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Rodolfo D. Fahrer
Alfredo Ortíz Fragola

La práctica asistencial y docente de muchos años en el Departamento de Salud Mental del Hospital de Clínicas "J. de San Martín" es el origen de este libro. Se trata de un manual para alumnos y jóvenes profesionales sobre los fundamentos de la salud mental, con una concepción integral del hombre y la matriz ambiental en la que está inserto.

MANUAL DE NUTRICIÓN Y DIABETES

Adolfo Zavala y cols.

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Enfermedad por arañazo de gato con compromiso hepato-esplénico en adultos inmunocompetentes.

Reporte de casos

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ABSTRACT

Cat scratch disease (CSD) is an emerging zoonosis caused by *Bartonella henselae*. It can occur atypically including meningitis, neuroretinitis, endocarditis and hepatosplenic involvement, a rare occurrence in immunocompetent adults. Therapeutic management is controversial, supported by case series and retrospective data published literature. Five cases of CSD with hepatosplenic involvement are described. The correct clinical and epidemiological anamnesis allow the diagnostic and avoid the performance of invasive procedures in most cases. The possibility of performing *Bartonella* spp PCR and serology is crucial.

Keywords: cat-scratch disease, liver abscess, splenic disease, *Bartonella Henselae*

Introducción

La infección por *Bartonella henselae* es una zoonosis emergente de distribución mundial, cuya presentación habitual es la enfermedad por arañazo de gato (EAG).[1] Su reservorio es el gato, que desarrolla una infección crónica y asintomática, más frecuente en animales menores de un año.[2] La transmisión se produce por mordedura, lamedura o rasguño de gatos y por picadura de la pulga *Ctenocephalides felis*, que actúa como vector. La seroprevalencia de *B. henselae* en estos animales varía según la región, siendo hasta del 50%.[1] En Buenos Aires se ha reportado en un 11,9%.[3]

La EAG se presenta frecuentemente en la infancia, caracterizándose por linfadenopatías regionales relacionadas anatómicamente con el sitio de inoculación, que suelen autolimitarse. En un 10-15% de los adultos inmunocompetentes se presenta de forma atípica incluyendo síndrome oculoglandular de Parinaud, neuroretinitis, meningoencefalitis, lesiones hepato-esplénicas, osteomielitis, endocarditis y fiebre de origen desconocido. En pacientes con trastornos de la inmunidad celular puede manifestarse con lesiones angioproliferativas que comprometen hígado, bazo, ganglios, piel y mucosas, conocidas como angiomatosis bacilar y peliosis hepato-esplénica.[4] Los diferentes escenarios clínicos producidos por la infección por *B. henselae* plantean un desafío diagnóstico.

A continuación, se reportan cinco casos de EAG con compromiso hepatoesplénico en adultos inmunocompetentes, diagnosticados entre 2017 y 2019.

Casos clínicos (Ver Tabla 1)

Caso 1

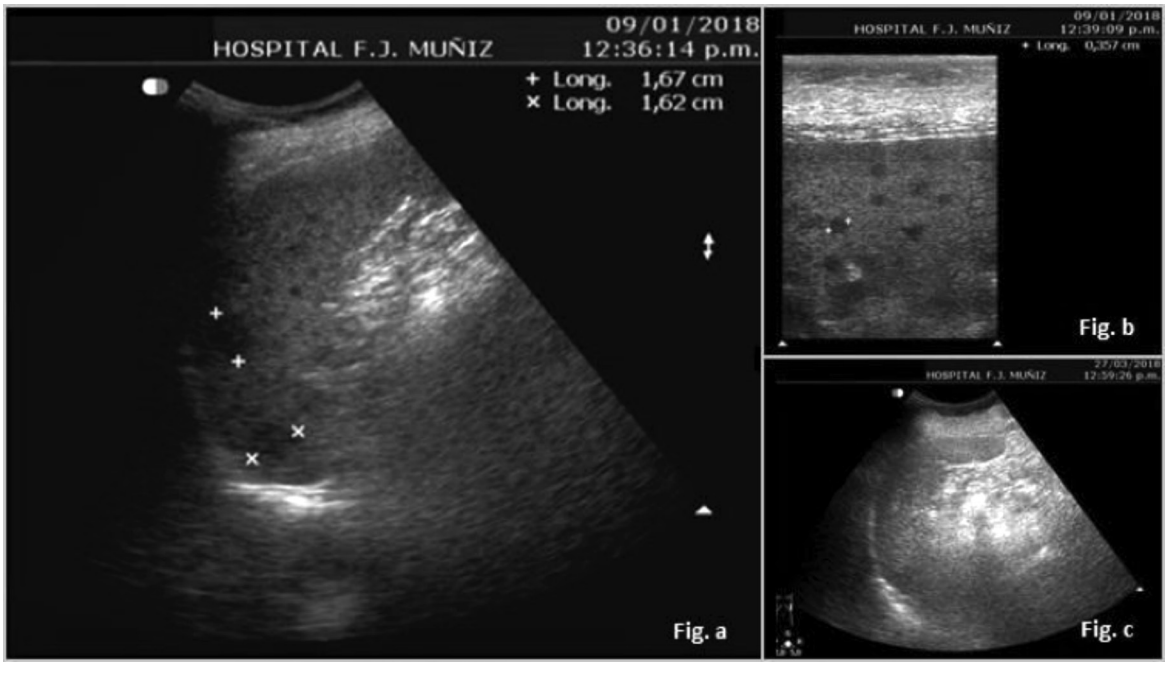
Paciente femenina de 64 años. Consultó por fiebre de 8 días acompañado cefalea retro-ocular y artralgias en ambas muñecas. Refería contacto con gato joven y picadura de pulgas.

Se realizó laboratorio evidenciando eritrosedimentación (VSG) de 75 mm 1er hora, TGO 36 UI/L, TGP 38 UI/L y FAL 347 UI/L. Se realizaron radiografía y ecocardiograma transtorácico sin hallazgos patológicos, y hemocultivos que resultaron negativos. En ecografía abdominal se

Tabla I **Características clínicas y epidemiológicas**

	CASO 1	CASO 2	CASO 3	CASO 4	CASO 5
Contacto con gatos	Picadura de pulgas	Lamedura	Arañazo	Mordedura	Contacto estrecho
Tiempo de evolución clínico	8 días	30 días	7 días	21 días	15 días
Dolor abdominal	+	+	+	+	-
Adenopatías palpables	-	-	+	+	-
Cefalea	+	+	+	+	+
Hepatoesplenomegalia	+	+	+	+	+
Lesiones hipoecoicas hepáticas y/o esplénicas	+	+	+	+	+
VSG	75 mm	40 mm	32 mm	3 mm	48mm
FAL	347 UI/l	Normal	Normal	Normal	278 U/l
TGO/TGP	36/38 UI/l	26/80 UI/l	53/58 UI/l	30/40 UI/l	201/115 U/l
Ig M	> 1/20	No se realizó	No se realizó	> 1/20	No se realizó
Ig G 1era muestra	> 1/512	Negativa	Negativa	>1/512	Negativa
Ig G 2da muestra	No se realizó	Positiva	Positiva	No se realizó	Positiva
PCR sangre	+	-	-	No se realizó	+
Tratamiento	Azitromicina + rifampicina 6 semanas	Doxiciclina + rifampicina 8 semanas	Doxiciclina 4 semanas	Rifampicina + azitromicina 3 semanas	Azitromicina 10 días, Luego azitromicina +doxiciclina 6 semanas
Defervescencia	72 hs	48 hs	48 hs	72 hs	72 hs (de biterapia)
Control ecográfico con mejoría de lesiones	+	+	+	+	+

(presente/positivo), - (negativo/ausente)



Caso 1: Ecografía esplénica. Fig. a: imágenes hipoecoicas. Fig. b: con transductor de alta frecuencia. Imágenes hipoecoicas subcentimétricas. Fig. c: control postratamiento. Bazo normal.

constató hepatomegalia con hiperecogenicidad y leve esplenomegalia con múltiples lesiones focales hipoecoicas de hasta 16mm. Se realizaron pruebas serológicas que descartaron HIV, sífilis, brucelosis, Chagas y hepatitis virales. Se realizó serología para *Bartonella henselae* resultando IgM positiva >1/20 e IgG >1/512 con PCR en sangre periférica positiva. Completó 6 semanas de tratamiento con rifampicina y azitromicina observándose defervescencia a las 72 horas y desaparición de lesiones esplénicas en ecografía de control posterior.

Caso 2

Paciente masculino de 21 años que consultó por fiebre y odinofagia de 1 mes de evolución, habiendo sido evaluado en otros centros donde recibió antibióticos sin respuesta clínica. Refería lameduras por gato joven.

En examen de laboratorio presentó VSG de 40 mm en 1er hora, TGO 26 U/I, TGP 80 U/I y ELISA HIV negativo. Al examen físico se constató dolor abdominal leve. Se realizó ecografía abdominal donde se observaban en hígado 3 imágenes hipoecoicas, la mayor de 19 mm, leve esplenomegalia con imágenes focales hipoecoicas de hasta 20 mm y adenopatía precava de 40mm. Los hemocultivos fueron negativos. Se solicitó serología y PCR para *Bartonella henselae* siendo ambas negativas. Dada la fuerte sospecha clínica, imagenológica y epidemiológica, se inició tratamiento empírico con rifampicina y doxiciclina durante 8 semanas. El paciente evolucionó de forma favorable, con defervescencia a las 48hs. Se realizó nueva serología para *B. henselae* al mes, evidenciando seroconversión de IgG. Se solicitó ecografía control a los dos meses de iniciado el tratamiento, constatando desaparición de las lesiones.

Caso 3

Paciente masculino de 48 años sano que refería contacto con gato joven. Consultó por fiebre, tumoración submandibular y dolor abdominal en hipocondrio derecho de una semana de evolución. Al examen físico presentaba lesiones en mano derecha producto de arañazos de gato de coloración rojiza, adenopatía submandibular derecha duroelástica, móvil, no dolorosa de 5 cm y esplenomegalia de 3 cm por debajo del reborde costal.

En examen de laboratorio presentó VSG 32 mm/hora, TGO 53 UI/l y TGP 58 UI/l. Se realizó ecografía abdominal observándose imagen focal e hipoecoica hepática de 10 mm con adenopatía isoecoica de 9 mm en ligamento hepatoduodenal y esplenomegalia heterogénea con imágenes hipoecoicas menores de 5mm. En la ecografía de partes blandas evidenció adenopatías de 15 y 16mm en región parotídea derecha.

Los hemocultivos y las serologías de VIH, hepatitis virales y brucelosis fueron negativas. Se realizó PCR y serología para *Bartonella henselae* con resultado negativo, constatando seroconversión de IgG al día 20. Realizó tratamiento con doxiciclina 6 semanas con defervescencia a las 48 hs de iniciado el mismo. Se observó en ecografía control resolución ad-integrum.

Caso 4

Paciente masculino de 48 años sin antecedentes. Consultó por cuadro de 3 semanas evolución con fiebre, astenia, adinamia, sudoración profusa, cefalea y dolor abdominal, acompañado de adenopatías dolorosas epitroclear y axilar izquierda. Refería mordedura de gato cachorro en mano izquierda 3 meses atrás. Había consultado en otro centro, donde se realizó una ecografía abdominal que revelaba esplenomegalia e inició tratamiento con azitromicina por sospecha de EAG. Evolucionó con defervescencia a las 72 hs de iniciado el mismo, pero continuó con síntomas constitucionales. Se repitió la ecografía abdominal y de partes blandas, evidenciando esplenomegalia de 163 mm con imagen hipoecoica de 8mm, hígado hiperecogénico, adenopatía axilar izquierda de 36 mm y epitroclear izquierda de 22mm. No se observaron alteraciones en los parámetros de laboratorio. Se descartó infección por VIH y los hemocultivos fueron negativos.

Se solicitó IgM *Bartonella henselae* con título mayor 1/20 e IgG con título mayor 1/516. Se decidió tratamiento combinado con rifampicina, doxiciclina y corticosteroides adyuvantes. Debido a intolerancia gastrointestinal por doxiciclina, completó 3 semanas de tratamiento

con rifampicina y azitromicina con resolución del cuadro clínico y mejoría ecográfica.

Caso 5

Paciente femenina de 29 años con antecedente de contacto con gato, no recordaba haber sido arañada.

Consultó por fiebre, cefalea, astenia y adinamia de 15 días de evolución. Se constató hepatoesplenomegalia leve. Se realizó laboratorio evidenciando leucocitos 2400/mm3, Hto 35%, Hb 11,5 g/dl, TGO 201 U/l, TGP 115 U/L, FAL 278 U/l, LDH 1762 U/l y VSG 48 mm/hora. Los hemocultivos resultaron negativos.

Se realizó ecografía abdominal que presentó hepatomegalia homogénea, bazo de 128 mm con imágenes hipoecoicas menores de 4 mm, adenopatías isoecoicas de 14.3 mm en ligamento hepatoduodenal y aisladas adenopatías isoecoicas retroperitoneales, menores a 12.4 mm. En tomografía con contraste de cerebro, tórax, abdomen y pelvis que no se observaron hallazgos adicionales.

Las serologías para *M. pneumoniae, C. pneumoniae*, VEB, CMV, VIH, hepatitis virales y lues resultaron negativas.

Se realizó biopsia hepática con hallazgo de mínimo infiltrado linfocitario constituido por linfocitos pequeños cd3+. No se reconocieron elementos linfoides B. Sin evidencia de compromiso por enfermedad linfoproliferativa.

La PCR en sangre entera para *Bartonella henselae* resultó positiva, mientras que la inmunofluorescencia IgG fue negativa. Se inició tratamiento con azitromicina y debido a persistencia de fiebre al día 10 de tratamiento, se adicionó doxiciclina con buena respuesta clínica y normalización de parámetros de laboratorio. Se solicitó nueva determinación de IgG para *Bartonella henselae* con resultado positivo, confirmándose el diagnóstico por seroconversión.

