80 +	16.6	11.6	24.4	
Spoke an indigenous language (yes)		33.1	30	39.2	0.009
Household Wealth Index					
	Tertile 1	33.3	29.4	40.6	
	Tertile 2	34.8	36.3	32.2	0.005
	Tertile 3	31.9	34.3	27.3	
Body Mass Index					
	Normal	20.4	17.1	32.2	
	Overweigth	38.1	40.2	33.6	<0.001
	Obesity	40.6	42.7	34.3	
Functional disability					
	ADL	29.3	23.8	39.2	<0.001
	IADL	42.2	36.2	52.9	<0.001
NSAID consumption		13.1	13.1	19.6	0.016
CRP category					
	0-5 mg/L	48.7	69	61.3	
	>5mg/L	36.5	31	38.7	0.028
AGP (>1g/dL)		7.6	5.2	11.8	0.001
Frailty					
	Non frail	40	44.8	30.6	
	Pre-frail	46.9	45	51.2	<0.001
	Frail	13	10.1	18.2	
Sarcopenia		15.7	12.4	22.1	<0.001
Medical condition previously diagnosed by a physician					
	T2 Diabetes	30	27.6	34.4	0.047
	Hypertension	50	48.8	52.2	0.382
	Dyslipidemia	293	34.5	37.5	0.400
	Renal disease	12.1	11.8	12.7	0.738
	Arthritis	20.3	18	24.4	0.037

Cirrhosis	2.54	1.9	3.4	0.235
Cancer	4.2	2.6	6.9	0.005
Serum micronutrient deficiency[1]				
B12 deficiency	9	8.1	10.5	0.302
Iron deficiency	5.2	4.6	6.3	0.326
Vitamin A deficiency	3.4	1.7	6.3	0.002
Adjusted vitamin A deficiency	2.4	1.6	5	0.007
Vitamin D deficiency	9.7	7.8	13.2	0.042

* X^2 test

Abbreviations: ADL: Basic Activity of daily living; IALD: Instrumental Activity of Daily Living; NSAID: Non-steroid anti-inflammatory drugs; CRP: C Reactive Protein; AGP: Alpha glycoprotein 1 acid.

[1] Vitamin B12 deficiency was defined as <-0.5 SD, considering homocysteine and folate concentration ccording to Fedosov's equation.(42) Iron deficiency was defined as serum ferritin <15 ng/mL after correcting for inflammation according Turnham,(40) or if sTfR >28 nmol/L. Vitamin A deficiency if serum retinol <20 ug/dL.(37) VAD prevalence was corrected by inflammation. Vitamin D deficiency (VDD) if 25(OH)D<50 nmol/L.(36)

Table 2. Anemia and distribution of causes in OA

	n	**Proportion (%)**
Anemia		**35.3**
Mild		*85.9*
Moderate		*13.8*
Severe		*0.3*
Nutritional deficiencies		**7.0**
ID	*3*	1.1
B12D	*16*	5.6
ID + B12	*3*	1.1
Chronic Renal Disease *84*		**29.3**
Anemia of inflammation	*42*	**14.6**
Multiple causes	*9*	**3.1**
Unexplained Anemia	*130*	**45.0**

Table 3. Descriptive characteristics of older adult population by cause of anemia

	Non anemics	Renal disease		Iron deficiency	Inflammation		B12-deficiency	Multiples causas	Unknow
Variable / n sample	516	84		6	42		16	9	130
Sex (women)	60.5	59.5		50	52.4		56.3	66.7	66.2
Indigenous	30.4	35.7		0	47.6		43.8	22.2	42.3
Age group (years)									
60-69	54.3	34.5		50	28.6		31.3	44.4	47.7
70-79	33.9	34.5		50	40.5		37.5	44.4	33.1
80 +	11.8	31		0	31		31.3	11.1	19.2
Household Wealth Index									
Tertil 1	29.9	43.9		16.7	39		37.5	66.7	37.5
Tertil 2	36.1	25.6		33.3	34.1		31.3	22.2	36.7
Tertil 3	34	30.5		50	26.8		31.3	11.1	25.8
Body Mass Index									
Normal	17.4	24.4		40	46.2		37.5	22.2	32.8
Overweigth / Obesity	82.6	75.6		60	53.8	*	62.5	77.8	67.2
Sarcopenia	16.4	24.7		20	50	*	25	22.2	25.2
Comorbidities previously diagnosed by a physician									
Type 2 Diabetes	28.1	48.8	*	0	26.2		37.5	33.3	29.2
Hypertension	49	64.3		66.7	35.7		50	77.8	46.2
Dyslipidemia	34.1	46.4		50	16.7		25	22.2	40
Cancer	2.7	9.5	*	0	23.8	*	0	22.2	0
Cirrhosis	1.7	3.6		0	14.3	*	0	11.1	0
Renal disease	12.2	39.3	*	0	0		0	33.3	0
Arthritis	18.4	25		50	28.6		6.3	11.1	23.8
Alcohol consumption	31.4	19		16.7	26.2		25	22.2	22.3
Tobacco consumption	26.7	20.2		33.3	19		25	44.4	20
Functional Disability									

	ALD	24.2	44	*	16.7	57.1	*	25	66.7		30.8
	IALD	36.6	57.1	*	16.7	66.7	*	50	88.9		44.6
No Frail		44.6	25		66.7	33.3		25	11.1		34.6
	Pre-frail	45.3	52.4		0	38.1		56.3	33.3		56.2
	Frail	10.1	22.6	*	33.3	28.6		18.8	55.6	*	9.2
Drugs		71.5	94	*	66.7	61.9		75	77.8		74.6
NSAID		14	19		50	14.3		18.8	22.2		19.2
SAID		2.7	2.4		0	2.4		6.3	11.1		0.8
Category of anemia											
Mild		0	77.1		83.3	88.1		81.3	55.6		93.8
Moderate		0	22.9		16.7	11.9		12.5	44.4		6.2
Severe		0	0		0	0		6.3	0		0

Abbreviations: Activity of daily living (ADL), Instrumental Activities of daily living (IADL), Non anti-inflammatory steroids drugs (NSAID), anti-inflammatory steroids drugs (AISD), C reactive protein (CRP), Alpha glycoprotein 1 acid (AGP), .

* Statistically different from non anemic group, $p<0.05$ adjusted by Bonferroni's test

Table 4. Serum biomarkers of older adult population by cause of anemia

Variable / n sample	Non anemics 516	Chronic Renal disease 84		Iron deficiency 6		Inflammation 42		B12-deficiency 16	Multiples causas 9		Unknow 130
Vitamin D categories											
25(OH)D ≥75 nmol/L	50.4	41.7		66.7		52.4		31.3	44.4		47.7
25(OH)D 50-74 nmol/L	41.9	42.9		0		38.1		56.3	11.1		42.3
25(OH)D <50nmol/L	7.8	15.5		33.3		9.5		12.5	44.4	*	10
Retinol <20 ug/dL	1.7	6		0		19	*	6.3	0		3.1
Adj Retinol <20 ug/dL	1.6	4.6		0		11.4	*	5.1	0		2.9
Vitamin B12 deficiency	8.1	6		50	*	0		100	66.7	*	0
Iron deficiency	4.5	7.1		100		7.1		0	33.3	*	0
Low ferritin (<15 ng/mL)	1.6	0		33.3	*	4.8		0	0		0
Low serum iron (<60 ug/dL)	4.1	16.7	*	50	*	54.8	*	18.8	44.4	*	0
High Ferritin (>300 ng/mL)	7	11.9		0		11.9		0	33.3		3.8
CRP categories											
0-3 mg/L	49.6	44		100		26.2		50	33.3		53.8
3-10 mg/L	36.8	36.9		0		28.6		31.3	33.3		40
>10 mg/L	13.6	19		0		45.2	*	18.8	33.3		6.2
AGP (>1g/dL)	5.2	16.7	*	0		28.6	*	12.5	44.4	*	1.5
IL6 (>10 pg/dL)	7.6	17.9	*	0		42.9	*	12.5	33.3		6.9
Median of bioquimichal biomarkers[a]											
Edad (years)[b]	69.8 ± 7.5	75 ± 9.5	*	71.3 ± 4.1		75.2 ± 9.3	*	73.1 ± 7.6	74.2 ± 9.1		71.7 ± 8.5
25(OH)D (ng/mL)	30.1 (25.3- 36.5)	27.9 (23.6- 33.4)		33.9 (18.2- 43.3)		30.9 (23.7- 42.4)		28.3 (25.7- 38.1)	26.7 (17.9- 38.1)		29.6 (24.2- 34.2)
Retinol (nmol/L)	45 (36.3- 55.6)	52.8 (42.6- 73)	*	39.4 (26.3- 49.3)		37.8 (28.5- 44.8)		42.9 (29.2- 47.7)	31.3 (28.5- 33.1)		45.3 (37.6- 52.3)
Hepcidin (mg/dL)	13.1 (5.3- 27.3)	15.2 (5.2- 35.6)		16.7 (10.7- 32.8)		23 (5.9- 43.1)	*	12.3 (7.2- 30.6)	18.8 (13.2- 42.7)		9.2 (4.2- 22)
CRP (mg/L)	3 (1.6- 6.5)	3.6 (1.2- 8.8)		1.2 (0.8- 1.6)		7.7 (2.6- 37.7)	*	3.1 (0.9- 7.6)	6.4 (2.1- 15.5)		2.8 (1.6- 6.4)

