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Queratosis folicular invertida del conducto auditivo externo: Una patología benigna poco frecuente y de localización inusual.

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ABSTRACT

Inverted follicular keratosis is a rare benign neoplasm originating from the infundibulum of the follicular epithelium. Although it does not have a characteristic or pathognomonic clinic, it usually presents as a solitary, asymptomatic, slow-growing lesion on the face. The certain diagnosis is made with the histopathological study, which shows a proliferation of basaloid cells and squamous keratotic cells with squamous eddies, which extend to the dermis. The treatment of choice is surgical removal, with a good prognosis and little tendency to recur. Localization in the external auditory canal is extremely rare (in the reviewed literature we have found only three reported cases) and represents both a diagnostic and therapeutic challenge.

We present a patient with an inverted follicular keratosis located in the left external auditory canal of 5 years of evolution that significantly compromised his quality of life due to the symptoms (pain, otorrhea, hearing loss) and that evolved satisfactorily with the established treatment (surgical excision and subsequent topication of the area with 50% trichloroacetic acid).

Key words: inverted follicular keratosis, external auditory canal.

Introducción:

La Queratosis folicular invertida (QFI) es un tumor anexial benigno poco frecuente originado en la vaina radicular externa del infundíbulo folicular. Descrito por primera vez en 1954 por Helwig y col., suele presentarse como una lesión única, preferentemente en cara, a nivel de mejillas y labio superior. La localización en conducto auditivo externo (CAE) es extremadamente infrecuente (sólo tres casos comunicados). El diagnóstico se confirma con el estudio histopatológico. La resección completa es la terapéutica de elección, aunque existen también casos comunicados de tratamiento con imiquimod 5%, con buenos resultados[1, 2].

Se presenta un paciente con una queratosis folicular invertida localizada en conducto auditivo externo izquierdo de 5 años de evolución que comprometía significativamente su calidad de vida por la sintomatología (dolor, otorrea, hipoacusia) y que evolucionó satisfactoriamente con el tratamiento instaurado (extirpación quirúrgica y posterior topicación de la zona con ácido tricloroacético 50% -TCA-).

Caso clínico:

Varón de 39 años, sin antecedentes de relevancia, derivado del Servicio de Otorrinolaringología por dermatosis pruriginosa en CAE izquierdo de 5 años de evolución que en los últimos dos años agregó dolor, otorrea intermitente e hipoacusia progresiva. Refería el paciente que había sido tratado en dos oportunidades con aspiración de las lesiones y posterior topicación con nitrato de plata, sin mejoría.

Al examen físico dermatológico presentaba tumoración eritematosa, lobulada, de superficie lisa, que obstruía la luz del CAE izquierdo (Foto 1).

Se realizó tomografía axial computarizada (TAC) de cráneo y hueso temporal, que informó: tejido isodenso a nivel de conducto auditivo externo, sin compromiso óseo ni de membrana timpánica (Foto 2). La audiometría evidenció hipoacusia de tipo conductivo.

En conjunto con el servicio de Otorrinolaringología se realizó exéresis de la lesión bajo visión microscópica con posterior topicación del CAE con TCA 50%.

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Foto 1. Tumoración eritematosa multilobulada que obstruye la luz del CAE.

El estudio histopatológico de la pieza mostró en la epidermis una proliferación endofítica de queratinocitos basaloides benignos con remolinos escamosos, pseudoquistes córneos y queratinocitos apoptóticos; y en la dermis papilar, edema y vasodilatación (Foto 3).

Se envió una muestra al Laboratorio Nacional de Referencia de Papilomavirus del Instituto Nacional de Enfermedades Infecciosas- AN-LIS "Dr. Malbrán" para detección del genoma de VPH e hibridación reversa en línea (RLB) con oligosondas tipo específicas para los tipos virales: 4, 5,8, 9,12,14,15,17,19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 48, 49,50, 60, 65,75, 76, 80, 92, 93 y 96, que resultó no detectable.

Luego de 6 meses de seguimiento el paciente se encuentra asintomático y sin evidencia de nuevas lesiones.