En control ecográfico luego de 2 meses se observó mejoría de la hepato- esplenomegalia y disminución del número de lesiones.

Discusión

La EAG atípica es infrecuente en adultos, la mayoría de los casos reportados son en población pediátrica e inmunocomprometidos, mientras que en el adulto inmunocompetente puede presentarse con compromiso hepatoesplénico, manifestándose como un síndrome febril con síntomas constitucionales y microabscesos en hígado y bazo.[5, 6, 7] Existen pocos reportes de esta afectación por *B. henselae* en la literatura.[1, 2, 5, 6] En los casos presentados los signos y síntomas más frecuentes fueron fiebre, cefalea y dolor abdominal; dos de los casos se acompañaron de linfoadenopatías regionales. La presencia de afectación hepatoesplénica con presencia de microabscesos, observados en los cinco casos, resultaron de importancia para la sospecha clínica. En el laboratorio, se observó aumento de VSG junto a discreto aumento de transaminasas hepáticas en cuatro de los casos.

Todos los pacientes referían contacto estrecho con gatos, hecho que pone de manifiesto la importancia de una adecuada anamnesis. Es importante definir el tipo de contacto con el felino e incluir además de los arañazos, mordeduras, lameduras y picaduras de sus pulgas como antecedente epidemiológico de relevancia.

El diagnóstico se realizó a través de IFI y PCR en sangre, sin requerir la obtención de material por biopsias excepto en uno de los casos. Las dos técnicas serológicas más utilizadas son ELISA e IFI. La sensibilidad y especificidad de las mismas son variables y pueden arrojar falsos positivos por reacción cruzada con otras especies de *Bartonella* spp, *Coxiella burnetti y Chlamydophila pneumoniae*. [8] A pesar de esto, resulta una herramienta útil cuando se suma el título IgG > 1/256, IgM > 1/20 y al analizarse muestras pareadas.[9] Armitano y col. analizaron las serologías IgG e IgM en pacientes con sospecha clínica, observando que un 10% de los casos presentaban Ig M >1/20 con IgG negativa.[8] Esto resalta la importancia de solicitar una nueva muestra serológica durante la convalecencia. Las técnicas de biología molecular resultan más específicas, pero tienen una menor sensibilidad (43- 76%), dato que concuerda con lo observado en nuestros pacientes.[1]

El tratamiento de la EAG es controvertido y existen pocos estudios al respecto. En su presentación típica, al tratarse de una enfermedad autolimitada puede mantenerse conducta

expectante, aunque algunos autores recomiendan drenaje y tratamiento antibiótico.[10] Bass y col. observaron que un curso de azitromicina de 5 días logró disminuir el tamaño ganglionar, sin reducir el tiempo de duración de los síntomas.[11] En un estudio retrospectivo que evaluó pacientes con compromiso ocular, el uso de doxiciclina más rifampicina y corticoides evidenció un mejor resultado en la agudeza visual posterior en los casos moderados y severos. La duración del tratamiento fue de 4 a 6 semanas en todos los casos.[12] Si bien no hay ensayos clínicos controlados sobre el tratamiento de la afectación hepatoesplénica, existe en los reportes de casos una preferencia hacia los tratamientos combinados con rifampicina, incluyendo gentamicina, trimetoprima-sulfametoxazol y fluoroquinolonas en pediátricos, y a doxiciclina en adultos.[6, 10]

La defervescencia ocurrió dentro de las 72 hs en 4 de los 5 pacientes, hallazgo similar al reportado en la bibliografía.[6] Esto permite la utilización de pruebas terapéuticas cuando existan demoras o dificultades en la confirmación diagnóstica. El control posterior se realizó con ecografía abdominal encontrándose resolución ad-integrum dentro de los 2 meses de inicio de antibióticos en 4 pacientes.

El diagnóstico de EAG requiere de una fuerte sospecha clínica. Debe considerarse dentro de los diagnósticos diferenciales de síndrome febril con afectación hepato-esplénica. Resulta de importancia la anamnesis exhaustiva sobre contacto con gatos incluyendo lamedura, mordedura y/o picadura de pulgas. La realización de pruebas serológicas pareadas y PCR en sangre periférica permite confirmar el diagnóstico evitando la realización de procedimientos invasivos.

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Declaraciones

Los autores declaran no tener conflictos de interés de ninguna clase, que el trabajo ha sido aprobado por el comité de ética responsable en el lugar de trabajo y no declaran medios de financiación del trabajo realizado.

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RESUMEN

La enfermedad por arañazo de gato (EAG) es una zoonosis emergente causada por *Bartonella henselae*. Puede presentarse de forma atípica, incluyendo meningitis, neuroretinitis, endocarditis y compromiso hepatoesplénico, lo cual es poco frecuente en adultos inmunocompetentes. Su manejo terapéutico es controvertido dada la ausencia de ensayos aleatorizados al respecto. Se describen 5 casos de EAG con compromiso hepato-esplénico, donde la correcta anamnesis epidemiológica permitió la sospecha diagnóstica, evitando la realización de procedimientos invasivos en la mayoría de los casos. La posibilidad de realización de PCR y serología para *Bartonella* spp. fueron de vital importancia.

Palabras claves: Enfermedad por arañazo de gato, absceso hepático, enfermedad esplénica, *Bartonella henselae*

Methylenetetrahydrofolate Reductase C677T Polymorphisms as a Risk Factor for Polycystic Ovarian Syndrome in a Sample of Jordanian Women

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine reproductive disorder, it can be identified by hyperandrogenism, oligomenorrhea or anovulation and polycystic ovaries on ultrasound. Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphisms associated with hyperhomocysteinemia are among the risk factors for PCOS.
Objective: The present case control study aims to explore the relationship between Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphisms as a risk factor and PCOS among Jordanian patients suffering from this disease.
Methods: 306 subjects (146 PCOS patients and 160 healthy subjects as a control group) were enrolled in the study. DNA was extracted from venous blood sample withdrawn from each participant for analyzing MTHFR C677T polymorphisms using Polymerase Chain Reaction (PCR) in combination with restriction enzyme fragment length polymorphism (PCR-RFLP). Later, PCR-RFLP products were digested with *hinfI* enzyme, then, electrophoresed on a 2% agarose gel, stained and examined under UV light. Plasma homocysteine levels were assayed using ELISA method.
Results: A significant difference was observed in plasma homocysteine levels among PCOS patients versus the control subjects and in between the different polymorphisms of PCOS patients. No significant difference was detected in the distribution and allelic frequency of MTHFR C677T polymorphisms in PCOS patients compared to the controls. 677/TT genotype and T allele were associated with 1.54 and 1.46 folds increase in the susceptibility for PCOS.
Conclusion: The study has shown that MTHFR T677T polymorphism and T allele are possible risk factors for PCOS among Jordanian women and may play a role in the pathogenesis of the disease.
Keywords: Homocysteine, Polycystic Ovarian Syndrome, Hyperandrogenism, MTHFR C677T Polymorphisms, Risk factor, PCR-RFLP.

Introduction

According to Polycystic ovary syndrome (PCOS) Consensus Workshop Group criteria, PCOS is the most common heterogeneous endocrine disorder among women throughout the reproductive life in agreement with those of the National Institute of Health for diagnosis of PCOS, it is characterized by a confirmed hyperandrogenism by clinical, laboratory investigations and an ultrasound image revealing polycystic ovaries with the exclusion of the other pathologies having similar characters, not having excess androgen secretion, or those who are not showing typical

ultrasonographic evidence of polycystic ovaries.[1] It is defined as a multifactorial disorder with various metabolic, endocrine, environmental and partly genetic factors. However, more than a hundred candidate genes have been investigated to play significant roles in pathogenesis of PCOS on one hand and in improving the diagnosis and treatment of the disease on the other hand.[2]

The full manifestation of PCOS can be detected at adolescence, when the hypothalamo-pituitary-gonadal axis becomes functioning, which is associated with some metabolic changes. The metabolic changes are associated with fat distribution within the body that increase insulin levels as a result of increased impact of circulating androgens and stimulation of steroidogenesis in ovaries.[3] Such a condition of increased insulin in women with PCOS resulting in hyperandrogenism and anovulation. Overweight girls with insulin-resistance are subjected to early adrenal over activity and PCOS at adulthood.[3]

PCOS is also known as a familial disorder to be inherited from one generation to the next one. The genetic effects on PCOS pathogenesis include those revealing high familial heritability of the disease.[4] Recently, various loci associated with PCOS risk had been identified by genome-wide association studies (GWASs) that included; acute regulatory gene of steroidogenesis polymorphisms, gonadotropin-releasing hormone receptor gene polymorphisms, follicle-stimulating hormone receptor gene polymorphisms, insulin receptor polymorphisms, vitamin D3 receptor polymorphisms, methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms.[5, 6]

MTHFR is the enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate for the remethylation of homocysteine into methionine.[7] Few studies have investigated the effect of low folates level in inhibiting the ovulation in immature super-ovulated rats and the degradation of Griffian follicles with increased number of cystic follicles in female rhesus monkeys.[8] Among humans, PCOS and the high plasma homocysteine level are correlated, which can be corrected by supplementing the patients with folic acid.[9]

MTHFR C677T polymorphism (cytosine to thymine transition at nucleotide 677 resulting in an alanine to valine substitution at codon 222) are involved in regulating folate metabolism and affecting homocysteine pathway, furthermore had been shown to be associated with 50% decrease in the activity of the enzyme (thermolabile MTHFR) and elevated levels of plasma homocysteine.[10]

This is suggesting MTHFR as a candidate gene for increasing the risk for PCOS.[11] Homocysteine is a cysteine homologue, which can be converted into methionine or cysteine for the reactions that require B-complex vitamins especially B6. There is an inverse relationship between high plasma and follicular fluid levels of homocysteine, oocyte, and embryo quality, suppressing the expression of of the gene encoding for elongation factor 2 (E2) which participates in the elongation stage of protein synthesis resulting in impaired follicle growth and oocyte maturation among the PCOS patients.[12]

More recently, a meta-analysis was performed to evalue the effect of MTHFR C677T polymorphisms as risk factors for PCOS, the results obtained from the analysis of 1478 PCOS cases from 16 different ethnicities showed that the MTHFR C667T genotypes had variable effects that may lead to increase the risk of PCOS.[13]

The aim of the present study is to explore the possible correlation between MTHFR C677T polymorphisms as a risk factor and PCOS among the Jordanian women having the disease. To the best of our knowledge, this is the first time for such a study to be conducted in Jordan.

Methodology

Subjects

A case control study was conducted on 146 Jordanian patients with PCOS, who attended the Gynecology and Obstetrics outpatient clinics of the Health center, Mutah University and Al-Karak Governmental Hospital,

Jordan. Rotterdam consensus criteris was applied to confirm the diagnosis of PCOS.[14] 160 normal healthy women, age-matched, free of menstrual cycle disturbances with no PCOS symptoms, no history of autoimmune or endocrine disorders and no surgery in the pelvis were enrolled in the study as a control group.

Exclusion criteria

All patients with diabetes mellitus, hypertension, thyroid diseases, Cushing's syndrome, ovarian tumors, acromegaly and oral contraceptive pills use during the last 6 months prior the study were excluded.

Blood sampling

A 5 ml venous blood sample was withdrawn from each participant in the study in EDTA treated tubes after overnight fasting. Plasma was separated immediately by centrifugation at 1800 x g for 15 minutes,then, stored at -20°C for homocysteine assay. The whole EDTA blood was used for DNA extraction.

Assay of plasma homocysteine concentration

It was done by commercially Enzyme-Linked Immunosorbent Assay (ELISA) supplied by Diagnostic Automation/USA according to the method of Engvall et al.[15]

Analysis of MTHFR C677T polymorphisms

DNA was extracted from all samples using the method of Sambrook et al.[16] They were analyzed by PCR method coupled with the restriction enzyme fragment length polymorphism (PCR-RFLP) as described by Jacques et al.[17] The sequences of primers for MTHFR C677T polymorphisms were as follows: sense-5'- TGA AGG AGA AGG TGT CTG CGG GA -3' and antisense-5'- AGG ACG GTG CGG TGA GAG TG -3'. These primers amplified 198 bp fragment of DNA. The PCR began with an initial denaturation at 94°C for two minutes, then, 35 cycles of denaturation at 94°C for one minute, annealing at 62°C for one minute, and extension at 72°C for one minute. The final extension was at 72°C for ten minutes. The amplicons were digested with 5 units of *hinfI* restriction enzyme (Promega, Madison, WI, USA) at 37°C for two hours. The digested amplicons were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide. Later, the amplicons were visualized using a UV transilluminator, which resulted in a 198 bp band for the homozygous wild type (C677C), 175 bp and 23 bp bands for the heterozygous mutants (C677T), and 198, 175 and 23 bp bands for the homozygous mutants (T677T) of MTHFR C677T polymorphisms. The 23 bp band was not seen because of its small size.

Statistical analysis

Data was analyzed using SPSS statistics version 21 software (IBM Corp. Armonk, NY). The numerical data were expressed as mean±SD and the difference between groups was assessed using ANOVA. The qualitative data was expressed as frequency, percentage, and odds ratio. The possible connection between two variables was assessed using Pearson's χ^2. Allele and genotype frequencies were determined by allele counting. The concordance of the polymorphisms frequencies with the Hardy-Weinberg equilibrium was evaluated using Pearson's χ^2.

Results

The results have shown significant difference between the mean values of plasma homocysteine level in the group of PCOS patients when compared to the control subjects (Table 1). The distribution of MTHFR C677T polymorphisms and the frequency of C and T alleles have been shown in table 2.