AGP (g/L)	0.5 (0.4- 0.7)	0.6 (0.5- 0.8)		0.6 (0.5- 0.7)		0.7 (0.4- 1.1)	*	0.6 (0.4- 0.8)	0.9 (0.8- 1.2)	*	0.6 (0.4- 0.7)
IL6 (pg/ml)	2.5 (1.4- 4.5)	4.5 (2.6- 8)	*	2.3 (0.2- 3.7)		5.7 (2.3- 21.4)	*	3.8 (1.7- 5.2)	8.8 (7.8- 18.4)	*	2.6 (1.6- 4.6)
Iron (ug/dL)	100.5 (83.9- 123.6)	79.8 (65.3- 109.7)	*	72 (53.4- 88.9)		58.8 (48- 86.1)	*	88.3 (63.2- 111.7)	88.7 (55.6- 108)		99.2 (79.3- 129)
Eritropoietin (miu/ml)	10 (7.8- 13.4)	11.1 (8.2- 16.1)		18.6 (15.1- 28.5)	*	12.3 (8.3- 17.5)		12.9 (9.5- 16.8)	10.4 (7.1- 14.3)		10.5 (7.4- 13.4)
Ferritin (ng/mL)	120.8 (75.7- 198.6)	122.1 (77.7- 203.1)		54.5 (13.1- 84.6)		121.7 (44.5- 184.2)		96.3 (72.7- 129.6)	244.8 (145.3- 355.6)	*	118.8 (74.7- 171.5)
Number of drugs	3.3 ± 2.3	4.3 ± 2.7	*	4.8 ± 3.6		4 ± 3.2		3.6 ± 2.1	4.4 ± 3.2		3.7 ± 2.4
Number of chronic comorbidities	2 ± 1.6	3.1 ± 1.7	*	2.5 ± 0.8		2.1 ± 2		1.8 ± 1	3.1 ± 1.7		2 ± 1.7
Hemoglobin (g/dL)	13.7 ± 1.2	10.9 ± 1.1		11 ± 1.3		11.2 ± 1.1		11 ± 1.7	10.2 ± 1.2		11.3 ± 0.9

[a] Median (interquartile range)

[b] Mean ± SD

[*] Statistically different from non anemic group, $p<0.05$ adjusted by Bonferroni's test.

Abbreviations: C reactive protein (CRP), Alpha glycoprotein 1 acid (AGP), Interleukine-6 (IL6).

Table 3. Multinomial regression model of variables associated to each cause of anemia

	Renal	Nutritional	Inflammation	Multiple causes	Unknow
MODEL 1	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**
Hepcidin (ng/mL)	1.01 (1.01,1.02)	1.01 (1,1.01)	1.02 (1.02,1.02)	1.02 (1.01,1.02)	0.99 (0.98,1.01)
Age (years)	1.08 (1.05,1.11)	1.05 (0.98,1.11)	1.08 (1.05,1.11)	1.07 (0.97,1.17)	1.03 (1.01,1.05)
Sex (female)	1.01 (0.74,1.38)	0.81 (0.09,7.44)	0.71 (0.49,1.01)	1.27 (0.27,5.99)	1.27 (1,1.62)
Indigenous	1.02 (0.7,1.5)	1.05 (0.96,1.14)	2.02 (1.82,2.24)	0.46 (0.35,0.6)	1.49 (1.21,1.84)
Tertile of household wealth index					
2	0.52 (0.21,1.28)	0.87 (0.81,0.93)	0.89 (0.34,2.32)	0.27 (0.25,0.29)	0.89 (0.63,1.26)
3	0.76 (0.71,0.83)	1.14 (0.22,5.9)	0.9 (0.88,0.91)	0.16 (0.04,0.56)	0.73 (0.5,1.06)
MODEL 2	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**
Hepcidin (ng/mL)	1.01 (1.01 ,1.02)	1.01 (1.01 ,1.01)	1.02 (1 ,1.03)	1.01 (1 ,1.03)	0.99 (0.98 ,1.01)
VA deficiency	1.92 (1.54 ,2.39)	2.72 (0.03 ,261.66)	3.98 (1.41 ,11.23)	0 (0 ,0)	1.43 (0.37 ,5.58)
VD deficiency	1.93 (1.05 ,3.57)	2.76 (1 ,7.72)	1.13 (0.43 ,2.98)	8.65 (4.76 ,15.72)	1.18 (1.06 ,1.3)
IL-6 (Log)	1.36 (1.29 ,1.44)	0.96 (0.79 ,1.17)	1.94 (1.68 ,2.25)	3.46 (2.16 ,5.54)	1.06 (1.06 ,1.06)
Age	1.07 (1.05 ,1.1)	1.04 (0.98 ,1.11)	1.07 (1.04 ,1.1)	1.05 (0.98 ,1.13)	1.03 (1 ,1.05)
Sex	0.95 (0.77 ,1.16)	0.73 (0.1 ,5.56)	0.76 (0.65 ,0.89)	0.77 (0.18 ,3.23)	1.24 (0.95 ,1.64)
Indigenous	0.92 (0.7 ,1.22)	0.99 (0.63 ,1.58)	1.65 (0.98 ,2.77)	0.42 (0.22 ,0.79)	1.46 (1.14 ,1.87)
Tertile of household wealth index					
2	0.54 (0.19 ,1.53)	0.89 (0.81 ,0.98)	1.1 (0.31 ,3.97)	0.21 (0.12 ,0.37)	0.9 (0.6 ,1.36)
3	0.76 (0.6 ,0.95)	1.15 (0.2 ,6.48)	1.12 (0.94 ,1.33)	0.1 (0 ,2.26)	0.73 (0.47 ,1.13)
MODEL 3	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**	**OR (CI95%)**
Hepcidin (ng/mL)	1.01 (1.01 ,1.01)	1.01 (1 ,1.02)	1.01 (1 ,1.02)	1.01 (0.99 ,1.03)	0.99 (0.97 ,1.01)
VA deficiency	2.26 (0.95 ,5.41)	2.44 (0.02 ,300.57)	3.2 (2.31 ,4.44)	0 (0 ,0)	1.35 (0.31 ,5.81)
VD deficiency	1.4 (0.47 ,4.2)	1.8 (1.04 ,3.1)	0.86 (0.07 ,9.96)	5.76 (4.78 ,6.93)	1.11 (0.81 ,1.53)
IL-6 (Log)	1.29 (1.17 ,1.43)	0.97 (0.72 ,1.32)	1.91 (1.3 ,2.82)	3.68 (1.45 ,9.35)	1.08 (1.04 ,1.11)

Frailty						
	1	1.33 (0.89 ,1.97)	0.95 (0.53 ,1.68)	0.81 (0.09 ,7.59)	1.02 (0.83 ,1.25)	1.39 (1.07 ,1.82)
	2	1.51 (0.26 ,8.64)	1.51 (0.78 ,2.93)	1.15 (0.03 ,47.32)	5.76 (5.39 ,6.17)	0.83 (0.78 ,0.88)
Overweight/Ob		0.99 (0.81 ,1.22)	0.36 (0.08 ,1.68)	0.44 (0.15 ,1.29)	1.94 (0.55 ,6.87)	0.42 (0.25 ,0.72)
Age		1.08 (1.06 ,1.09)	1.02 (0.93 ,1.13)	1.05 (1 ,1.1)	1.03 (0.9 ,1.18)	1.02 (1.01 ,1.02)
Sex		0.82 (0.73 ,0.91)	0.66 (0.08 ,5.22)	0.88 (0.51 ,1.53)	0.55 (0.11 ,2.63)	1.26 (1.05 ,1.51)
Indigenous		0.83 (0.6 ,1.14)	0.98 (0.71 ,1.36)	1.48 (1.13 ,1.95)	0.48 (0.16 ,1.46)	1.47 (1.22 ,1.77)
Tertile of household wealth index						
	2	0.47 (0.22 ,0.99)	0.86 (0.64 ,1.14)	1.26 (0.55 ,2.9)	0.15 (0.08 ,0.26)	0.93 (0.57 ,1.51)
	3	0.72 (0.58 ,0.89)	1.05 (0.19 ,5.76)	1.41 (1.23 ,1.62)	0.08 (0 ,2.43)	0.82 (0.44 ,1.53)
NSAID		1.85 (1.06 ,3.24)	3.24 (0.72 ,14.49)	1.19 (1.16 ,1.22)	1.38 (0.37 ,5.17)	1.6 (1.17 ,2.2)
Type 2 diabetes		2.87 (1.16 ,7.11)	1.16 (0.65 ,2.09)	0.72 (0.16 ,3.24)	0.99 (0.63 ,1.56)	1.1 (0.32 ,3.82)

Abbreviations: Vitamin A (VA), Vitamin D (VD), Non-steroid anti-inflammatory drugs (NSAID)
For each categorical variable references are: non deficiency of VA nor VD, for frailty: non frail, sex male, first tertile of HWI, normal BMI for BMI condition, non indigenous, non consumption of NSAID and finally for diabetes condition: non diabetes previously diagnosed by a physician

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4. **Artículo 2.** Serum Retinol But Not 25(OH)D Status Is Associated With Serum Hepcidin Levels In Older Mexican Adults.

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Nutrients

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Article

Serum Retinol But Not 25(OH)D Status Is Associated With Serum Hepcidin Levels In Older Mexican Adults

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Abstract: 1) Background: Elevated hepcidin levels have been linked to anemia of inflammation (AI). Retinol deficiency has shown to upregulate hepcidin expression in animals; while conflicting evidence link VD status with hepcidin concentration in humans. The purpose of the study is to explore if VA and VD status are associated with hepcidin concentrations in older Mexican adults (OA). 2) Methods: A cross-sectional study was conducted in summer 2015, using serum samples from 803 fasting OA ages 60 and above residents from Campeche and Yucatán. VA deficiency (VAD) was defined as serum retinol concentration<20 ug/dL and VD deficiency (VDD) as 25(OH)D<50 nmol/L. The Log-hepcidin was the outcome variable expressed as continuos and tertiles of its distribution. Linear and ordinal regression models were used. 3) Results: VAD was present in 3.2% and VDD in 9% of OA. Log-retinol was inversely associated to Log-hepcidin (coef: -0.15, 95%CI -0.18,-0.12). VAD status shown a higher probability than non-VAD for higher hepcidin tertiles (OR=2.34, 95%CI: 1.27, 4.33). VDD states was not associated with hepcidin in the linear (coefficient: -0.13, CI=-0.31, 0.11) nor the ordinal model (OR=0.82, 95%CI: 0.54, 1.25). 4) Conclusions: VAD but not VDD status was inversely associated to hepcidin concentrations in OA.

Keywords: hepcidin, vitamin A, vitamin D, older adults

1. Introduction

Hepcidin is the main hormone that regulates iron homeostasis and has been considered the main mediator of anemia of inflammation (AI) or anemia of chronic disease [1]. Hepcidin causes a block in iron recycling through the hepcidin-ferroportin axis, thus diminishing iron export from macrophages during iron overload or infection despite sufficient iron stores [2]. In older adults (OA), inflammaging, metaflammation, inmunescence and frailty are all conditions that conferred a dysregulation of the immune system, promoting a low and chronic proinflammatory profile [3–5]. Although the pathological mechanism is still poorly understood, interleukin (IL)-6 seems to induce hepcidin expression under inflammatory conditions, therefore serving as a risk factor for AI development in OA [1,2,6].

Vitamins A (VA) and D (VD) play a crucial role on the immune response, since both exert their effects on target cells by binding to nuclear-hormone receptors. In interaction with either RAR or RXR nuclear receptors, both vitamins may directly regulate gene expression [7]. A vitamin D receptor element (VDRE) in the promoter region of Hepcidin Antimicrobial Peptide (HAMP) RNA gene has been identified, suggesting that VD may downregulate hepcidin expression independently from immunomodulation of pro-inflammatory cytokines [8]. VD deficiency (VDD) has been associated to anemia in healthy and diseased populations [7][9][10]. Additionally, some studies have explored the effect of VD supplementation on hepcidin levels in adults, most of which showed a reduction in hepcidin levels after the improvement in VD status [8,11,12].

VA deficiency (VAD) has been found to induce upregulation of HAMP expression in rodents [13,14]. Although no data currently exist to link VA status to circulating hepcidin levels in adult population, associations between VA status and the effect of VA supplementation on anemia has been well documented in infants and women [15]. It is likely that iron mobilization, in addition to the different actions of VA on erythropoiesis, may be one of the mechanisms through which VA exerts its role to maintain iron homeostasis [16].

VDD is highly prevalent in older adults across different populations, while VAD exists principally in developing countries [10,17,18]. Previously, we found that hepcidin levels in OA where higher in those with AI and CKD anemia in comparison to non-anemics, and that VA status, but not VD, showed an association with AI [19]. Identifying those metabolites immunomodulatory that might be associated to higher hepcidin concentrations, are relevant for the prevention of anemia with an inflammatory component. The aim of the study was to explore if serum retinol levels and 25(OH)D levels are both associated to hepcidin concentrations in OA, in an independent way of anemia status.

2. Materials and Methods

From July through September 2015, we recruited 829 OA (ages ≥ 60y) for a cross-sectional study to understand the causes of anemia. Participants were recruited from four localities in the southern region of México, including Champotón, Campeche, Mérida and Valladolid, and interviewed at their homes [19].

Of total study subjects, 803 had available serum and hematological parameters that were included in our analysis. Information on sociodemographic characteristics, chronic comorbidities, anthropometry, diet, nutritional status, and education was gathered by trained research assistants during home visits. The study was approved by the Ethics, Biosecurity and Research committee at the INSP. All participants gave their informed oral and written consent.

Biochemical analysis

Fasting venous blood samples were drawn and centrifuged in situ. The serum was then separated and stored in coded cryovials, and preserved in liquid nitrogen until delivery to a central laboratory in Cuernavaca, Mexico, where it was stored at −70 °C.

VD (serum 25(OH)D) (nmol/L), C reactive protein (CRP mg/dL), Homocystein (umol/L), B12 (pg/ml) and Ferritin were measured by immunochemiluminescence method using commercials kits (Abbott Diagnostics) in an ArchitectCI8200 equipment. Serum retinol was determined in an HPLC HP1110 LCDAD (Agilent Technology Waldbronn, Germany), using NovaPack C18 4um 3.9 x 150 mm with a flux of 1.5 mL/min of the mobile phase methanol, after extraction with 99% ethanol. Hepcidin was measured through a quantitative immunoassay technique using a commercial immunoassay (My Biosource ELISA kit) with a detection range between 4.69ng/ml-300 ng/ml. Capillary hemoglobin was measured using a portable photometer (Hemocue). Serum iron, was measured with an atomic absorption analyzer (Beckton Dickinson). Soluble transferrin receptor (sTfR) was measured using a commercial immunoassay (Quantikine IVD sTFR ELISA kit; R&D

Systems Inc, Minneapolis, MN) using recombinant human sTFR as standards. Alpha glycoprotein 1-acid (AGP), erythropoietin (EPO) and IL-6 concentrations were measured through immunoassay ELISA, using commercials kits (R&D Systems Inc, Minneapolis, MN). Creatinine (mg/dl) was measured through a colorimetric method with an ArchitectCI8200 autoanalyzer (Abbott Diagnostics, Germany).

Biochemical analyses were performed at the Centro Médico Nacional Siglo XXI and at the Nutrition Laboratory at the National Institute of Public Health (INSP) in Mexico.