Table 1. Mean value±SD of the age and the plasma homocysteine level among PCOS patients and the controls

	PCOS patients (no. 146)	Control group (no. 160)	P value
Age	26.1 ± 2.34	26.9 ± 3.01	0.655
Homocysteine (μmol/l)	23.2 ± 0.87	6.42 ± 0.532	<0.001*

* P<0.001 is significant versus the control subjects

Table 2. MTHFR C677T polymorphisms distribution and allele frequency between PCOS patients and the controls.

Polymorphism/ allele	PCOS patients (no. 160)	Control (no. 160)	OR (95% CI)	P vaule
T677T	49 (33.56)	78 (48.75)	1.54 (1.11-2.56)	0.43
C677T	67 (45.89)	52 (32.50)	0.78 (0.89-1.78)	0.66
C677C	30 (20.55)	30 (18.75)	0.72 (0.91-1.74)	0.71
C	127 (43.49)	124 (38.75)	0.89 (0.87-1.94)	0.86
T	165 (56.51)	196 (61.25)	1.46 (1.13-1.91)	0.39

Regarding the prevalence of MTHFR C677T polymorphisms, there was no significant difference between PCOS patients compared to the control subjects, there was increased prevalence of the heterozygous polymorphism (677/CT) in the PCOS group. No statistically significant association between the different polymorphisms was detected in PCOS patients; however, the homozygous genotype (677/TT) and T allele were associated with increased risk for PCOS (OR= 1.54 and 1.46,respectively) (Table 3). A statistically significant association was found between the mean homocysteine level of PCOS patients with the different MTHFR C677T polymorphisms (p =0.039).

Table 3. Association between the mean values of plasma homocysteine levels among PCOS patients with the different types of MTHFR C677T polymorphisms

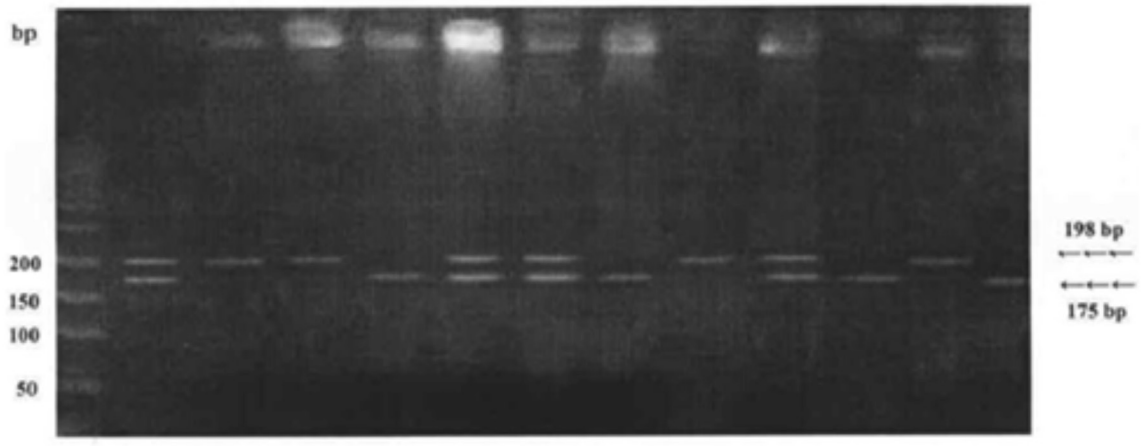
Polymorphisms	Plasma homocysteine level (µmol/l) (Mean±SD)	P value
677/CC	18.32 ± 2.01	
677/CT	24.31 ± 1.93	0.039*
677/TT	18.73 ± 1.66	

Discussion

PCOS is the most prevalent multifactorial endocrine pathology, associated with infertility, obesity, and insulin resistance caused by complex interactions between environmental and predisposing polygenic background.[18] However, the heterogeneity and the inability to realize the exact etiology and pathophysiology of PCOS have made it difficult to identify the candidate genes with understandable clinical significance involved in causing PCOS.[19] HapMap project of human genome was used to describe the pattern of human genetic variation, especially those affecting health. Several candidate genes play role in PCOS development, including genes involved in homocysteine-methionine metabolism particularly, MTHFR gene.[20]

The present study has investigated the level of plasma homocysteine as a consequence for MTHFR C677T polymorphisms resulting in reduction of enzyme activity among the patients with PCOS. The results have revealed a statistically significant difference in plasma

Figure 1. PCOS patients with the different MTHFR C677T polymorphisms. A 2% agarose gel electrophoresis showed the amplicons PCR-RFLP of C667T polymorphisms after being digested by hinfI. Later, it showed 198 bp fragment for the homozygous C677C polymorphism, three fragments 198, 175 and 23 bp for the heterozygous C677T polymorphism, and two fragments 175 and 23 bp for the homozygous T677T polymorphism. The 100 bp DNA ladder was indicated to the left side of the gel.



homocysteine level among PCOS patients as compared to the control subjects. Hyperhomocysteinemia is associated with atherogenesis and chronic vascular damage leading to arterial stiffness, which could be a risk factor for PCOS. Li et al. showed that serum homocysteine levels were greater among PCOS patients as compared to control subjects, which provides an evidence for playing the role of impaired liver function in PCOS.[21, 22] Agilli M, et al, and Salehpour S. have shown that the mean homocysteine levels were significantly higher among PCOS patients as compared to the healthy control subjects.[23, 24] The treatment of hyperhomocysteinemia in those patients is essential to improve reproductive functions. de la Calle et al. reported an increase in the levels of homocysteine in PCOS patients, which is negatively associated with the levels of folic acid.[25]

In the present study, the comparison of homocysteine levels of PCOS patients within different MTHFR C677T polymorphisms revealed a statistically significant difference (p =0.039), which is confirmed by the study of Grodnitskaya EE.[26] The genetic variants of MTHFR could affect the metabolism of folate and homocystiene resulting in elevated plasma levels of homocysteine, and playing an important role in PCOS pathogenesis.[27] Orio Jr F et al reported in a study including women with PCOS and healthy controls that the polymorphisms of MTHFR C677T do not influence the serum levels of homocysteine and PCOS development.[28]

The evaluation of MTHFR C677T polymorphisms frequency between the two study groups in our study revealed no statistically significant difference. In the PCOS group, there was increased frequency of the heterozygous polymorphism (677C/T), but no statistically significant association between C677T genotype and the increase in the susceptibility for PCOS (OR=0.78). The homozygous genotype (T677T) and T allele had elevated risk of PCOS when compared to the other genotypes (C677T and C677C) and C allele (OR= 1.54 and 1.46, respectively). Those findings are consistent with the results of (10), who reported statistically insignificant difference among the different MTHFR C677T polymorphisms in PCOS patients with a slightly higher prevalence of heterozygous (C677T) polymorphism among the women with PCOS.

The genetic association studies are essential as they provide a clear idea about the role MTHFR C677T polymorphism in the pathogenesis of PCOS.[29] An Indian study shows a relative risk of PCOS (OR=1.32) for the heterozygous (C677T) genotype. Another study found a relative high risk for PCOS.[30] Majority of the studies did not find any significant association between PCOS and MTHFR C677T polymorphisms.[28] It was noticed that high plasma levels of homocysteine were observed among PCOS patients; however, the presence of MTHFR C677T polymorphisms was not found to affect the high levels of homocysteine.[30] Palep-Singh M et al has found that Caucasian women with PCOS had higher plasma levels of homocysteine and a 1.9 times higher frequency of the T allele as compared to South Asian PCOS group.[31]

The obtained results in the present study revealed that the frequency was higher for the heterozygous model C677T than the other two models C677C and T677T which is inconsistent with the results obtained by Naghavi et al. who reported that the homozygous model C677C was higher in prevalence among Iranian patients with PCOS when compared to the other two models.[13, 18] Wang et al. studied the association between MTHFR C677T polymorphisms and the risk of PCOS, the study results revealed that the T allele was not significantly associated with the risk of PCOS. The analysis of the association by ethnicity showed that the T allele significantly increases risk of PCOS among the Asian population. There was no association for the Middle Eastern population and interestingly the T allele was found to be protective against PCOS among the Caucasian population. Moreover, the combined genotypes form (CT+TT) was significantly associated with increased risk of PCOS among the Asian and Middle Eastern population.

The results of the study of Carlus et al. had shown no association between MTHFR

C667T polymorphisms and the susceptibility of PCOS, which was inconsistent with the obtained results of Fu et al. who reported C667T polymorphism as a risk factor for PCOS.[32, 33] Qi et al. found that MTHFR C677T genetic mutation could influence the occurrence of PCOS risk among the Chinese population. [34] Jain M, Pandey P et al stated that the CT genotype of MTHFR C677T was associated with 1.32-fold increase in the risk of developing PCOS.[10] Choi et al. indicated that there was no correlation between the MTHFR C677T polymorphism and PCOS among the Korean population. Among the Turkish population, Karadeniz et al.[29] Karadeniz M et al reported that MTHFR C677T gene variants have no effect on plasma homocysteine levels in PCOS patients. The inconsistency of these results may be due to differences in ethnicities, selection of study subjects, and sample size.[30]

Limitations of the study

The current findings mare limited by the relatively small study subjects, other factors that may play various roles in developing the disease including the environment, behavior and diet were not evaluated, also, PCOS patients and the controls were selected from a health center and a hospital. Therefore multicenter studies should be included with recuirting more study subjects for verifying the obtained results in the current study and environmental-genetic interactions should be considered.

Conclusion

The study has investigated the correlation between MTHFR C677T polymorphisms as risk factors and PCOS among Jordanian patients suffering from this disease. There was statistically significant difference ($p = 0.039$) in homocysteine levels of PCOS patients with different genotypes. The results showed the association between homozygote mutant (TT) and T allele of MTHFR C677T polymorphism and increasing the susceptibility for PCOS among Jordanian women.

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Declarations

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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RESUMEN

Antecedentes: el síndrome de ovario poliquístico (SOP) es un trastorno endocrino reproductivo común, se puede identificar por hiperandrogenismo, oligomenorrea o anovulación y ovarios poliquísticos en la ecografía. Los polimorfismos de la metilentetrahidrofolato reductasa (MTHFR) C677T asociados con la hiperhomocisteinemia se encuentran entre los factores de riesgo del síndrome de ovario poliquístico.

Objetivo: El presente estudio de casos y controles tiene como objetivo explorar la relación entre los polimorfismos C677T de la metilenotetrahidrofolato reductasa (MTHFR) como factor de riesgo y el síndrome de ovario poliquístico entre los pacientes jordanos que padecen esta enfermedad.

Métodos: Se inscribieron en el estudio 306 sujetos (146 pacientes con SOP y 160 sujetos sanos como grupo de control). Se extrajo ADN de una muestra de sangre venosa extraída de cada participante para analizar los polimorfismos de MTHFR C677T utilizando la reacción en cadena de la polimerasa (PCR) en combinación con digestión con enzima de restricción (PCR-RFLP). Posteriormente, los productos de PCR-RFLP se digirieron con la enzima HinfI, luego se sometieron a electroforesis en un gel de agarosa al 2%, se tiñeron y se examinaron bajo luz ultravioleta. Los niveles de homocisteína en plasma se analizaron utilizando el método ELISA.

Resultados: Se observó una diferencia significativa en los niveles plasmáticos de homocisteína entre los pacientes con SOP frente a los sujetos de control y entre los diferentes polimorfismos de los pacientes con SOP. No se detectaron diferencias significativas en la distribución y frecuencia alélica de los polimorfismos MTHFR C677T en pacientes con SOP en comparación con los controles. El genotipo 677 / TT y el alelo T se asociaron con un aumento de 1,54 y 1,46 veces en la susceptibilidad al síndrome de ovario poliquístico.

Conclusión: El estudio ha demostrado que el polimorfismo MTHFR T677T y el alelo T son posibles factores de riesgo de SOP entre las mujeres jordanas y pueden desempeñar un papel en la patogenia de la enfermedad.

Is there an association between - 2549 Insertion/ Deletion Polymorphisms in the Promotor Region of the Gene Encoding for VEGFA as a Risk Factor and the Idiopathic Recurrent Spontaneous Miscarriage in a sample of Jordanian Women?

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ABSTRACT

Background: At least 50% of the cases of recurrent spontaneous miscarriage are aetiologically idiopathic. Recently various genetic polymorphisms have been proposed as susceptibility risk factors for pregnancy loss. **Objective**: The aim of the present case control study is to establish the association between the functional –2549 I/D polymorphisms in the promoter region of the vascular endothelial growth factor A (VEGFA) gene and idiopathic recurrent spontaneous miscarriage (IRSM) in a sample of Jordanian women. **Subjects and methods**: 328 subjects were recruited, 103 and 98 women with primary and secondary IRSM, respectively, 127 normal women were selected as a control group. Genomic DNA was isolated from a blood sample withdrawn from each participant, then, -2549 I/D polymorphisms of VEGFA gene were genotyped by Polymerase Chain Reaction (PCR). **Results**: The obtained results revealed that ID polymorphism and D allele of VEGFA -2549 I/D polymorphisms have the highest frequencies in both primary and secondary IRSM patients, no significant difference between the three groups regarding polymorphisms and allele frequencies, patients with DD+ID genetic models have positive association with high risk of IRSM versus II model, and patients with D allele are more liable to have IRSM than those having I allele, no significant difference in the association of VEGFA -2549 I/D polymorphisms with IRSM in the three genetic models of the primary and secondary IRSM patients. **Conclusion**: patients with ID genetic model of -2549 I/D polymorphisms in the VEGFA gene's promotor region and D allele have higher risk for IRSM.

Keywords: Polymorphisms, Miscarriage, VEGFA, Idiopathic, Insertion/Deletion**,** recurrent spontaneous miscarriage are aetiologically idiopathic**,** PCR.