Definition of variables

VD status was classified as deficient if serum 25(OH)D was <50 nmol/l [20] and VA was considered deficient if serum retinol <20ug/dL [21]. Anemia was classified according to WHO criteria as Hb <13 g/dL in men and <12 g/L in women [22]. Iron deficiency (ID) was defined as sTfR >28 nmol/L or serum ferritin concentration <15 ng/mL, adjusting for inflammation [23]. High ferritin status was defined as ferritin≥350 ng/mL, and low serum iron as <60mcg/dL. Vitamin B12 deficiency (B12D) was defined if Fedosov's equation fell below -0.5 SD, accounting for serum homocysteine levels and folate [24]. Glomerular filtration rate (GFR) was estimated from serum creatinine with the use of the CKD-EPI. Chronic kidney disease (CKD) was defined if estimated GFR<60 mL/min/1.73m2 [25] or a previously-diagnosed kidney disease. Categories of inflammation were considered using AGP and CRP combination according Turnham: non-inflammation, incubation, early convalescence and late convalescence [23].

BMI was calculated as weight in kilograms divided by the squared height in meters, and study participants were classified as normal (18-24.9 kg/m2) or overweight/obese (≥25 kg/m2). Presence of chronic diseases (hypertension, diabetes, dyslipidemia, myocardial infarction, angina pectoris, heart disease, cirrhosis, arthritis, stroke, chronic lung disease, osteoporosis, and cancer) were obtained by self-report if previously diagnosed by a physician. Use of medication was registered and classified as either non-steroid anti-inflammatory drugs (NSAID) and steroid anti-inflammatory drugs (SAID). Ethnicity was defined based on which an indigenous language was spoken in the household. An asset index was considered using a principal component analysis, the first component gave a total variance of 33%. This was divided into tertiles with the uppermost tertile indicating the highest socioeconomic status (SES).

The phenotype of frailty was the proposed by Fried [26]. To define sarcopenia we used the criteria from the European Working Group on Sarcopenia in Older People [27]. Functional performance was evaluated through Katz's index for activities of daily living (ADLs) [28] and Lawton's scale for instrumental activities of daily living (AIDL) [29].

Information on consumption of VD and other micronutrient supplements was obtained by a semi-quantitative FFQ during the last 7 days prior the survey.

Statistics

Data are reported as frequencies for categorical variables and means, standard deviations, medians, or interquartile ranges for continuous variables. Bivariate analysis was conducted using a chi-squared test for categorical variables and Kruskal–Wallis test for non-normally distributed parameters in order to compare the characteristics of OA by VA and VD status.

We adjusted for retinol and ferritin levels by using inflammatory markers (AGP and CRP), given that inflammatory or infectious conditions could affect ferritin and retinol levels. For estimating the VAD prevalence, we used a regression correction approach, using marginal estimation [30].

To assess the independent association of 25(OH)D and retinol categories with hepcidin levels, we performed linear and ordinal regression analyses. Hepcidin was the outcome variable and was expressed using both the log of hepcidin and tertiles of hepcidin levels. Since hepcidin levels were

non-normally distributed, their values were logarithmically transformed. In the linear models we used bootstrap-based clustered errors to account for the correlation between state-level observations [31].

Independent variables (VA and VD levels) were explored as follows: by unit of change, by a change in -10 units, using log transformed VA or VD levels, and by categorical status (VAD and VDD), where those with 25(OH)D levels ≥50nmol/l and retinol ≥20ug/dL were considered the reference.

For each outcome variable (log and tertiles of hepcidin), we fitted a set of models: Model 1: unadjusted data; Model 2: adjusted for sex, age, indigenous and socioeconomic status, vitamin A or vitamin D status; Model 3: adjustments made in model 1 plus ferritin, sTFR, BMI, renal disease, anemia, frailty, inflammation, AINES, AIE use, vitamin D and other micronutrient supplements; Model 4: same as model 3, plus IL-6.

3. Results

Characteristics of OA are presented in Table 1. Overall, 60.9% were women, 42.2% had a functional disability as defined by IADL, 35.5% had anemia, 9.7% had VDD, 2.4% had VAD after correcting for inflammation, 5.2% were ID, 9% were B12D, and 2.8% of OA were taking supplements of VD.

Table 1. Descriptive characteristics of Older Mexican Adults sample

Characteristic	Frequency (%)
N sample	829
Sex (% women)	60.9%
Age group (years)	
60-69	49.1
70-79	34.7
80 +	16.6
Speak an indigenous language (yes)	33.1
Tertile of SES (asset index)	
Tertile 1	33.3
Tertile 2	34.8
Tertile 3	31.9
Body Mass Index	
Normal	20.4
Overweight	38.1
Obesity	40.6
CRP category	
0-5 mg/L	48.7
>5mg/L	33.7
AGP (>1g/dL)	7.6
IL6 >10 mg/L	10.7
Functional disability	
ADL	29.3
IADL	42.2
Frailty	
Not frail	40
Pre-frail	46.9
Frail	13
Sarcopenia	15.7
Medical condition	

T2 Diabetes	30
Hypertension	50
Renal disease	12.1
Arthritis	20.3
Cirrhosis	2.54
Cancer	4.2
NSAID consumption	13.1
Anemia	35.5
Serum micronutrient deficiency[1]	
B12 deficiency	9
Iron deficiency	5.2
Vitamin A Deficiency	3.4
Adjusted Vitamin A Deficiency*	2.4
Vitamin D Deficiency	9.7
Supplement of VD	2.8

Abbreviations: ADL: Basic Activity of daily living; IALD: Instrumental Activity of Daily Living; NSAID: non steroid anti-inflammatory drugs; CRP: C Reactive Protein; AGP: Alpha glycoprotein 1 acid.

[1] Vitamin B12 deficiency was defined as <-0.5 SD, considering homocysteine and folate concentration according to Fedosov's equation [24]. Iron deficiency was defined as serum ferritin <15 ng/mL after correcting for inflammation according Turnham [23] or if sTfR >28 nmol/L. Vitamin A deficiency if serum retinol <20 ug/dL [21]. Vitamin D deficiency (VDD) if 25(OH)D<50 nmol/L [20].

*VAD prevalence was adjusted considering inflammation

Table 2 shows the characteristics of OA by VD and VA status. Those with VDD were more likely to be female, ages 80 and older, and suffer from anemia, B12D, sarcopenia, diabetes, hypertension, functional disability, frail and drugs consumption, as compared to those OA with 25(OH)D levels ≥50nmol ($p<0.05$). Those with VAD were more likely to be of lower SES, have low prevalence of VDD, and suffer from anemia, B12D, low serum iron, high ferritin, high frequency of inflammation, normal BMI, sarcopenia, cirrhosis, frailty and higher medication consumption, in comparison with those OA with normal retinol levels ($p<0.05$).

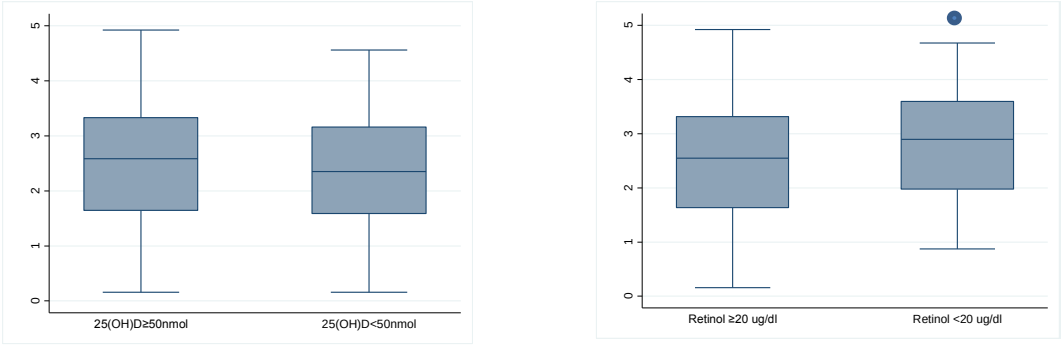
Table 2. Characteristics of Mexican Older Adults by Vitamin A and D status

	Vitamin D			**Vitamin A**		
	25(OH) D ≥50nmol	**25(OH) D <50nmol**	**P value**	**Retinol ≥20 ug/dl**	**Retinol <20 ug/dl**	**P value**
Sex (women)	58.8	79.5	<0.001	61.2	48.1	0.172
Indigenous	33.5	35.9	0.673	32.6	66.7	<0.001
Age group (years)						
60-69	50.9	33.3		49.6	37	
70-79	33.7	42.3		34.4	37	
80 and older	15.4	24.4	0.009	16	25.9	0.29
Tertile of SES (asset index)						
Tertile 1	33.2	35.9		32.5	63	
Tertil 2	35.2	30.8		35.2	22.2	
Tertil 3	31.6	33.3	0.736	32.3	14.8	0.004
Anemia	34.3	48.7	0.012	34.7	66.7	0.001

Vitamin D status	-	-	-			
25(OH)D ≥75 nmol/L	-	-	-	47.9	74.1	
25(OH)D 50-74 nmol/L	-	-	-	42.3	18.5	
25(OH)D <50nmol/L	-	-	-	9.8	7.4	0.025
Retinol <20 ug/dl	3.4	2.6	0.681	-	-	-
Vitamin B12 deficiency	8.1	16.7	0.012	8.5	22.2	0.014
Iron deficiency	4.83	7.69	0.275	5	7.4	0.58
Low ferritin (<15 ng/mL)	1.38	2.56	0.413	1.5	0	0.515
Low serum iron (<60 ug/dL)	8.4	9	0.866	7.5	37	<0.001
High Ferritin (≥350 ng/mL)	7.4	6.4	0.738	6.8	22.2	0.003
CRP categories						
0-3 mg/L	47.9	56.4		49.5	25.9	
3-10 mg/L	37.4	28.2		36.6	33.3	
>10 mg/L	14.8	15.4	0.259	13.9	40.7	<0.001
AGP (>1g/L)	7.3	10.3	0.351	7	25.9	<0.001
IL6 (> 10pg/mL)	10.2	15.4	0.16	9.1	55.6	<0.001
BMI						
Normal	22.6	23.3		21.7	50	
Overweight / Obese	77.4	76.7	0.893	78.3	50	0.001
Sarcopenia	19.7	29.6	0.049	19.8	42.3	0.005
Comorbidities previously diagnosed by a physician						
Type 2 Diabetes	29.1	42.3	0.016	30.4	29.6	0.931
Hypertension	48.8	60.3	0.055	50.1	44.4	0.561
Dislypidemia	34.6	41	0.261	36	14.8	0.024
Cancer	4	6.4	0.315	4.4	0	0.266
Cirrhosis	2.2	3.8	0.365	1.9	14.8	<0.001
Renal disease	12	15.4	0.388	12.4	11.1	0.845
Arthritis	20.3	21.8	0.752	20.9	7.4	0.088
Functional Disability						
ALD	27.3	50	<0.001	29.3	37	0.383
IALD	40	64.1	<0.001	41.9	55.6	0.157
No Frail	41.9	19.2		40.1	29.6	
Pre-frail	47.4	44.9		47.4	40.7	
Frail	10.6	35.9	<0.001	12.5	29.6	0.033
Drugs	72.8	84.6	0.024	74.7	51.9	0.008
NSAID	15	23.1	0.064	16.1	7.4	0.223
SAID	2.3	3.8	0.419	2.4	3.7	0.681
Supplement of VD	2.2	5.1	0.119	2.6	0	0.406

Abbreviations: CRP: C Reactive Protein; AGP: Alpha glycoprotein 1 acid; IL-6 Interleukin 6; BMI: Body Mass Index; ADL: Basic Activity of daily living; IALD: Instrumental Activity of Daily Living; NSAID: non steroid anti-inflammatory drugs; SAID: steroid anti-inflammatory drugs

As hypothesized, serum hepcidin levels were significantly lower in OA with VAD than in OA with normal serum retinol (2.4 ± 1.08 vs 2.8 ± 1.09, p=0.16) (Fig.1. A) Log-normalized hepcidin levels were not statistically significantly different between those with normal levels of VD (25(OH)D≥50nmol) (2.4 ± 1.08) versus those with VDD (25(OH)D<50nmol) (2.3 ± 1.05; p=0.147) (Fig 1 B).

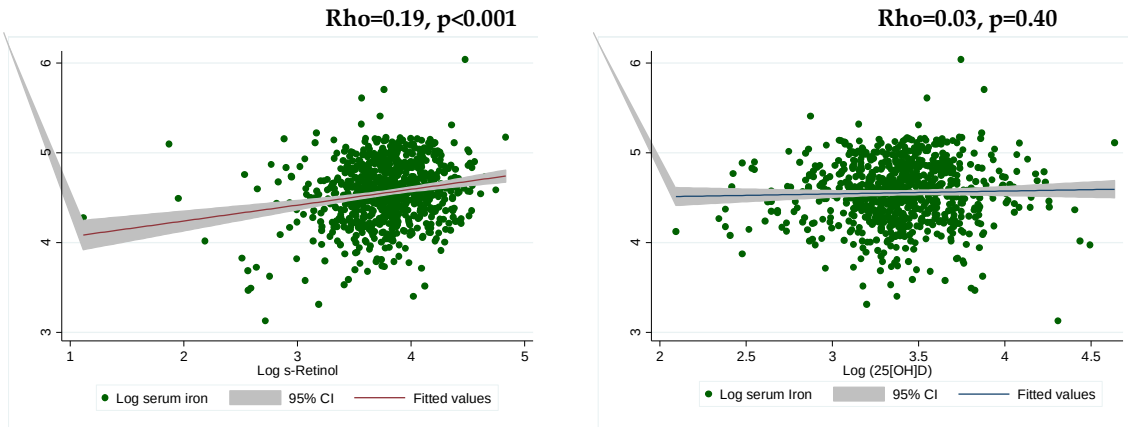


(**a**) (**b**)

Figure 1. Logarithmic normalized hepcidin levels by Vitamin D and Vitamin A status in Mexican Older Adults. (**a**) Mean of Log Hepcidin by vitamin D deficiency; (**b**) Mean of Log Hepcidin by vitamin A deficiency. * p<0.05

When looking at the descriptive association between biomarkers and VDD and VAD status, the log normalized levels of retinol were correlated with biomarkers of inflammation, such as s-IL6 (rho= -0.32, p<0.001) and CRP (rho= -0.25, p<0.001); but not with AGP (rho=0.05, p=0.16). For VD, 25(OH)D had a weak negative correlation with IL-6 (IL-6 rho= -0.07, p=0.03), but was not significant for CRP (rho=0.01, p=0.72) or AGP (rho= -0.04, p=0.23). When stratifying by anemia, the correlation between retinol and biomarkers was stronger for anemics (rho= -0.38, p<0.001 for IL6; rho= -0.29, p<0.001 for CRP; and rho=0.06 p=0.28 for AGP) than non anemics (rho= -0.27, p<0.001 for IL6; rho= -0.21, p<0.001 for CRP; and rho= 0.04 p=0.26 for AGP). For 25(OH)D levels, correlation with biomarkers were stronger in non anemics rho= -0.12, p=0.005 for IL6; rho= -0.07, p=0.01 for CRP; and rho= -0.04 p=0.34 for AGP; than in anemics: rho=0.02, p=0.73 for IL6; rho=0.13, p=0.02 for CRP; and rho=-0.02 p=0.66 for AGP.

Figure 2 demonstrates the positive correlation between log retinol and serum iron levels (rho= 0.19, p<0.001); while logged 25(OH)D levels were not correlated with log serum iron levels (rho=0.03, p=0.40).



(**a**) (**b**)

Figure 2. . Smoothed correlation of serum retinol and 25(OH)D with serum iron in OA. (**a**) Correlation between Log serum retinol and Log serum iron; (**b**) Correlation between Log serum 25(OH)D and Log serum iron.

Table 3 shows that retinol values in all presentations (non-transformed, logarithmically transformed, and grouped by 10 units or VAD status), were inversely associated with log hepcidin levels in both the unadjusted and adjusted models. In the adjusted model (model 2), those with VAD has an increased odds of higher hepcidin levels (Coefficient =0.45, 95%CI: 0.19, 0.7) (Model 2, for VA deficiency and Log of hepcidin). In the ordinal model using tertiles of hepcidin levels as outcome variable, OA with VAD had a higher probability of falling within the highest tertile of hepcidin (OR=2.34, 95%CI: 1.27, 4.33) (Model 2, for VA deficiency). Model 4 considered the adjustment by IL-6; although the association previously observed diminished in magnitude, VA still remained significantly and inversely associated with hepcidin levels ($p<0.05$) (Table 3).

Neither 25(OH)D nor VD status were associated with hepcidin levels. The unadjusted and adjusted models did not show a significant association of VD and hepcidin levels in this population ($p>0.05$) (Table 3).

4. Discussion

In this study, we found that retinol levels were associated with hepcidin concentrations, independent of the inflammatory process, since VAD were associated to higher hepcidin levels in OA. This association occurred in a population living in the southern region of Mexico, where the prevalence of anemia is high, mainly caused by inflammation [19]. Meanwhile, 25(OH)D, was not significantly associated with hepcidin levels in this population.