Introduction

IRSM, the frequent obstetric complication, can be explained as loss of three or more successive pregnancy prior to the 20th weeks of gestation. Up to 50% of IRSM patients have unknown underlying etiology and it affects 1-5% of the women who are seeking bear children.[1] Different factors like endocrine dysfunctions, autoimmune diseases, and uterine pathologies in addition to environmental and nutritional factors have been demonstrated for their direct and/or indirect effects on miscarriage.[2] Placental circulation in addition to fetal vasculature should be sufficient for maintaining normal pregnancy which is requiring angiogenesis. Such a process is dependent upon multiple factors, including VEGFA.[3]

VEGFA, the potent angiogenic factor, is secreted by both fetal and maternal trophoblastic cells for enhancing vascular permeability, hematopoiesis, endothelial cell proliferation and survival.[4] VEGF has receptors (VEGFR1/Flt-1, VEGFR2/KDR/Flk-1), its functions are related to human reproduction, including placental organization and fetal angiogenesis, in addition to pre-decidualization and gametogenesis.[5, 6] The rate of expression of VEGFA/VEGFR was the highest in the maternal side of the placenta, which is different under conditions of normal pregnancy versus the complicated one.[7] This is because of the abnormalities of angiogenesis during implantation and placenta formation early in pregnancy which may lead to IRSM.[3]

The possible role of VEGF has enticed the interest of researchers and clinicians. Thus far, several single nucleotide polymorphisms (SNPs) based on nucleotides substitutions and those dependent on insertion (I) or deletion (D) or both of one or more nucleotides (indels) have been described in the VEGF gene. Several SNPs are identified to be associated with the susceptibility to several cancers, metabolic or vascular disorders. [8] Although several SNPs were discussed in IRSM and other reproductive disorders, the interests in insertion/deletion (I/D) polymorphisms researches become more prominent, due to their participation in genetic and phenotypic divergence and diversity.[9, 10]

Human VEGF, the highly polymorphic, cell specific mitogenic gene especially in the promoter, 5'-and 3'-untranslated regions, located on chromosome 6p21.3 is consisted of seven introns and eight exons, spanning approximately 14 kb.[11] More than twenty-five different polymorphisms have been identified in the gene encoding for VEGFA which were suggested to influence the levels of its expression.[12]

The most common genetic association with IRSM that was investigated for 20 polymorphisms are –1154 G/A, +936 C/T, –2578 C/A, and –634 G/C SNPs, the obtained results from these studies are inconsistent.[13, 14, 15] n the VEGFA gene, a functional I/D polymorphism is located at position –2549 in the promoter region.[16] Deletion of an 18 base pair (bp) long sequence (D allele) results in a 1.95-fold elevated transcriptional activity compared to the allele containing the insertion (I allele).[17]

VEGF gene -2549 I/D polymorphisms may be related to other diseases. In a recent study, it had been shown that uterine leiomyoma was linked to VEGF gene -2549 I/D polymorphisms, while, another study concluded that raised susceptibility to diabetic nephropathy in north Indian population has an association with DD genotype and D allele in I/D polymorphism at –2549 position of VEGF gene. [18, 19] Previous literatures had evaluated the effect of VEGF gene polymorphisms on IRSM and revealed novel data.[15, 20, 21, 22, 23, 24] Our study made a comparison with the previous meta-analysis and to the best of authors' knowledge; it is the first study to be conducted in Jordan.

The aim of our study is to explore whether there is an association between the functional –2549 I/D polymorphism in the promoter region of the VEGFA gene and IRSM in a sample of Jordanian women.

METHODOLOGY

Subjects

Two hundred and one women who had experienced at least two successive spontaneous abortions and stillbirths were recruited from out-patient clinics attendants of the Obstetrical Clinic, Obstetrics and Gynecology Department, Al-Karak governmental Hospital. One hundred and twenty seven women who experienced at least two live births and no abortion and who have no history of infertility were included in the study as a control group. A written informed consent was obtained from each participant in the study. The study was approved from the Medical Ethics, Committee of the Faculty of Medicine, Mutah University.

Exclusion criteria

Women with chromosomal anomalies in either partner, endocrine or metabolic disorders, anti-phospholipid syndrome, autoimmune dis-

ease or other systemic diseases, previous arterial or venous thrombosis, or structural uterine anomalies detected by ultrasonography and/or hysteroscopy were excluded from the study.

The subjects enrolled in the study were subdivided into three groups:

1- Group I: primary IRSM (n=103), with a history of two or more pregnancy losses but no live birth.

2- Group II: secondary IRSM (n=98), experienced three or more pregnancy losses after one live birth.

3- Group III: control (n=127) with at least two live births with no abortion nor history of infertility.

The cases with the history of recurrent pregnancy loss (primary and secondary) were subjected to ultrasound and hysterosalpingography for detection of anatomic abnormalities of the genital tract such as the woman with septate uterus were excluded from the study.

-2549 Insertion/Deletion polymorphisms analysis

Five ml of blood was withdrawn from each participant in the study (IRSM patients and the healthy controls) for DNA extraction using the method adopted by Sambrook et al.[25] The analysis of 18 bp I/D polymorphisms of -2549 in the promotor region of VEGFA gene was determined by PCR in a thermal cycler (Perkin Elmer Cetus, Norwalk, CT, USA) using a pair of primers (sense-5′-CCTGGAGCGTTTTGGTTAAA-3′ and antisense-5′-ATATAGGAAGCAGCTTGGAA-3′).[16] The reaction total volume was 50 µl containing 100 ng DNA template, 50 mM KCl, 10 mM TrisHCl, 0.1% Triton X-100, 200 mM each of dATP, dCTP, dGTP and dTTP (Integrated DNA Technologies/ USA), 2.5 mM $MgCl_2$, 0.5 mM of each primer, and 1 U Taq DNA Polymerase (iNtRON Biotechnolgoy/ Korea). The reaction started with an intial denaturation for 2 min at 95°C, then, the samples were subjected to 30 cycles of three file program in the thermal cycler (45 sec at 95°C, 30 sec at 55°C) and 1 min at 72°C, followed by a final extension at 72°C for 5 min. The 18 bp I/D polymorphism PCR products were electrophoresed on 2% agarose gel, stained and visualized under UV light. The 100 bp DNA ladder was indicated to the left side of gel, two fragments were revealed two bands 234 bp and 18 bp (not seen) for I allele, and one band 216 bp for D allele.

Statistical analysis

Data were analyzed by IBM SPSS version 20. Pearson's $\chi 2$ and ANOVA tests were used to assess differences between the studied groups. Allele and genotype frequencies were determined by allele counting, Odds ratio (OR) and 95% confidence interval (CI) were also determined. Concordance of the polymorphisms frequencies with Hardy-Weinberg equilibrium was evaluated using $\chi 2$.

Results

The study of VEGFA -2459 I/D polymorphisms among the different groups (Table 1) revealed that I/D polymorphism is the highest in frequency, while, II gene is the lowest one and there is no statistically significant difference among the three groups ($\chi 2$= 0.625, p = 0.960). The frequency of D allele was the highest in patients with secondary IRSM (61.1%) and the least among the controls (56.3%), while, I allele frequency was higher in the control group (43.7%) than in patients with primary IRSM (43.6%) and secondary IRSM (38.9%) and no statistically significant difference among the three groups ($\chi 2$= 0.341, p = 529) (Table 2).

Table I Polymorphisms' frequency of the VEGFA -2459 I/D in the IRSM groups (primary and secondary) of women and the controls

Group/ polymorphism	DD (no. %)	ID (no. %)	II (no. %)	$\chi 2$, P value
Control	46 (36.22%)	51 (40.15%)	30 (23.63%)	0.625, 0.960
Primary IRSM	36 (34.95%)	44 (42.72%)	23 (23.33%)	
Secondary IRSM	39 (39.80%)	42 (42.86%)	17 (17.34%)	
Total IRSM	75 (37.31%)	86 (42.79%)	40 (19.90%)	

Table II Alleles frequency of -2549 I/D polymorphisms in IRSM groups (primary and secondary) and the controls

Group/allele	D (no. %)	I (no. %)	χ2, P value
Control	142 (55.90%)	112 (44.10%)	0.341, 0.529
Primary IRSM	116 (56.31%)	90 (43.69%)	
Secondary IRSM	120 (61.22%)	76 (38.78%)	
Total IRSM	236 (58.70%)	166 (41.30%)	

The obtained results revealed an OR of 1.28 with 95% CI = 0.94 to 1.85) suggesting that the patients with dominant and co-dominant genetic models (DD+ID) had positively associated with the risk of IRSM compared to the recessive genetic model (II). Another comparison between dominant gene (DD) on one side with recessive and co-dominant genes (ID+II) on the other side, showing an OR of 1.24 with 95% CI = 0.88 to 1.93 which is still indicating that IRSM is more likely to occur in the patients with the dominant gene. Patients with DD polymorphism had more liability (Odds ratio = 1.34 with 95% CI= 0.99 to 1.97) to have IRSM than those with recessive gene (II). In contrast, Patients with DD and II polymorphisms are less likely to have IRSM than patients with ID polymorphism (Odds ratio = 0.94 with 95% CI= 0.98 to 1.75and Odds ratio = 0.96 with 95% CI= 0.83 to 1.44, respectively). The assessment of D and I alleles revealed that patients with D allele are more liable to have IRSM than patients with I allele (Odds ratio = 1.33, 95 % CI =0.94 to 1.97) (Table 3).

Table III The association of VEGFA -2549 I/D polymorphisms with IRSM in patients versus the controls

VEGFA polymorphism	OR	95% confidence interval	χ2
DD+ID versus II	1.28	0.94 to 1.85	0.108
DD versus ID + II	1.24	0.88 to 1.93	0.087
DD versus II	1.34	0.99 to 1.97	0.126
DD versus ID	0.94	0.98 to 1.75	0.212
II versus ID	0.96	0.83 to 1.44	0.263
D versus I	1.31	0.98 to 1.94	0.171

Additionally, the results revealed that there is no statistically significant difference in the association of VEGFA -2549 I/D polymorphisms with IRSM under dominant, recessive and co-dominant genetic models of the primary and secondary IRSM patients, while, the assessment of D and I alleles revealed that IRSM is more likely to occur in patients with D allele more than patients with I allele (Odds ratio = 1.33, 95 % CI=0.94 to 1.97) (Table 4). (Figure 1)

Table IV The association of VEGFA -2549 I/D polymorphisms with the primary IRSM patients versus the secondary IRSM patients

VEGFA polymorphism	OR	95% confidence interval	χ2 *P* value
DD+ID versus II	0.69	0.72 to 1.47	0.407 0.523
DD versus ID + II	0.81	0.82 to 1.46	0.200 0.655
DD versus II	0.94	0.94 to 1.58	0.014 0.906
DD versus ID	0.87	0.79 to 1.73	0.393 0.531
II versus ID	0.71	0.91 to 1.44	0.299 0.584
D versus I	1.33	0.94 to 1.97	0.131 0.538

Discussion

IRSM is a disorder affected by factors that are related to genetics and non genetics. Certain studies have revealed an association between genetic variants as risk factors and IRSM.[26, 27, 28, 29] VEGF is a main factor for vasculogenesis in both pathological and physiological conditions; it is overexpressed in a variety of tissues, including the reproductive system of women, ischemic tissues, cancers and during cellular transformation, the enhancement of angiogenesis up regulates the plasma level of VEGF.[30, 31, 32, 33, 34]

The role of VEGF in both placental and fetal angiogenesis has been concluded from gene studies.[35, 36] VEGF is necessary for the oocytes maturation, trophoblasts proliferation, embryo implantation, the placental angiogenesis, and the maternal and fetal blood vessels growth.[13, 37] According to the above, it is reasonable that VEGF may be involved in the pathogenesis of IRSM in women.

Genetic polymorphisms may affect the production of VEGF, like I/D 18 base pair (bp) polymorphism at site -2549 of the VEGF gene

Figure 1 IRSM patients with the different VEGFA -2459 I/D polymorphisms

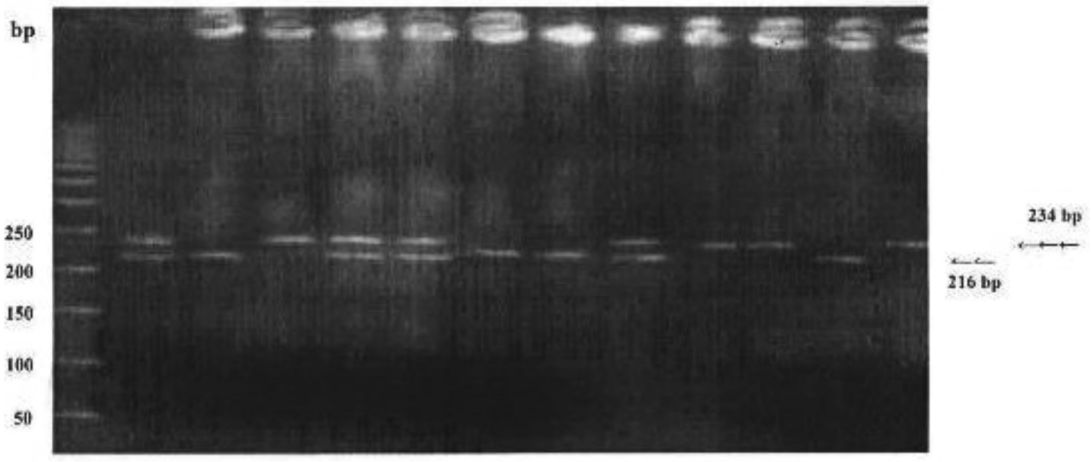

Figure 1: 2% agarose gel electrophoresis for PCR amplicons of VEGFA -2549 I/D polymorphisms, the dominant gene (DD) (234 bp), the recessive gene (II) (216 bp), the co-dominant gene (ID) (216, 234 bp), 100 bp DNA ladder is indicated to the left side of the gel.

which was demonstrated to be involved in the development of many diseases, especially those based on angiogenesis, including breast cancer, Alzheimer's disease and preeclampsia.[38, 39, 40, 41, 42] The homozygous (DD) and heterozygous deletion (DI) genotypes have more transcriptional activity of VEGF gene and therefore more production.[16]

The obtained results in the present study revealed that the frequency of ID polymorphic form of VEGFA -2459 I/D genetic models among primary, secondary and total IRSM groups of women was higher than II and DD models, while, II polymorphism was the lowest one. The assessment of the frequency of D and I alleles showed that D allele was the highest in frequency in secondary IRSM women and the least among the controls, I allele frequency was higher in the control group than in women with primary and secondary IRSM. There was no statistically significant difference between the three groups regarding frequency of polymorphisms and alleles.