As we previously documented [19], VAD and VDD were both associated with anemia but through different etiologies. VAD but not VDD showed a strong association to anemia of inflammation (AI) in this population, after considering confounders. VDD was associated to anemia of nutritional deficiencies and multiple causes. Although, a misclassification can occur because the criteria used, AI has been defined as low serum iron in absence of ID concomitant with hyperferritinemia that occur during an inflammatory process (malignancy, infections, renal disease and autoimmune disease) [32]. This data suggests that vitamin A may play a role in the pathophysiology of AI, with hepcidin as an important mediator.

The regulation of hepcidin synthesis is stimulated by iron overload, hypoxia, erythropoiesis and inflammation. In the setting of inflammation, hepcidin expression is driven mainly by an increase in the pro-inflammatory interleukin-6, that occurs via the JAK-STAT3 pathway [33]. The resulting higher hepcidin levels cause iron retention in macrophages, hepactocytes and enterocytes with reduced availability of iron for erythropoiesis, impairing the proliferation of erythroid progenitor cells affecting the heme synthesis [32].

In humans, the ability of both (VA and VD) liposoluble vitamins to modulate hepcidin expression is unknown; nevertheless, it is well documented that retinol and vitamin D interact due to their role as ligand-regulated transcription factors for multiple gene expression in a wide range of biological processes (i.e systemic inflammation) [34][35]. Retinoid acid receptors (RARs) and retinoid X receptors (RXRs) are transcriptional factors of the nuclear receptor (NR) superfamily. RARs and RXRs form RAR/RXR heterodimers and bind to RARE's located in the gene promoters, modulating expressions of target genes through activation by their cognate ligands.[34] RXRs may also heterodimerize with other members of the NR, as the nuclear vitamin D receptor (VDR), binding to VD3 response elements (VDREs) in the promoters of VD3-responsive genes [35]. According to Bacheta et al, hepcidin have VDREs in the promoter region of HAMP gene in monocytes [8]. This may suggest that the interaction between these two vitamins may activate or repress hepcidin expression. In our study, OA did not share both vitamin deficiencies (with exception of 2 OA).

Therefore, it was not possible to explore the synergism interaction between both vitamin deficiencies with hepcidin levels.

Most of the experimental evidence suggests that both VA and VD may downregulate hepcidin expression in an independent way of their suppression activity of inflammatory pathways. The evidence regarding the effect of VAD on hepcidin expression came from experimental studies in rodents. Some authors have found a direct link between VA depletion and higher HAMP expression. Arruda et al [14] first showed that VAD rats had increased liver hepcidin mRNA level, higher iron spleen concentration and a higher oxidative status than control rats. In 2012, Citelli et al [13] found that VAD mice, liver hepcidin and ferritin mRNA levels were upregulated, suggesting that VAD affected hepatic iron mobilization as well the transcription factors of protein genes involved in the iron bioavailability, but without affecting iron absorption. In contrast, Da Cunha et al. [36] found that VAD rats had a lower hepatic HAMP mRNA levels to half those of the control levels and suggested that VAD modulate iron metabolism via ineffective erythropoiesis by down-regulated renal Epo mRNA and up-regulated Hmox1 in the spleen [36]. By using the same protocol, Ribeiro Mendez et al [37] found that VAD up-regulated hepatic Bmp6 and Hfe mRNA levels and down-regulated hepatic HAMP mRNA levels, impairing HJV-BMP6-SMAD signaling pathway that normally activates the expression of hepcidin in ID. Differences in the results between these two studies may be due in part to the rats' diet treatment as well as the baseline levels of VA stored in their livers.

In populations with a high risk of anemia and micronutrient deficiency, vitamin A supplementation (VA-Sup) has been suggested as a strategy for improving Hb levels and ameliorating anemia. A recent meta-analysis of VA-Sup on anemia and iron status in different life stages (not including OA) showed that VA-Sup reduced the risk of anemia by 26% by improving hemoglobin and ferritin levels in individuals with low serum retinol (but without effects on the prevalence of ID by using serum ferritin as biomarker) [38]. Even though the exact mechanism is unknown, diminishing Hb through an elevation in hepcidin levels appears to be one of several biological pathways by which VA status can induce anemia.

Contrary to our hypothesis, VDD was not associated with hepcidin levels in our sample, even after stratifying by anemia (data not shown). Few experimental studies in cell lines and pilot studies of VD supplementation (VD-Sup) in humans have shown a link between hepcidin and VD. Bacchetta et al [8] identified a VDRE binding site on human hepcidin promoter and 1,25-dihydroxyvitamin D (1,25(OH)2D3) directly downregulated HAMP gene transcription (by 0.5-fold) and ferritin, while increased expression of ferroportin. A pilot study of a single oral dose of VD2 (100,000 IU) in 7 healthy adults, showed that VD-Sup increased serum 25D-hydroxyvitamin D (25(OH)D)] and decreased hepcidin levels by 34% within 24hrs. Zughaier et al [11] showed that (1,25(OH)2D3) was associated with reduced hepcidin expression and increased ferroportin and NRAMP1 expression in vitro and in vivo in inflammatory conditions by regulation of hepcidin-ferroportin axis in macrophages. A randomized, double-blind, placebo-controlled trial pilot study of VD-sup (n= 38, cholecalciferol, 50,000 IU weekly for 12 weeks) showed an increase in serum 25(OH)D and a decrease in serum hepcidin in subjects with early stage CKD [11]. Smith et al's study [12] demonstrated a 73% decrease of plasma hepcidin in 28 healthy adults treated with vitamin D3 compared with placebo, after a single oral dose of 250,000 IU for 1 week. In contrast, Panwar et al [39] did not find any association of VD-Sup on hepcidin levels in 40 adults with CKD (stage 3 or 4) by using a randomized, placebo controlled double-blinded study design (calcitriol 0.5 mcg daily for 6 weeks). A distinction from the other studies is that CKD patients were eligible irrespective of their baseline vitamin D status, authors's discusses that the effects of VD-Supl on hepcidin are more robust in VDD status. Observational studies that explore the association of VDD on hepcidin levels are scarce across all population groups. Recently, lower hepcidin levels were associated to higher 25(OH)D status in children with inflammatory bowel disease.[40] In our study, OA were ambulatory, and they had diverse chronic comorbidities with a diverse pro-inflammatory profile, but not necessarily ill enough to promote a systemic immune response. It is possible that in systemic inflammatory conditions, the optimum

25(OH)D levels may preserve hepcidin levels, but not in chronic condition or with a low proinflammatory profile.

The results from our study are likely different from other VD studies due to a number of factors. First, the target population. We are analyzing OA with a high prevalence of anemia and inflammation (50%), in comparison to healthy, young adults with a high prevalence of VDD that respond to VD-Supl or with early stages of CKD. Second, the inflammatory response is different in OA versus healthy young people due to dysregulation of the immune system, the immunesenescence and inflammageing that course in normal aging (increased levels 2–4 fold of pro-inflammatory cytokine and decreased level of anti-inflammatory cytokine in comparison with young adults) [3,4,41], the frailty condition [42] and metaflammation[5][43] (due to high prevalence of chronic comorbidities like DM and HTA). In addition, most of these studies highlight the VD-Sup effect over hepcidin levels, rather than basal 25(OH)D levels. In our study, adjusting for VD supplement did not change the results. Third, VDD was not as prevalent in this population as OA from other national surveys [10,17,44], likely because sun exposure is high in the southern region in comparison with other regions of Mexico. A larger sample or higher prevalence of VDD might have altered the results. Fourth, we used 25(OH)D levels to determine VD status, and it is possible that the active form of VD [1,25(OH2)D3], could be a better indicator of their activity as a suppressor of immune systemic inflammation [9,45], despite the fact that 25(OH)D is the gold standard biomarker for assessing vitamin D status.

Our results were based-population of OA from urban areas in a region where predominate multiple nutritional deficiencies, anemia, chronic diseases and obesity in all age groups [46]. Regional differences may account for differences in etnias, environment, culture, and sociodemografic characteristics in comparison with other regions of Mexico and from other OA populations.