VEGFA variants-IRSM interrelationship was confirmed by the following studies, which illustrated the association of labor before 37th weeks of gestation and VEGF 936C/T polymorphisms in Greeks and North Indians study which claimed that subjects with VEGF gene polymorphisms –1154 G/A and +936C/T have higher risk of abortion, but Lee et al. who studied Koreans and concluded that VEGF polymorphisms of in IRSM are inconclusive, therefore, further studies are needed to be done with more sample sizes and in different races or ethnicities.[43, 44, 45] Another Brazilian study involving -634, 936 VEGF polymorphisms suggested that no correlations were found in any of the investigated polymorphisms, even among co-dominant, dominant and alleles.[46]

Our results suggested that -2549 I/D polymorphisms of the dominant (DD) and co-dominant (ID) models had positively associated with IRSM risk versus the recessive one (II). Also, the comparison between the dominant gene compared to the co-dominant and the recessive ones indicated that IRSM is more likely to occur in the patients with the dominant gene. In contrast, patients with dominant and recessive polymorphisms are less likely to have IRSM than patients with co-dominant polymorphism. Furthermore, it is shown from the results of the present study that patients with D allele are more likely to have IRSM compared to those having I allele, but, no significant difference in the association between the three genetic models and IRSM in primary and secondary patients.

Pereza et al (24)studied –2549 I/D polymorphisms of the VEGFA gene in the promoter region in couples and found that the frequency of ID gene is the highest in both

diseased and control patients. This is consistent with the findings of this study, however, the comparison between DD and ID genetic models was less than the results in the present research. Additionally, Hashemi et al. found that the Or of ins/ins versus del/del and del/ins+ins/ins compared to del/del were 2.85 and 2.19, respectively, while, the allelic OR was higher than the values obtained in the present study.[21] However, this inconsistency in the results can be explained by the racial and ethnicity difference of patients.

VEGF -2549 polymorphisms were studied in patients with diabetic retinopathy, where ID genotype was also identified as a potential risk factor for the disease, whereas in other studies, DD polymorphism was detected as a risk factor for diabetic retinopathy , where ID genotype was also identified as a potential risk factor for the disease, whereas in other studies, DD polymorphism was detected as a risk factor for diabetic retinopathy.[47, 48]

VEGF gene polymorphisms in patients with diabetes mellitus were evaluated; the study identified a statistically significant association between DD and DD+ID genotypes as risk factors on one hand, D allele on the other hand, and the development of diabetes mellitus and its neuropathic complication, while, the frequency of II genotype was more in the controls suggesting the role of this polymorphism and I allele which was detected to be more frequent than D allele in the controls in the prevention of diabetes mellitus pathogenesis. Those findings are proving the association between the existence of D allele and the increase in the risk of vascular complications of diabetes mellitus.[49]

An additional study clarified the role of VEGF SNPs in metabolic syndrome pathogenesis, stating that the association between remains to be evaluated this should be done using functional studies by enrolling larger homogenous populations in various racial and ethnic populations.[9]

The limitations to this study are including, a relatively small number of IRSM patients. It should be carried out on a larger group of patients to provide the data for explaining the role of -2549 I/D VEGFA gene polymorphisms in the development of IRSM. Also, the correlation between the levels of VEGFA and the different polymorphisms was not included in the present study which is requiring further investigations in the future.

Conclusion

The results of the present study showed that women with ID model of the -2549 I/D polymorphisms in the promotor region of the gene encoding for VEGFA and those who carry D allele are at higher risk to have IRSM. More genetic studies in different ethnic populations for the VEGFA gene polymorphisms regarding larger loci for more SNPs are needed to shed the light on the association between the studied polymorphisms of VEGFA gene including -2549 I/D genotypes and IRSM in the Jordanian women for the accurate diagnosis and proper management.

Declarations

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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RESUMEN

Antecedentes: al menos el 50% de los casos de aborto espontáneo recurrente son etiológicamente idiopáticos. Recientemente se han propuesto varios polimorfismos genéticos como factores de riesgo de susceptibilidad a la pérdida del embarazo. Objetivo: El objetivo del presente estudio de casos y controles es establecer la asociación entre los polimorfismos funcionales –2549 I / D en la región promotora del gen del factor de crecimiento endotelial vascular A (VEGFA) y el aborto espontáneo recurrente idiopático (IRSM) en una muestra de las mujeres jordanas. Sujetos y métodos: Se reclutaron 328 sujetos, 103 y 98 mujeres con IRSM primario y secundario, respectivamente, se seleccionaron 127 mujeres normales como grupo de control. Se aisló ADN genómico de una muestra de sangre extraída de cada participante, luego, se genotipificaron los polimorfismos I / D -2549 del gen VEGFA mediante la reacción en cadena de la polimerasa (PCR). Resultados: Los resultados obtenidos revelaron que el polimorfismo ID y el alelo D de VEGFA -2549 polimorfismos I / D tienen las frecuencias más altas en pacientes IRSM tanto primario como secundario, sin diferencia significativa entre los tres grupos en cuanto a polimorfismos y frecuencias alélicas, pacientes con DD + ID Los modelos genéticos tienen una asociación positiva con un alto riesgo de IRSM versus el modelo II, y los pacientes con alelo D son más propensos a tener IRSM que los que tienen el alelo I, no hay diferencia significativa en la asociación de polimorfismos VEGFA -2549 I / D con IRSM en los tres modelos genéticos de los pacientes con IRSM primario y secundario. Conclusión: los pacientes con modelo genético ID de polimorfismos I / D -2549 en la región promotora del gen VEGFA y el alelo D tienen mayor riesgo de IRSM.

Clinical study of Erythrasma in diabetic patients

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ABSTRACT

This study was conducted to characterize the frequency of occurrence, extent, age, and sex incidence of Erythrasma in diabetic patients according to the type, duration, and state. A cross-sectional and case-control combined study of 200 diabetic patients and 160 non-diabetic groups visiting the outpatient clinic of Al-Saddar Teaching Hospital, Department of Medicine and Dermatology, from the period of December 2019 to July 2020. Among the diabetic group, their ages range from 12-60 years with a mean age of 37.6 years. 148 patients were non-insulin-dependent diabetes mellitus type (NIDDM) and 52 patients were IDDM. Among all the diabetic patients examined by wood's light to detected Erythrasma infection 34(17%) were found to be affected, from 26 males (76.5% of the affected) and 8 females (23.5%) were affected. Among the 52 patients with IDDM, 15(28.8%) were affected and only 19(12.8%) from the remainder with NIDDM affected. The peak age incidence was found to be in the fourth decade (30-40 y). The site of greatest propensity of the lesions appeared to be the groin was 100%. The extensive or generalized form was found only in 3(8.8%) patients and the least affected site was the toe webs only in 2(5.9%). The presentation of the patients was found to be asymptomatic in 22(64.7%), and the color change (red brown) was found in all of the patients. In the conclusion, the occurrence of Erythrasma in diabetic patients is more frequent than its occurrence in non-diabetic patients. There is a significant association between the occurrence of Erythrasma and the IDDM. The frequency of occurrence of Erythrasma increase with the long duration of DM and more with the uncontrolled DM.

Keywords: Erythrasma, Dermatology, Corynebacterium minutissimum, NIDDM, IDDM

Introduction

Erythrasma is a mild chronic localized superficial infection of the skin caused by a closely related aerobic coryneform bacteria usually knows as Corynebacterium minutissimum.[1] It is more prevalent in humid and warm climates and characterized by a half-moon shaped plaque uniformly brown and scaly and has no advancing border. [2, 3] Erythrasma can occur at any age but its commoner among adults than children with the peak incidence is in early adult life, males and commonly affected more than females.[1, 4] Erythrasma as detected by woods light examination. Involve the toe clefts more frequently than any other site. As clinically manifested lesions, they occur most commonly in the groin.

Diabetes mellitus has been cited as a predisposing condition for Erythrasma. The presence of hyperglycemia which impairs leucocyte function makes the infection more severe in diabetes. [5] Usually, diabetic patients with Erythrasma are of adult type (NIDDM) and after undiagnosed or inadequately controlled, thus inadequate treatment may be of etiological impotent is the development of Erythrasma. Erythrasma rarely present is the Juvenile or insulin-dependent diabetes mellitus (IDDM).[6]

Erythrasma responds well to a wide variety of systemic and topical antimicrobial agents.[7-9]

Methods

A cross-sectional and case-control combined study of 200 diabetic and 160 nondiabetic control patients (**Supp 1, 2, 3, 4**) visiting the outpatient clinic of the Department of Dermatology, from the period of December 2019 to July 2020.

Supp 1. The control group was examined and was affected by Erythrasma.

Control group examined	Male	Female	No. of affected	%	Male	Female
					No. (%)	
160	120	40	12	7.5	8 (75)	4 (25)

Supp 2. Age incidence of the control group (n=12).

Age (years)	No.	%
10 - 20	-	-
20 - 30	1	8.3
30 - 40	7	58.3
40 - 50	3	25
50 - 60	1	8.3

Supp 3. Site of predilection.

Site	No.	%
Generalized	-	-
Groin	12	100
Axilla	2	16.7
Toe web	-	-
Others	-	-

Supp 4. The presentation of the affected patients.

Presentation	No.	%
Asymptomatic	12	100
Mild itching	-	-
Moderate itching	-	-
Discoloration (red-brown)	12	100
Others	-	-

Wood's lamp examination

The invisible long-wave ultraviolet radiation (365 nm) produced by a wood's lamp is used to induce visible fluorescence to make help a diagnosis. Wood's light is produced by filtering the UV light source with barium silicate glass containing approximately 9% nickel oxide.[10] The fluorescence technique with wood's light has been used as a preventive measure to monitor and quantify skin protection at the workplace in high-risk occupations.[11].

Laboratory investigation

Gram stain of the scales showed gram-positive rod-like organisms in long, and filaments. The scales are collected by pressing small pieces of scotch tape (about 4cm X 2cm). On to the lesion and following withdrawal, the ferulaceous scales will remain on the glue side. These pieces are then immersed for some minutes in lactophenol cotton blue stain. Following absorption of the stain, the scales are washed in current water to remove the excess of blue stain, dried with filter paper dehydrated via a passage in two bottles containing absolute alcohol, and then placed in xylene in centrifugation tube, the xylene dissolves the scotch tape glue, and the scales fall free in the tube. After centrifugation and decantation, the scales concentrated on the bottom of the tube are collected with a platinum loop placed in Canada balsam on a microscopy slide and closed with a coverslip, the preparation is then ready to be examined.[12]

Another procedure to strip the epidermis with cyanoacrylate on a glass slide with permit easier handling and examination.[13]

Culture

Medium containing 20% fetal bovine serum, 78% tissue culture media No.199 in 2% sugar, and 0.05% tris. PH 6.8-7.2, the culture on the media yields growth which occurs as small shiny moist whitish-grey translucent colonies which fluoresce coral red under wood's light.[14]

Histopathology

Light and electron microscopy examination of the skin biopsy specimen from the affected area showed numerous bacteria in the superficial stratum corneum. So unless the C. minutissimum is detected the histopathology is not diagnostic, also there is a minimal inflammatory reaction.[15]

STATISTICAL ANALYSIS

Results

Erythrasma presented in 17% of diabetic patients. The affected males were (76.5%), while females were (23.5%), and the M: F ratio was 3.23:1. While among non-diabetic volunteers were 7.5%, 8 males (75%) and 4 females (25%). Those findings were significant differences (P = 0.007). (Table 1)

Among NIDDM, 19(12.8%) patients were affected by Erythrasma, while 15(28.8%) IDDM patients were affected, with significant differences (P = 0.008). (Table 2).

There were 18(52.9%) of the affected patients had DM of >5 years duration, while 12(35.3%) had a duration of <5 years, and 4(11.8%) were newly diagnosed. (Table 3).

There were 20(58.8%) of the affected with Erythrasma had the criteria of uncontrolled DM. While the 14(41.2%) fulfilled the criteria of controlled DM, which was statistically significant differences (P=0.00002). (Table 4).

The peak age incidence was recorded in the fourth decade (30-40 years) of 29.4% of patients. (Table 5).

Erythrasma was generalized or extensive in 3(8.8%) patients. The groin was affected in all the patients followed by the axilla (29.4%) and the least affected was the toe webs (5.9%). (Table 6).

The asymptomatic presentation had appeared in 22(64.7%), while the mild to moderate itching presented in (23.5%-11.8%). Discoloration of brown-red color was presented in all the affected patients. (Table 7).

Table 1. The Erythrasma of diabetic patients examined (n=200).

No.	%	Male	Female	P-value
		No. (%)		
34	17	26 (76.5)	8 (23.5)	0.007

Table 2. The type of DM associated with the affected patients.

Type of DM	No.	No. (affected)	%	Male	Female	P-value
				No. (%)		
NIDDM	148	19	12.8	12 (63.2)	7 (36.8)	0.008
IDDM	52	15	28.8	14 (93.3)	1 (6.7)	

Table 3. Duration of DM and the patients affected.

Duration	No.	%
More than 5 years	18	52.9
Less than 5 years	12	35.3
Newly diagnosed	4	11.8
Total	34	

Table 4. The state of DM and the percentage of patients affected.

State of DM	No.	%	*P*-value
Uncontrolled	20	58.8	0.00002
Controlled	14	41.2	

Table 5. Age incidence of Erythrasma in diabetic patients (n=34).

Age (years)	No.	%
10 – 19	3	8.8
20 – 29	6	17.6
30 – 39	10	29.4
40 – 49	7	20.6
50 – 59	8	23.6

Table 6. The predilection site of the lesions.

Site	No.	%
Generalized	3	8.8
Groin	34	100
Axilla	10	29.4
Toe web	2	5.9

Table 7. Presentation of affected patients.

Presentation	No.	%
Asymptomatic	22	64.7
Mild itching	8	23.5
Moderate itching	4	11.8
Discoloration (red brown)	34	100

Discussion

There is a high prevalence of Erythrasma among diabetic patients and at the same time not denying the importance of other predisposing factors like heat, humidity, obesity, maceration, and poor hygiene.

Despite the findings above, the patients affected by extensive (generalized) Erythrasma were low and in most cases, the Erythrasma appeared to be localized to one site and/or another. These findings suggested that DM is not only the factor that determines the extent of Erythrasma, and other factors mentioned above may contribute.

The younger age group of those patients with more physical activities and the high exposure to the predisposing factors, and probably the difficult treatment (insulin injection) and inadequate control of DM. Also, there was a significant association between Erythrasma affection and the state of DM (high prevalence in an uncontrolled group of patients), this may support that

the hyperglycaemic state found in those patients impair the leucocyte function and make the patient more susceptible to the infection and in turn make the infection more severe and hence we found that all the extensive form of infection was in the uncontrolled group of patients.

The peak age of incidence was found to be in the fourth decade, and this also may reflect probably the more exposure of those active age group to the predisposing factors.

The male predominance of the disease may indicate the higher physical activity, and these make the male exposed to the common protective factors especially sweating friction and maceration which are aggravated by the hot humid environment of our living place.

Regarding the site of Erythrasma, the groin was involved in all the cases and this may explain that this area is more subjected to sweating and friction aggravated by the type of clothing and the environment.

The controversial result of toe web infection (least side affected) may reflect that the foot of most of our patients was uncovered and those mostly used open shoes or another simple footwear (sandal).

Most patients were asymptomatic, and this supports the previous finding that Erythrasma is a superficial asymptomatic infection of the skin and may only cause slight irritation and itching.

Conclusions

The occurrence of Erythrasma in diabetic patients is more frequent than its occurrence in non-diabetic patients. There is a significant association between the occurrence of Erythrasma and the IDDM. The frequency of occurrence of Erythrasma increase with the long duration of DM and more with the uncontrolled DM.

Declarations:

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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RESUMEN

Este estudio se realizó para caracterizar la frecuencia de aparición, extensión, edad y sexo de la incidencia del eritrasma en pacientes diabéticos según el tipo, la duración y el estado. Estudio transversal y combinado de casos y controles de 200 pacientes diabéticos y 160 grupos no diabéticos que visitaron la consulta externa del Hospital Docente Al-Saddar, Departamento de Medicina y Dermatología, desde el período de diciembre de 2019 a julio de 2020. Dentro del grupo con Diabetes las edades oscilan entre los 12 y los 60 años con una edad media de 37,6 años. 148 pacientes padecían diabetes mellitus tipo no insulinodependiente (NIDDM) y 52 pacientes padecían IDDM. Entre todos los pacientes diabéticos examinados con la lámpara de Wood, para detectar infección por eritrasma, 34 (17%) resultaron afectados, de 26 hombres (76,5% de los afectados) y 8 mujeres (23,5%) se vieron afectados. Entre los 52 pacientes con DMID, 15 (28,8%) se vieron afectados y sólo 19 (12,8%) del resto con DMID se vieron afectados. Se encontró que la incidencia máxima de edad se encuentra en la cuarta década (30-40 años). El sitio de mayor propensión de las lesiones parecía ser la ingle en un 100%. La forma extensa o generalizada se encontró solo en 3 (8,8%) pacientes y el sitio menos afectado fue la membrana de los dedos solo en 2 (5,9%). La presentación de los pacientes fue asintomática en 22 (64,7%) y el cambio de color (marrón rojizo) se encontró en todos los pacientes. En conclusión, la aparición de eritrasma en pacientes diabéticos es más frecuente que su aparición en pacientes no diabéticos. Existe una asociación significativa entre la aparición de eritrasma y la IDDM. La frecuencia de aparición de eritrasma aumenta con la larga duración de la DM y más con la DM incontrolada.

Antimetabolite drug in patients with sickle cell diseases in hematological center of kerbalaa training hospital

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ABSTRACT

Background: the antimetabolite drug increase fetal hemoglobin level and reduce the frequency of crisis in sickle cell disease patients. **Aim:** To evaluate the effect of antimetabolites (hydroxyurea) in cases with frequent sickling crisis of sickle cell disease and non-transfusion dependent thalassemia in Karbala training hospital from APRIL 2016 till December 2020. **Patient and methods:** from eighty-one patients conducted in this case control study, forty were received hydroxyurea and the other forty-one patients were not. Monitoring every two weeks in the first three months by sending for investigations (Hb, WBC, platelet count and blood urea and serum creatinine) in addition to assessment of drug side effects. The remaining forty-one patients who refused drug therapy we consider them as a control group. **Result:** the case group who received hydroxylurea had crisis mostly after 12 weeks from last crisis, whereas the control group had crisis mostly each 3 to 7 weeks in P value 0.0001. There was no side effect in 77.5% of cases received hydroxyurea.The remaining 22.5% of cases had less or nonspecific side effects. **Conclusion:** In patient with sickle cell diseases who suffered from recurrent episodes of crisis, Hydroxyurea therapy significantly decreases the frequency of the painful crisis, with low level of side effects in comparison with control group.

Key words: Antimetabolites, Sickle cell disease, Hemoglubinopathies, Hydroxyurea.

Introduction

Sickle cell disease is generally mean the conditions associated with the sickling process, whereas the term sickle cell anemia is usually used to describe homozygosity of hemoglobin S (i.e. HbSS). The HbSS is more severe form, HbSC of intermediate severity (combined heterozygosity for hemoglobin S and C), and less severe in those with sickle cell trait (heterozygosity for HbS).[1]

In sickle cell-beta thalassemia: the disease varies with the quantity of hemoglobin A, often being quite severe in patients with sickle cell-beta (0) thalassemia and less severe in patients with sickle cell-beta (+) thalassemia. There are an estimated 54,736 Childs born with combined heterozygosity HbSC disease each year worldwide.[2]

Sickle cell crisis (Acute painful crisis) is the episodes of acute pain are the most common type of vasoocclusive events.[3]

It is described as unremitting discomfort that can occur in any part of the body, but mostly occurs in the chest, abdomen, or any extremities. This painful episode is often abrupt and causes difficulty of doing the daily life activities and it is heavy on children and their caregivers.

The etiology of pain is unknown exactly, and the pathogenesis are started when blood flow is disrupted in the microvasculature by sickled cells, resulting in ischemia to the tissue that supplied by it. the risk factors for development and initiation of it may be physical stress, infection, dehydration, hypoxia, local or systemic acidosis, exposure to cold, and longtime swimming.[3]

Hydroxyurea is part from an antimetabolite drugs that shown in adults with sickle cell disease (SCD) to increase fetal hemoglobin levels and reduce the symptoms of SCD. We hypothesized that antimetabolites therapy in children with severe (equal or more than three crises per year) sickle cell disease could improve hematologic parameters and decrease frequency of it.[4]

Hydroxyurea is the only drug proven effective in decreasing the frequency of the crisis events. In children the hydroxyurea is safe and well tolerated in children over 5 years of age child.

the hydroxyurea may be considered in the certain groups of nontransfusion dependent thalas-

semia diseases which includes β-thalassemia intermedia, pulmonary hypertension, alloimmunized patients, extra medullary hematopoietic pseudo neoplasms and leg ulcers.[5]

the beneficial effects in patients with sickle cell disease are uncertain exactly. The Known mechanism for its pharmacological effects that may contribute to its beneficial effects include:

1. increasing hemoglobin F levels in red blood cells.
2. decreasing neutrophils.
3. increasing the water content of RBCs.
4. increasing deformability of sickle cells.
5. altering the adhesion of RBCS of endothelium.

The metabolism of hydroxyurea is 60% by liver and gastrointestinal tract and the half-life of it is 2-4 hrs and it excreted mainly in urine.[6] The starting dose should be 15 -20 mg/kg once each day with an incremental dosage increase every 8 weeks of 5 mg/kg, and and may reach a maximum of 35 mg/kg per dose if not reach the toxic level.[3] The patient should be checked for complete blood counts every 2 weeks for the first three months. After that; each month with hepatic and renal function studies every 2 weeks for first three months then monthly. History and physical examination regarding GIT, CNS, dermatological side effects should be evaluated monthly.[5]

Hydroxyurea should be temporarily stopped, and adjustment of the dose is indicated if:

1. the absolute neutrophil count decrease below 2000/μL
   Or
2. platelets below 80000/μL. (3)

The side effect of hydroxyurea are nausea, vomiting, constipation, diarrhea, mucositis, Acute pulmonary reactions, genetic mutation, myelosuppression, secondary leukemia, hyperuricemia and renal failure, Dermatological changes (hyperpigmentation), azoospermia, infertility.[6]

Contraindication of usage of hydroxyurea are severe anemia, bone marrow depression (WBC less than 2500/mm3, platelets are less than 100000/mm3, pregnancy and lactation.[6]

Patients and Method.

The study started at April 2015 and continue till December 2020, 81 patients conducted in case control study who had sickle cell diseases. The cases included in our study had frequent painful crisis (more than three episodes per year). From these 81 patients, 40 patients started with hydroxyurea in a dose of 10 – 20 mg/kg/day as 500mg capsule taken orally after meal. 18 0f them diagnosed as sickle cell anemia,15 patients diagnosed as a sickle thalassemia syndrome and 7 had thalassemia intermedia. The remaining 41 patients who refused drug therapy considered as control and they were as the following: Monitoring of Patients on hydroxyurea every 2 weeks in the first 3 months by taking a blood sample (3 ml for each patient) investigated for HB, WBC total and differentials, platelet count (this was measured by Systemax XT -2000 i) and blood urea and creatinine (measured by Cobas Integra 400 plus). After these three months of treatment, the cases followed up according to their routine visit, or when they were seeking for medical consultation.

From whole study only 3 patients discontinue treatments because of appearance of side effect of drugs as the following: Sickle thalassemic patient stopped treatment due to complaining from nonspecific vomiting and abdominal pain after 8 days from onset of treatment, Sickle thalassemic patient discontinue treatment of unknown cause, Appearance of azoospermia after 6 months of treatment.

Results:

Forty cases (25 males and 15 female) were received hydroxyurea. From which, 18 sickle cell anemia, 15 sickle thalassemia,7 thalassemia intermedia cases. Forty-one control patients from which ,24 sickle cell anemia,13 sickle thalassemia and four cases were thalassemia intermedia as shown in table 1.

Table 1: Descriptive analysis of cases and controls according to diagnosis.

	cases		control	
	Number	percentage	Number	percentage
Sickle cell disease	18	22.2 %	24	29.6 %
Sickle thalassemia	15	18.5 %	13	16 %
Thalassemia intermedia	7	8.64 %	4	4.93 %
Total	40	49.34%	41	50.53 %

The painful crisis in case group before receiving hydroxyurea were mainly 7-12weeks and in control group 3-7 weeks. After receiving hydroxyurea painful crisis were mainly > 12 weeks in P value 0.0001 as shown in table2 and 3.

Table 2: frequency of crisis in cases and control groups before starting hydroxyurea.

		2-3 wks	3-7 wks	7-12 wks	<12 wks
number of crisis	control	5-10	***20-25***	5-10	0-5
	cases	10-15	10-15	***15-20***	0-5

Table 3: frequency of crisis after introducing hydroxyurea. (P value = 0.0001)

		2-3 wks	3-7 wks	7-12 wks	<12 wks
Number of crisis	control	5-10	***20-25***	5-10	0-5
	cases	0-5	5-10	10-15	***15-20***

Mainly There were no side effects of hydroxyurea in case group 31 patient (77.5%) others had nonspecific side effects (nausea, vomiting or fatigue) in 4 patients (10%), agranulocytosis 2 patients (5%) the remaining side effects (thrombocytopenia, asthenia, and azoospermia) were of 2.5% for each one.as shown in table 4.

Table 4. Number and percentage of patients in cases group with side effects of hydroxyurea.

	number	percentage
No side effects	31	77.5 %
Nonspecific side effects	4	10 %
Thrombocytopenia	1	2.5%
agranulocytosis	2	5%
azotemia	1	2.5%
Azoospermia	1	2.5%

DISCUSSION

The significant decrease in painful crisis syndrome case group in our research goes with the research of JAIN DL (2012) in asian children with sickle cell disease in which it shows that there is significant decrease in no. of vasoocclusive crisis and hospitalization with hydroxyurea despite increase baseline HbF.[9]

A multicenter randomized controlled trial 2011 (Wang WC) shows that there were decrease in pain and dactylitis as well as a significant decrease in acute chest syndrome, hospitalization rate.[10] Other studies that had the same conclusion with our result were Sharef SW(2013), Rigano P (2013), Gilmore A (2011), Nzouakou R (2011), Italia k (2009), Voskandou E (2010) and Charache S 1995). [12, 13, 14, 15, 16, 17]

Recommendations.