No studies have previously documented the association between VAD and hepcidin in OA, largely because VAD only continues to be a public health issue in developing countries. Even when prevalence of VAD was lower than VDD, the association was significant mainly because serum retinol is the active form of VA, homeostatic ally controlled that drop until liver reserves are very low [21]; while for VD status, the active form 1, 25(OH2)D3 was not measure. Higher levels of hepcidin concentration in OA may be the result of multiple stimulus rather than one cause (iron overload plus inflammation, inmunescense, metaflammation) and the lack of association with 25(OH)D status in optimum levels could be the result of a reduced renal function or a failure into incorporation to cell [2]. Some polymorphism associated with biomarkers of inflammation as well as those for VD pathway may better reflect the risk rather than serum baseline levels, since the prevalence of these polymorphs may vary across different ethnicities and populations [47]. The polymorphism associated to VDR in Mexican population is higher in the southern region of Mexico (30%), being present in the 29% of Mayan ethnic population [personal communication]. This risk profile may explain, that even in normal 1,25(OH2)D3 and 25(OH)D serum concentration, VD is not properly used by the cell to execute their multiple action, (in this case, to inhibit HAMP gene expression).

One limitation of the present study is the cross-sectional design that limits the ability to infer causation. The significant association between retinol and hepcidin levels may be the result of residual confounding due to serum retinol drops under inflammatory conditions. Additionally, we included adjusted for IL-6 in the model, a known mediator in the association, despite the fact we did not use structural models. As result, the magnitude of association was reduced but still remained statistically significant. In addition, reverse causality is common in biomarker measurement, and therefore VAD and VDD may be the result of chronic inflammatory pathways that underlie the higher hepcidin levels. Moreover, the sample was restricted to OA from urban areas and they were not representative of OA from the southern region nor from the State of Campeche nor Yucatan. Our sample of OA with VAD had limited power and thus we were not able to explore stratified analysis for anemia condition and other covariables.

355 **Table 3.** Adjusted linear and ordinal regression model for Vitamin A and D status and its association to Log hepcidin levels in OA.

	Model 1, n=803		Model 2, n=797		Model 3, n=780		Model 4, n=780	
VITAMIN A Outcome: Log of Hepcidin								
	β	**95CI%**	**β**	**95CI%**	**β**	**95CI%**	**β**	**95CI%**
Retinol (ug/dl)	-0.002	(-0.004 , -0.001)	-0.003	(-0.005 , -0.01)	-0.004	(-0.01 , -0.01)	-0.003	(-0.005 , -0.01)
Log retinol	-0.10	(-0.16 , -0.03)	-0.15	(-0.18 , -0.12)	-0.16	(-0.19 , -0.13)	-0.11	(-0.15 , -0.07)
Decrement (10 u)	0.02	(0 , 0.04)	0.03	(0.01 , 0.05)	0.04	(0.03 , 0.05)	0.03	(0.01 , 0.05)
VA deficiency	0.35	(0.06 , 0.63)	0.45	(0.19 , 0.71)	0.42	(0.18 , 0.65)	0.29	(0.17 , 0.41)
Outcome: Tertile of hepcidin								
	OR	**95CI%**	**OR**	**95CI%**	**OR**	**95CI%**	**OR**	**95CI%**
Retinol (ug/dl)	1.0	(0.99 , 1)	0.99	(0.99 , 1)	0.99	(0.99 , 1)	0.99	(0.99 , 1)
Log retinol	0.84	(0.81 , 0.87)	0.75	(0.72 , 0.79)	0.74	(0.7 , 0.78)	0.8	(0.71 , 0.9)
Decrement (10 u)	1.04	(1.01 , 1.07)	1.06	(1.02 , 1.1)	1.07	(1.02 , 1.13)	1.06	(1 , 1.12)
VA deficiency	1.87	(1.03 , 3.39)	2.34	(1.27 , 4.33)	2.47	(1.48 , 4.1)	2.06	(1.47 , 2.89)
VITAMIN D Outcome: Log of Hepcidin								
	β	**95CI%**	**β**	**95CI%**	**β**	**95CI%**	**β**	**95CI%**
25(OH)D (ng/ml)	0.004	(0.0 , 0.01)	0.004	(-0.002 , 0.01)	0.004	(-0.001 , 0.01)	0.004	(-0.002 , 0.01)
Log vitamin D	0.15	(-0.02 , 0.32)	0.14	(-0.07 , 0.35)	0.15	(-0.03 , 0.32)	0.15	(-0.03 , 0.33)
Decrement (10 u)	-0.04	(-0.09 , 0.01)	-0.04	(-0.1 , 0.02)	-0.04	(-0.1 , 0.01)	-0.04	(-0.1 , 0.02)
VD deficiency	-0.13	(-0.31 , 0.05)	-0.1	(-0.31 , 0.11)	-0.14	(-0.37 , 0.09)	-0.15	(-0.35 , 0.05)
Outcome: Tertile of hepcidin								

	OR	**95CI%**	**OR**	**95CI%**	**OR**	**95CI%**	**OR**	**95CI%**
25(OH)D (ng/ml)	1.0	(1 , 1.01)	1.0	(0.99 , 1.02)	1.0	(0.99 , 1.02)	1.0	(0.99 , 1.02)
Log vitamin D	1.24	(0.97 , 1.58)	1.21	(0.78 , 1.88)	1.17	(0.76 , 1.79)	1.17	(0.73 , 1.87)
Decrement (10 u)	0.95	(0.88 , 1.03)	0.96	(0.84 , 1.09)	0.97	(0.85 , 1.1)	0.97	(0.83 , 1.12)
VD deficiency	0.80	(0.61 , 1.06)	0.82	(0.54 , 1.25)	0.78	(0.44 , 1.38)	0.78	(0.46 , 1.33)

Model 1 unadjusted

Model 2 adjusted for sex, age, indigenous and socioeconomic status, vitamin A or vitamin D status.

Model 3 adjusted for model 2 plus ferrritin, stfr, body mass index, chronic renal disease, anemia, frailty, status of inflammation (CRP and AGP), AINES and AIE consumption and supplement of VD.

Model 4 adjusted for model 3 plus IL-6.

In summary, the results of the present study shown that VA, yet not VD, is associated with hepcidin levels in OA with a high prevalence of anemia mainly due to inflammatory etiology. These finding have a public health implication since anemics had higher prevalence of VAD and VDD than non-anemics. Further longitudinal studies must explore the temporal trends between baseline levels of hepcidin, VD and VA status in settings where AI in OA is highly prevalent.

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5. Consideraciones generales

Este estudio es un primer acercamiento a conocer las causas de la anemia en los adultos mayores, cuya prevalencia fue alta en esta región en comparación a otras encuestas nacionales realizadas en México en mayores de 60 años (ENSANUT 2015, SAGE-Mex 2009; datos no publicados).

Los principales resultados destacan algunos aspectos relevantes:

1) La enfermedad renal crónica es la causa de mayor contribución de la anemia de etiología conocida, siendo la diabetes el más fuerte predictor de la anemia por enfermedad renal crónica. La diabetes autoreportada estuvo presente en una tercera parte de la población AM, por lo que las estrategias que conlleven a su prevención, impactarán sustancialmente en menores complicaciones microvasculares como el daño renal, y por ende la anemia por ERC.

2) La anemia de etiología conocida (enfermedad renal, múltiples causas, y la inflamatoria), tiene un componente inflamatorio que coexiste con las deficiencias nutricionales (de hierro y vitamina B12).

3) Una gran proporción de anemia no se explica por los criterios (robustos) previamente definidos.

4) La hepcidina parece tener un rol en la anemia por la inflamación, hallazgo que es consistente con otros estudios y con la fisiopatología de la AI pero no así, con la fisiopatología de la deficiencia de hierro.

5) El estatus de retinol sérico, la forma activa de la vitamina A, se asoció inversamente con las concentraciones de hepcidina sérica y con mayor probabilidad de AI.

6) El estatus de 25(OH)D se asoció con mayor probabilidad de anemia de origen nutricional y por múltiples causas, pero no con AI ni con los niveles de hepcidina.

Estos resultados deben ser interpretados en el contexto de un estudio transversal, por lo que la causalidad y dirección de las asociaciones están limitadas por diseño. No obstante, las asociaciones identificadas y las prevalencias observadas proveen un sustento, si bien descriptivo, de uno de los problemas de salud pública creciente en este grupo de población y cuyas oportunidades de prevención deben ser resaltadas y hechas visibles, no sólo en el ámbito académico sino para la sociedad en general.