1. the usage of hydroxyurea in the managements of of sickle cell disease .
2. further researchs regarding usage of hydroxyurea in young children with hemoglobinopathies .
3. We recommend further study for the reversibility of azoospermia and azosthenia after stopping hydroxyurea therapy.
4. further study to prove malignancy associated with hydroxy urea treatments.

Declarations.

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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RESUMEN

Antecedentes: el fármaco antimetabolito aumenta el nivel de hemoglobina fetal y reduce la frecuencia de crisis en pacientes con anemia de células falciformes. Objetivo: Evaluar el efecto de los antimetabolitos (hidroxiurea) en casos con crisis falciforme frecuente de anemia de células falciformes y talasemia no dependiente de transfusiones en el hospital de formación de Karbala desde abril de 2016 hasta diciembre de 2020. Pacientes y métodos: de 81 pacientes realizados en este estudio de casos y controles, cuarenta recibieron hidroxiurea y los otros cuarenta y un pacientes no. Se realizaron monitoreos cada dos semanas en los primeros tres meses mediante el envío para análisis (Hb, WBC, recuento de plaquetas y urea en sangre y creatinina sérica)

además de la evaluación de los efectos secundarios de los medicamentos. Los cuarenta y un pacientes restantes que rechazaron la terapia con medicamentos los consideramos un grupo de control. Resultado: el grupo de casos que recibió hidroxilurea tuvo crisis principalmente después de 12 semanas desde la última crisis, mientras que el grupo de control tuvo crisis principalmente cada 3 a 7 semanas con un valor P=0,0001. No hubo efectos secundarios en el 77,5% de los casos que recibieron hidroxiurea. El 22,5% restante de los casos tuvo efectos secundarios menores o inespecíficos. Conclusión: En pacientes con drepanocitosis que sufrieron episodios recurrentes de crisis, la terapia con Hidroxiurea disminuye significativamente la frecuencia de la crisis dolorosa, con un bajo nivel de efectos secundarios en comparación con el grupo control.

Body Mass Index as the Risk Factor Affecting Knee Osteoarthritis of the Elderly

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ABSTRACT

Introduction: Osteoarthritis is a disease that progresses over time and culminates in the destruction of articular and joints. Basic Health Research (RISKESDAS) 2013 shows that East Nusa Tenggara have the highest prevalence of the rheumatic disease in Indonesia, about 33,1 %. Method: This research is an observational-analytic study with a cross-sectional design. This research aims to determine the factors affecting Osteoarthritis of the Elderly at Sikumana Community Health Center, Maulafa District, Kupang City during the period of December 2018 to February 2019. Result: In this research, body mass index/BMI (PR=1,21, p=0,037) has a significant correlation to osteoarthritis of the elderly, yet gender (PR=1,02, p=0,839) and history of knee trauma (PR=1,08, p=0,453) have no significant correlation to osteoarthritis of the elderly. Conclusion: An overweight body increases the mechanical pressure of the knee joint, which causes osteoarthritis. In this research, women have a higher risk of osteoarthritis compared to men. The higher the BMI, the prevalence of osteoarthritis increases significantly. Around 41 % of the Elderly with Osteoarthritis have obesity. Amongst any other risks, obesity shows a correlation with the prevalence of Osteoarthritis. Patients' awareness of the body mass index (BMI) should be increased to reduce the prevalence of osteoarthritis.

Keywords: osteoarthritis, elderly, risk factors, body mass index

Introduction

Osteoarthritis is a disease that progresses over time and culminates in the destruction of articular and joints. Thus, with an increasing elderly population, the treatment of knee osteoarthritis has become a major healthcare issue. Osteoarthritis of the knee is very common, affecting 12.4 million (33.6 %) adults over the age of 65. Interestingly, women are more affected and burdened by osteoarthritis of the knee than men.[1]

In Indonesia, osteoarthritis is the most common rheumatic disease compared to others. According to the World Health Organization (WHO), the number of patients with osteoarthritis in Indonesia is about 8,1% of all the population.[2] Basic Health Research (RISKESDAS) 2013 states that East Nusa Tenggara province had the highest number of patients with rheumatic diseases (33,1%).[3]

Osteoarthritis of the knee is very common, affecting 12.4 million (33.6 %) adults over the age of 65. Osteoarthritis is one of the most disabling diseases in developed countries. Global estimates are that 9.6% of men and 18.0% of women >60 years of age have symptomatic (painful) osteoarthritis. Eighty percent of patients with osteoarthritis have limitations in movement and 25% cannot perform their major daily activities.

This research aims to determine the factors affecting osteoarthritis of the elderly at Sikumana Community Health Center, Maulafa District, Kupang City during the period of December 2018 to February 2019.

Materials and Method

This research is an observational-analytic study with a cross-sectional design conducted in Sikumana Community Health Center, Maulafa District, Kupang City, from December 2018 to February 2019. Consecutive sampling was used in this survey, with a total of 134 respondents. All those respondents went to Sikumana Community Health Center to do a check-up and get medication.

The variables in this research are osteoarthritis, gender, body mass index (BMI), and history of

knee trauma. The Chi-Square statistic in SPSS version 26.0, for the data analysis, was used in this research. Each variable is cross tabulated to get the fixed data.

All of the respondents had received the information that their data will be put in this research. Each of them had agreed and signed the informed consent, and none of the 134 respondents rejected it.

Result

From the cross-sectional study that was conducted at Sikumana Community Health Center, Maulafa District, Kupang City from December 2018 to February 2019, the total of patients with osteoarthritis were 105 from the total of 134 patients. From the gender data, there were 39 men & 95 women. From the body mass index (BMI) data, there were 55 obese patients & 79 non-obese patients. From the history of knee trauma data, there were 30 patients with a history of knee trauma & 104 patients with no history of knee trauma.

In this research, body mass index (BMI) has a significant correlation to osteoarthritis, while gender and history of knee trauma have no significant correlation to osteoarthritis of the elderly. Women have a higher risk (71%) of osteoarthritis compared to men (29%). Obese patients with osteoarthritis are about 41%. Patients with a history of knee trauma who have osteoarthritis are about 22%.

Table 1. The distribution of patients based on gender, body mass index (BMI), and history of knee trauma

Variable	Category	Frequency	Percentage (%)
Osteoarthritis	(+)	105	78
	(-)	29	22
Gender	Men	39	29
	Women	95	71
Body Mass Index (BMI)	Obese	55	41
	Non-Obese	79	59
History of Knee Trauma	(+)	30	22
	(-)	104	78

Table 2. Bivariate analysis between gender with osteoarthritis of the elderly

Variable	Category	Gender		PR (Prevalence Ratio)	p-value
		Men	Women		
Osteoartritis	(+)	31	74	1,02	0.839
	(-)	8	21		

Table 3. Bivariate analysis between body mass index (BMI) with osteoarthritis of the elderly

Variable	Category	BMI (Body Mass Index)		PR (Prevalence Ratio)	p-value
		Obesed	Non-Obesed		
Osteoartritis	(+)	48	57	1,21	0.037
	(-)	7	22		

Table 4. Bivariate analysis between the history of knee trauma with osteoarthritis of the elderly

Variable	Category	History of Knee Trauma		PR (Prevalence Ratio)	p-value
		(+)	(-)		
Osteoartritis	(+)	25	80	1,08	0.453
	(-)	5	24		

Discussion

Osteoarthritis is a disease that progresses over time and culminates in the destruction of articular and joints. Thus, with an increasing elderly population, the treatment of knee osteoarthritis has become a major healthcare issue. Osteoarthritis of the knee is very common, affecting 12.4 million (33.6 %) adults over the age of 65. Interestingly, women are more affected and burdened by osteoarthritis of the knee than men.[1] Osteoarthritis is classified into two groups, e.g., primary osteoarthritis & secondary osteoarthritis. The primary osteoarthritis is idiopathic and is caused by the genetic factor, which is the abnormality of the collagen that makes it easier to break. The secondary osteoarthritis is caused by endocrine abnormality, inflammation, metabolic, growth, micro and macro trauma, excess immobility, obesity, etc.[2]

In Indonesia, osteoarthritis is the most common rheumatic disease compared to others. According to the World Health Organization (WHO), the number of patients with osteoarthritis in Indonesia is about 8,1% of all the population. (2) Basic Health Research (RISKESDAS) 2013 states that East Nusa Tenggara province had the highest number of patients with rheumatic diseases (33,1%).[3]

Osteoarthritis is one of the most disabling diseases in developed countries. Global estimates are that 9.6% of men and 18.0% of women >60 years of age have symptomatic (painful) osteoarthritis. Eighty percent of patients with osteoarthritis have limitations in movement and 25% cannot perform their major daily activities. World Health Organization (WHO) data also demonstrated that osteoarthritis moved from the 12th to the 6th leading cause of years lost to disability or morbidity between 2002 and 2007. Increases in life expectancy and aging populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020.[4] According to WHO, the elderly is a classification of the people who are between 60-74 years old.

The decision tree format of the American College of Rheumatology (ACR) classification criteria for clinical knee osteoarthritis was applied to the collected examination data. Although the traditional "3 out of 6" format has been the more widely promulgated version, the decision tree format was recommended by the authors in their original publication. As all participants in our study satisfied the age criterion, the ACR clinical criteria for knee osteoarthritis were fulfilled in the following circumstances.[5]

- crepitus + and morning stiffness > 30 min and bony enlargement +; or
- crepitus + and morning stiffness <= 30 min; or
- crepitus - and bony enlargement + (where + = present, - = absent).

Osteoarthritis can affect men and women. Primary osteoarthritis mostly affects post-menopause women and Secondary osteoarthritis mostly affects men.[6] It is often described as a chronic degenerative disease and thought by many to be an inevitable consequence of growing old. Osteoarthritis was defined as the presence of knee symptoms in a patient with ipsilateral (Kellgren & Lawrence) grade 2 or greater radiographic changes. The prevalence of radiographic osteoarthritis increased with each decade of life from 33% among those aged 60–70 to 43.7% among those over 80 years of age. The prevalence of symptomatic knee osteoarthritis in all subjects was 9.5% and increased with age in women but not in men.[7] There were a significantly higher number of women with symptomatic disease.[1] In this research, the bivariate analysis between gender with osteoarthritis of the elderly shows the prevalence ratio (PR)=1,02. The p-value=0,839, which means there is no significant correlation between both variables.

Obesity can increase the mechanical pressure at the joints that bear the body, which commonly causes knee osteoarthritis. One study in Chingford shows that for every 2 units increasing of body mass index (BMI), or about 5 Kg of body weight, the odds ratio of radiographic knee osteoarthritis increases by 1,36 points. The study concludes that the risk of knee osteoarthritis increases with heavier bodyweight.[2] First documented in 1945, the strong association between obesity and knee osteoarthritis has been widely verified. Leach and colleagues found that 83% of their female subjects who had knee osteoarthritis were obese compared with 42% of the control group. In a case-controlled study of 675 matched pairs, Coggon and colleagues determined that the risk for knee osteoarthritis in people who had a body mass index of 30 kg/m2 or greater was 6.8 times that of normal-weight controls. Felson and colleagues showed that a 5.1-kg loss in body mass over 10 years reduced the odds of developing osteoarthritis by more than 50%.[8] In this research, the bivariate analysis between body mass index (BMI) with osteoarthritis of the elderly shows the prevalence ratio (PR)=1,21. The p-value=0,037, which means there is a significant correlation between both variables.

Previous knee trauma is a risk factor for knee osteoarthritis.[9] According to the study of Silverwood, et al., thirteen cohort studies were included in the meta-analysis of previous knee injury as a risk factor for the onset of knee

osteoarthritis with only one showing that those with a previous knee injury had a lower, though the non-significant risk of developing knee osteoarthritis. The other studies all showed an increased risk of knee osteoarthritis with a prior injury.[10] In this research, the bivariate analysis between the history of knee trauma with osteoarthritis of the elderly shows the prevalence ratio (PR)=1,08. The p-value=0,453, which means there is no significant correlation between both variables.

The limitation of this research is the lack of radiographic examination since it is important to diagnose knee osteoarthritis using X-ray to determine the Kellgen-Lawrence (KL) grading of knee osteoarthritis.

Conclusion

Body Mass Index has a significant correlation to Osteoarthritis at Sikumana Community Health Center, Maulafa District, Kupang City. Women have a higher risk of Osteoarthritis compared to men. Patients's awareness of body mass index (BMI) should be increased to reduce the prevalence of Osteoarthritis. The limitation of this research is the lack of radiographic examination at Sikumana Community Health Center.

Declarations:

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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RESUMEN

Introducción: La osteoartritis es una enfermedad que progresa con el tiempo y culmina en la destrucción de articulaciones y ligaduras. La Investigación Básica de Salud (RISKESDAS) 2013 muestra que East Nusa Tenggara tiene la prevalencia más alta de la enfermedad reumática en Indonesia, alrededor del 33,1%. Método: Esta investigación es un estudio observacional-analítico con un diseño transversal. Esta investigación tiene como objetivo determinar los factores que afectan la osteoartritis de los ancianos en el Centro de Salud Comunitario de Sikumana, distrito de Maulafa, ciudad de Kupang durante el período de diciembre de 2018 a febrero de 2019. Resultado: En esta investigación, índice de masa corporal / IMC (PR = 1,21, p = 0,037) tiene una correlación significativa con la osteoartritis de los ancianos, sin embargo, el género (RP = 1,02, p = 0,839) y los antecedentes de trauma de rodilla (RP = 1,08, p = 0,453) no tienen una correlación significativa con la osteoartritis de los ancianos. Conclusión: un cuerpo con sobrepeso aumenta la presión mecánica de la articulación de la rodilla, lo que provoca la osteoartritis. En esta investigación, las mujeres tienen un mayor riesgo de sufrir osteoartritis en comparación con los hombres. Cuanto mayor sea el IMC, la prevalencia de la osteoartritis aumenta significativamente. Alrededor del 41% de los ancianos con osteoartritis tienen obesidad. Entre otros riesgos, la obesidad muestra una correlación con la prevalencia de osteoartritis. Se debe aumentar la conciencia de los pacientes sobre el índice de masa corporal (IMC) para reducir la prevalencia de la osteoartritis.