Identificar la causa de la anemia en el AM y clasificarlo *ad hoc* con el menor error de medición en un estudio poblacional es un reto que conlleva varias aristas y que repercute en la interpretación de los datos. Varios de los criterios empleados para la definición de cada causa de anemia (deficiencia de hierro, B12, enfermedad renal) (Cuadro B) no han sido diseñados ni validados en la población de adultos mayores (incluyendo el criterio de anemia según la OMS), por lo que el error de medición y clasificación de "dichas etiologías" podría ser mayor en este grupo poblacional en comparación con otros grupos de menor edad. Además, al tratarse de población latina, y principalmente de la región sureste (con predominio de etnia maya) pueden existir ciertos riesgos asociados a las *posibles* variantes genéticas tanto para el metabolismo del hierro (y otras vitaminas), como a los moduladores de la respuesta inmune: citocinas pro y anti-inflamatorias, así como los involucrados al metabolismo de la vitamina D, entre otras. Al respecto, hay pocos estudios realizados en población mexicana e indígena que hayan analizado los polimorfismos de ciertas citocinas y el riesgo asociado a ciertos padecimientos (51); sin embargo, se desconoce cómo es el perfil proinflamatorio en este grupo de población.

La definición de la anemia por inflamación parece tener un constructo claro; sin embargo, su caracterización es compleja y no hay consenso en la literatura sobre "cómo medirla" a pesar de que varios autores han "estimado" su magnitud. La anemia por inflamación soporta una base inmunológica y ocurre principalmente en pacientes cuya activación aguda o crónica del sistema inmune sucede como parte de la fisiopatología de la enfermedad (ej. enfermedad renal crónica, infecciones [*como VIH, endocarditis, tuberculosis, osteomielitis],* cáncer y enfermedades autoinmunes).(52) Por lo que, siguiendo la definición anterior, las etiologías conocidas identificadas en este estudio (múltiples causas, enfermedad renal y anemia por inflamación), en su conjunto podrían clasificarse como "anemia por inflamación". No obstante, se desconoce cómo otras condiciones crónicas que conllevan una desregulación del sistema inmune como *diabetes, hipertensión y dislipidemia,* contribuyen a la AI; ya que no hay consistencia entre los grandes estudios epidemiológicos que han analizado las causas de anemia en los AM para considerarlas como criterio en su definición (cuadro B).

El uso de biomarcadores de inflamación (ej. CRP, AGP) como criterio único para su definición, podría conferir un potencial sesgo de información, al clasificar incorrectamente a un sujeto que cursa con un padecimiento agudo (o de corta duración) de un padecimiento crónico y que coexiste con anemia. Esto implica que la AI se sobreestime en magnitud y en

consecuencia, no se observe una asociación con los niveles de hepcidina que caracterizan a esta condición. Esto es relevante, porque los pocos estudios que han mostrado la caracterización de la AI con hepcidina, han empleado como criterio adicional, el bajo hierro sérico en presencia de inflamación y en ausencia de deficiencia de hierro, además de niveles elevados de ferritina (Cuadro B) (27). Esta definición, necesita mostrar mayor consistencia con otros estudios para poder establecer criterios de corte usando a la hepcidina como biomarcador de la AI y permitir la comparabilidad entre distintas poblaciones. En adición, en los otros estudios que emplean sólo a los biomarcadores de inflamación como criterio de AI, la proporción de anémicos "sin explicar" resulta más baja.(22,53) En nuestro estudio, la proporción de anémicos sin explicar fue alta y caracterizado por AM de un grupo de edad más joven que el resto de las otras causas de anemia y en el modelo ajustado, IL-6 fue un predictor positivo, además del consumo de AINES y el estatus de indigenismo. Lo anterior, sugiere que la anemia sin explicar conlleva un componente inflamatorio que no se refleja en los niveles de hepcidina. Una posible hipótesis, es que este grupo podría ser una categoría de transición de la etiología de la anemia a otra más definida (por inflamación, deficiencia nutricional o por ERC), ya que este grupo de anemia sin explicar está caracterizado además, por AM en condición de "pre-fragilidad". La posible contribución del status de fragilidad y el estado funcional del AM al desarrollo de la anemia,(54) no ha sido considerada en otros estudios que han caracterizado la etiología de la anemia, y dado su potencial rol en la fisiopatología de la inflamación crónica del AM(55), resulta de interés identificar su contribución por las posibles acciones de prevención en diversos desenlaces.

La deficiencia de vitamina A en los AM ha dejado de ser un tema de interés de salud pública en población americana y europea puesto que su prevalencia es muy baja.(56)(57) Por el contrario, los efectos conocidos de su toxicidad han sido ampliamente documentados mediante los estudios de suplementación. Al respecto, en América Latina, la evidencia de su deficiencia en AM había sido reportada para Chile en el año 2000, afectando al 13.7% en hombres y 15.9% de mujeres (58). Posterior a ese estudio, no se ha documentado la magnitud de la deficiencia de la vitamina A en población AM latina. Al respecto, hay que señalar que el retinol es la forma activa de la Vitamina A y cambios en su concentración pueden reflejar dos cosas: 1) un proceso inflamatorio o 2) un estado de real deficiencia. Al ser liposoluble y almacenarse en hígado, el estado de deficiencia se manifiesta en meses o años de exposición a la carencia de fuentes dietéticas de la VA; por lo que el ajuste por marcadores de inflamación es necesario para disminuir el posible efecto confusor en la

interpretación de los datos.(59) Además de sus múltiples funciones, la vitamina A es un elemento esencial en la expresión de diversos genes y en el fortalecimiento del sistema inmune.(60) Estimar el estatus de este nutriente en población adulta mayor, resultaba de interés para identificar su posible rol en la anemia, además de su frecuencia y magnitud como posible problema de salud pública.

La interacción biológica de las acciones de la vitamina D en conjunto con la vitamina A, replantean la necesidad de su estudio en poblaciones donde la prevalencia de deficiencia es alta; ya que el desbalance entre estos dos nutrientes puede dar lugar a reacciones de expresión o supresión de genes y contribuir a potencializar el riesgo si una de las deficiencias es predominante. (61)(62)(63) Dadas sus acciones inmunomoduladoras, es posible que las deficiencias de vitamina A y D observadas en el presente estudio sean resultado de un proceso inflamatorio y no el status *per se* de dicho nutriente. A pesar del ajuste por proteínas de inflamación (AGP, CRP e IL-6) en los modelos estadísticos, la causalidad reversa y confusión residual están presentes; por lo que un siguiente paso para dar consistencia en la interpretación de los datos es explorar el efecto de la ingesta dietética sobre las mismas variables desenlaces, de tal forma que la confusión por inflamación pudiera descartarse.

En resumen, este es el primer estudio en población de AM latinos que realiza una caracterización de las principales causas de anemia así como los factores que se asocian a dicha condición en los adultos mayores. Pocos estudios en la literatura han estudiado a la hepcidina en el AM, y no han documentado la potencial relación que tiene la vitamina D y retinol como moduladores de la respuesta inflamatoria en el AM. No obstante, es necesario comprender cómo son las trayectorias de hepcidina, considerando los estados basales tanto de VA como de VD y su contribución al desarrollo de la anemia en distintas etiologías así como los desenlaces asociados a cada etiología. Esto resalta la necesidad de estudios de cohorte en los AM que permitan entender estas relaciones y contribuir a recomendaciones mucho más precisas para la prevención o control de la anemia de etiología conocida y retrasar la aparición de desenlaces asociados.

6. Conclusiones

En conclusión, la anemia en el AM, tiene un fuerte componente inflamatorio como causa base. La enfermedad renal crónica fue la causa más frecuente de etiología conocida entre los anémicos afectando en promedio a 1 de cada 3; siendo la diabetes el más fuerte predictor asociado. Por el contrario, las deficiencias nutricionales tuvieron una baja contribución como causal de anemia, siendo la más frecuente la deficiencia de vitamina B12 y no así la deficiencia de hierro. Las elevadas concentraciones de hepcidina y de IL-6 caracterizaron las causas de anemia con un componente inflamatorio (ERC y AI). Por otro lado, una alta proporción de AM anémicos no fueron clasificados dentro de los criterios robustos definidos, no obstante, el estado de pre-fragilidad, IL-6, indigenismo y el consumo de AINES fueron predictores independientes de la anemia sin explicar, por lo que el componente inflamatorio podría estar asociado el desarrollo de la anemia en este grupo.

La deficiencia de vitamina A pero no así la deficiencia de vitamina D, se asoció con la anemia por inflamación y con mayores niveles de hepcidina; lo cual sugiere que la vitamina A, podría participar en los mecanismos involucrados en la movilización del hierro, de forma independiente a su acción en la eritropoyesis.

7. Referencias bibliográficas

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