The Impact of Routine Oesophagogastroduodenoscopy on the Management Plan before Cholecystectomy: A Prospective Study

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ABSTRACT

Introduction: Laparoscopic cholecystectomy has rapidly become the procedure of choice for routine gallbladder disease, and it is currently the most performed major abdominal procedure in Western countries, most authors suggest that it's safe to observe patients with asymptomatic gallstones, with cholecystectomy only being performed for those patients who develop symptoms. Fifteen percent of patients persist to have post cholecystectomy symptoms. This study aimed to evaluate the use of oesophagogastroduodenoscopy prior to laparoscopic cholecystectomy, and its impact on the management. Method: This was a prospective clinical study involving patients with gallstone admitted to the Al-Basra Teaching Hospital, Department of General Surgery from January 2016 to December 2019. All patients were followed up from the time of admission until six months later. These patients were divided into seven groups according to age. All patients were having an abdominal ultrasound examination in order to diagnose the presence of cholelithiasis and to exclude other abdominal problems. All patients scheduled for laparoscopic cholecystectomy underwent upper GIT endoscopy preoperatively. Results: A total of 1200 patient age range from 21 to 82 years were included (women, 83.33%, men, 16.66%) had cholelithiasis. Female to male ratio was 5:1. Positive endoscopic findings were observed in 380(31.6 %) patients. The management plan was changed in these patients with positive findings by endoscopy and their surgery was postponed until they received proper treatment. Conclusion: The routine use of oesophagogastroduodenoscopy prior to cholecystectomy would decrease the unneeded cholecystectomy in patients with cholelithiasis and positive endoscopic findings, which decrease post cholecystectomy persistence of symptoms.

Keyword: cholecystectomy, endoscopy before cholecystectomy, laparoscopic cholecystectomy, management of cholelithiasis.

Introduction

Laparoscopic cholecystectomy has rapidly become the procedure of choice for routine gallbladder disease and it's currently the most commonly performed major abdominal procedure in Western countries.[1-3] Laparoscopic cholecystectomy provides a safe and effective treatment for most patients with symptomatic gallstones and has become the treatment of choice for many patients.[4-9]

Most authors would suggest that it's safe to observe patients with asymptomatic gallstones, with cholecystectomy only being performed for those patients who develop symptoms or complications of their gallstones.[7] Laparoscopic cholecystectomy is the procedure of choice for the majority of patients with gallbladder disease.[1-9]

In 15% of patients, cholecystectomy fail to relieve the symptoms for which the operation was performed, such patients may be considered to have a post- cholecystectomy syndrome.[10, 11] However, such problems are usually related to the preoperative symptoms and are merely a continuation of those symptoms.[1, 12] This study aimed to evaluate the use of oesophagogastroduodenoscopy

prior to laparoscopic cholecystectomy, and its impact on the management plan of patients with symptomatic gallstones.

METHODS

This was a prospective clinical study which carried out from January 2016-December 2019 in Al-Basra Teaching Hospital Department of General Surgery. The Al-Basra Teaching Hospital is a 600-bedded public hospital with 700 to 1000 patients attending the outpatient clinics every day and about 1000-1250 patients attending the emergency unite every day. A total of 1200 patients were divided into six groups according to age, these patients with symptomatic cholelithiasis who scheduled for doing laparoscopic cholecystectomy by many surgeons are offered an OGD examination to rule out any other gastric, esophageal, or duodenal pathology that may give upper abdominal pain. Informed consent was obtained from each patient who enrolled in this study, and the study was approved by the ethics committee. All patients were followed up from the time of admission until six months later. All patients were having an abdominal ultrasound examination in order to diagnose the presence of cholelithiasis and to exclude other abdominal problems. All other preoperative investigation was done as routine cases. The results were entered data base and analyzed.

Exclusion criteria:

1-All patients with acute cholecystitis were excluded from the study.
2- Pediatric patients younger than 16 years of age were exclude from the study

RESULTS

A total of 1200 patient aged 21 to 82 years with female to male ratio 5:1, of these females were 1000 (83.4%), males were 200 (16.6%). Majority of the patients were in the fourth decade age as shown in table 1.

Table 1: Gender and age group distribution

Age groups	Female N. (%)	Male N. (%)	Total N. (%)
20-29	90 (7.5)	30 (2.5)	120 (10)
30-39	230 (19.2)	50 (4.1)	280 (23.3)
40-49	320 (26.7)	40 (3.3)	360 (30)
50-59	250 (20.8)	30 (2.5)	280 (23.3)
60-69	80 (6.7)	30 (2.5)	110 (9.2)
70+	30 (2.5)	20 (1.7)	50 (4.2)
Total N. (%)	1000 (83.4)	200 (16.6)	1200 (100)

Majority of patients 700 (58.3%) they presented with Epigastric pain, whereas the other presented with right hypochondrial pain in 200 (16.6%) patients, bloating in 150 (12.5%) patients, heart burn in 60 (5%) patients, vomiting in 50 (4.1%) patients, and indigestion in 40 (3.3%) patients. Fig.1

The preoperative abdominal ultrasound examination showed multiple gallstones in majority of patient 1040 (86.6%), single gallstone in 110 (9.1%) patients, empyema in 30 (2.5%) patients, and gallbladder polyp in 20 (1.6%) patients.

Positive endoscopic findings were found in 380 (31.6%) patients, and the main endoscopic findings were duodenal ulcer found in 120 (10%) patients, gastritis in 90 (7.5%) patients, esophagitis in 60 (5%) patients, benign gastric ulcer in 50 (4.1%) patients, hiatus hernia in 40 (3.3%) patients, and gastric outlet obstruction in 20 (2%) patients. (Table 2)

Table 2: OGD findings

Findings	N.	percent
Duodenal ulcer	120	10
Gastritis	90	7.5
Esophagitis	60	5
Benign gastric ulcer	50	4.1
Hiatus hernia	40	3.3
Gastric outlet obstruction	20	1.6
Total	380	31.6

All the patients with positive OGD findings postponed from surgery wait for medical treatment and follow up for a minimum period of 6 months, 180 (15%) patients of them had re-

currence or persistence of symptoms and they underwent OGD after they take medication and they allowed to do surgery only after normal OGD ensured, the remaining 200 patients (16%) showed no recurrence of symptoms for six months of follow up and surgery was cancelled.

Discussion

Laparoscopic cholecystectomy is indicated for patients with symptomatic cholelithiasis, and it is now the gold standard procedure and, but some of the patients had persistence of pain post cholecystectomy for unknown reason.[13] This pain may be due to peptic ulcer disease, gastritis or other upper gastrointestinal pathologies which have been missed because of overlapping of symptoms in the presentation of such patients and patients with symptomatic cholelithiasis.[14.15]

Although abdominal sonography and other routine preoperative investigation were usually done in all these patients with symptomatic cholelithiasis, but it cannot give a clear clinical picture about the presence of any of the upper gastrointestinal pathologies. So further confirmation by oesophagogastroduodenoscopy is mandatory to rule out such problems that may cause preoperative pain or other symptoms, which may persist postoperatively if not treated properly. Some authors suggested that before getting diagnosed with gallstones, patients may have previously undiagnosed functional gut disease.[16]

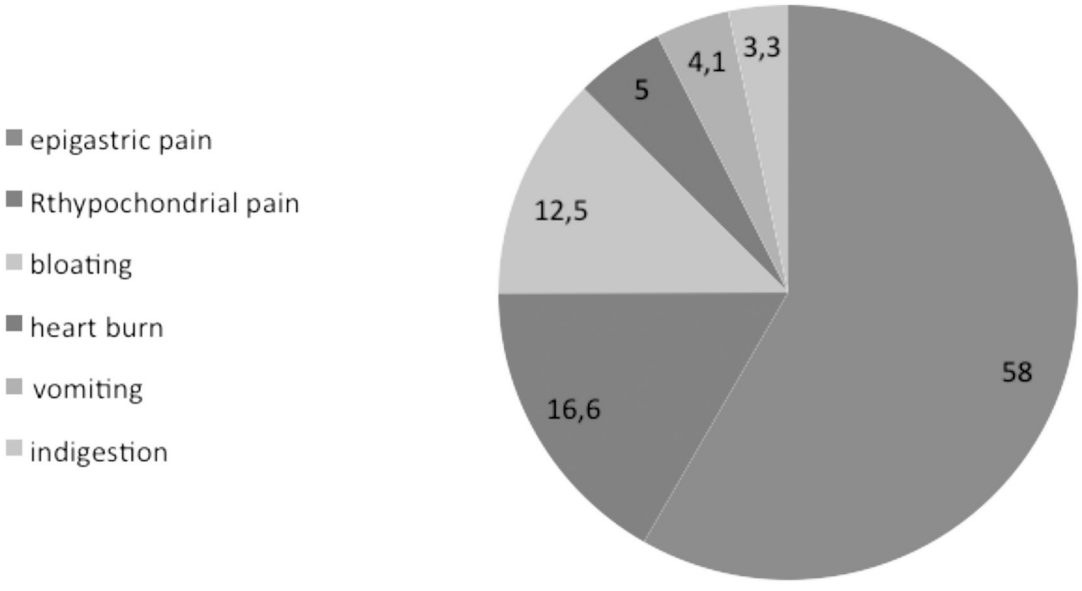
In this study 120 (10%) patients were found to have a peptic ulcer disease, and symptoms of these patients would aggravate by many reasons like fasting before surgery, stress of surgery and post-operative analgesia specially NSAIDS if needed as a pain killer. So, all these sufferings can be minimized, and their surgery postponed after they underwent a preoperative OGD to exclude these pathologies and the proper treatment is prescribed.

In other similar studies, researchers found that OGD must precede an elective cholecystectomy and they recommend a change in the plan of treatment because of OGD findings. [17-21]

In the present study 380 (31.6%) patients had a positive endoscopic finding, which is also observed in other similar study by Dietrich et al, who found that 31% of patient had a positive OGD findings resulting in changing the plan of therapy.[18] In a study performed by Schwenk et al they found that 345 (30.1%) patients) had a positive OGD findings of upper gastrointestinal pathologies.[17]

In our study peptic ulceration was found in 120 (10%) patients as the main OGD find-

Figure 1: presenting symptoms



ings, Sosada et al suggested that pain in asymptomatic cholelithiasis is due to peptic ulcer so he recommends routine use of OGD.[21] In other similar studies by Thybusch et al they found that gastritis is the main OGD findings in (25.7%), and he also recommends change of the plan of therapy by proper medication and cancellation of surgery.[22]

F. Rashid et al found that the routine use of preoperative OGD may reduce the post-operative persistence and recurrence of symptoms and decrease the overall cost of unneeded laparoscopic cholecystectomy.[15]

In summary the routine uses of preoperative OGD of our patients had changed the treatment plan due to the detection of other upper gastrointestinal pathologies which may give a similar clinical picture, thereby it ensures the patient safety and reduce the rate of unneeded laparoscopic cholecystectomy in patients with cholelithiasis.

Conclusion

The routine uses of OGD aid in the diagnosis of hidden upper gastrointestinal pathologies which lead to changes in the management plan in about one third of the patients with cholelithiasis as the clinical picture and the symptoms may overlap. This would help in decreasing the rate of unneeded laparoscopic cholecystectomy and thereby decreasing the expected postoperative persistence or recurrence of symptoms.

Declarations:

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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RESUMEN

Introducción: La colecistectomía laparoscópica se ha convertido rápidamente en el procedimiento de elección de rutina para la enfermedad de la vesícula biliar, y actualmente es el procedimiento abdominal mayor que se realiza con mayor frecuencia en los países occidentales; la mayoría de los autores sugieren que es seguro observar a pacientes con cálculos biliares asintomáticos, y que la colecistectomía solo se realiza por aquellos pacientes que desarrollan síntomas. El quince por ciento de los pacientes persiste teniendo síntomas posteriores a la colecistectomía. Este estudio tuvo como objetivo evaluar el uso de la esofagogastroduodenoscopía previa a la colecistectomía laparoscópica y su impacto en el manejo. Método: Este fue un estudio clínico prospectivo que involucró a pacientes con cálculos biliares ingresados en el Hospital Docente de Al-Basra, Departamento de Cirugía General desde enero de 2016 hasta diciembre de 2019. Todos los pacientes fueron seguidos desde el momento del ingreso hasta seis meses después. Estos pacientes se dividieron en siete grupos según la edad. A todos los pacientes se les realizó una ecografía abdominal para diagnosticar la presencia de colelitiasis y descartar otros problemas abdominales. Todos los pacientes programados para colecistectomía laparoscópica se sometieron a una endoscopia del tracto gastrointestinal superior antes de la operación.
Resultados: Se incluyeron un total de 1200 pacientes con rango de edad de 21 a 82 años (mujeres, 83,33%, hombres, 16,66%) con colelitiasis. La proporción de mujeres a hombres fue de 5:1. Se observaron hallazgos endoscópicos positivos en 380 (31,6%) pacientes. En estos pacientes se modificó el plan de manejo con hallazgos positivos por endoscopia y se pospuso su cirugía hasta recibir el tratamiento adecuado. Conclusión: El uso rutinario de esofagogastroduodenoscopia previa a la colecistectomía disminuiría la colecistectomía innecesaria en pacientes con colelitiasis y hallazgos endoscópicos positivos, lo que disminuye la persistencia de síntomas post colecistectomía.

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