COMENTARIOS

La QFI es un tumor anexial benigno y poco frecuente originado en la vaina radicular externa del folículo piloso, a nivel del infundíbulo. Fue descrito por Helwig y col. en 1954 como una pápula eritematosa de la cara con remo-
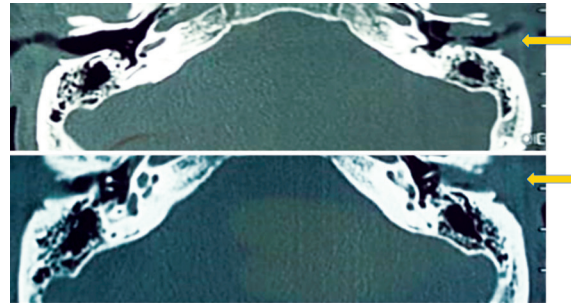
Foto 2. TAC: tejido isodenso a nivel de conducto auditivo externo, sin compromiso óseo ni de membrana timpánica.
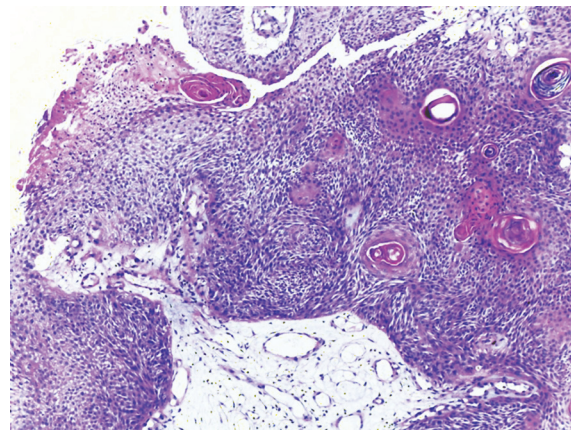
Foto 3. HyE 400 X. Proliferación de queratinocitos basaloides benignos con remolinos escamosos, pseudoquistes córneos y queratinocitos apoptóticos en epidermis y edema y vasodilatación en dermis papilar.

linos escamosos en el estudio histopatológico. Si bien se desconoce su causa, hoy en día se reconoce como un tumor anexial. En tiempos previos algunos autores habían postulado su posible relación con el HPV, interpretándolo como una variante de verruga vulgar, mientras que otros lo consideraban una forma de queratosis seborreica irritada[1-3]. En 1963 Duperrant y Mascaro fueron los primeros en sugerir que este tumor se origina en el infundíbulo del folículo piloso. Y en 1964 y luego nuevamente en 1983 Mehregan apoyó esta afirmación y dividió a la QFI en tres patrones de crecimiento: tipo queratoacantoma (formando una neoplasia exo-endofítica); tipo verruga (formando una lesión exofítica); y tipo sólido o nodular (formando lesiones endofíticas)[4-6].

La QFI afecta a personas en la edad media de la vida o mayores, con predilección por el género masculino en relación 2:1[1-3].

La presentación clínica más frecuente es una tumoración única y pequeña (menor a 1 cm), eritematosa o color piel, de superficie lisa o queratósica, asintomática, aunque también se han descrito lesiones múltiples, pigmentadas o grandes. La localización más frecuente es en la piel de cara y cuello (90%), en mejillas y labio superior, aunque también se han comunicado lesiones en frente, párpados, nariz, mentón y vulva[1-4, 7].

La localización en CAE es excepcional: sólo hemos encontrado tres casos en la literatura. El primero fue publicado por Soylu y cols.[1] en 1993. Se trataba de un hombre de 61 años con una tumoración queratósica de 4 cm en CAE derecho que simulaba un cuerno cutáneo gigante. En 2009 Halleck y col.[8] publicaron el segundo caso, un hombre de 74 años con una lesión que obstruía por completo el CAE y generaba hipoacusia parcial. Los autores encontraron en el estudio histopatológico coexistencia de queratosis seborreica hiperqueratósica con QFI. El tercer caso lo publicaron Hay y col.[2] en 2018. Se trataba de una mujer de 35 años con una tumoración verrugosa y sangrante que obstruía parcialmente la luz del CAE derecho. Todos los pacientes fueron tratados con resección completa de la lesión (el caso 2 realizó, además, laser CO2), con buena evolución y sin recidiva hasta el momento de su publicación. Nuestro paciente representa el cuarto caso comunicado de QFI de CAE, un varón adulto con una tumoración eritematosa única pero multilobulada que obstruía por completo el CAE izquierdo ocasionando dolor, otorrea e hipoacusia.

Debido a su presentación clínica poco específica, el diagnóstico de certeza de QFI se realiza con el estudio histopatológico, que evidencia lóbulos formados por células basaloides en la periferia y células escamosas y remolinos córneos en el centro, que se extienden hacia la dermis; ocasionalmente la epidermis presenta, además, hiperqueratosis y paraqueratosis y la dermis, vasodilatación, fibrosis y un leve infiltrado inflamatorio linfohitiocitario[2, 4, 6, 9]. El estudio histopatológico de la lesión de nuestro paciente reveló en la epidermis una proliferación benigna de queratinocitos basaloides con remolinos escamosos y pseudoquistes córneos y en la dermis, edema y vasodilatación.

Múltiples son los diagnósticos diferenciales clínicos a considerar ante una tumoración en CAE:

- queratosis seborreica: evidencia más la diferenciación folicular y muestra indicios de formación de centros germinativos foliculares. Hemos encontrado sólo 6 casos de queratosis seborreica en CAE en la literatura revisada, subtipos acantósico, adenoide e hiperqueratósico[3, 4, 10].
- verruga vulgar: si bien tanto la clínica como la histopatología pueden ser muy similares, en las verrugas se observan coilocitos (ausentes en la QFI)[3]. En el estudio histopatológico de nuestro paciente no se encontraron coilocitos. Tampoco se detectó genoma de HPV en el estudio realizado en el Instituto Nacional de Enfermedades Infecciosas-ANLIS "Dr. Malbrán".
- otras neoplasias: queratoacantoma, carcinoma basocelular, carcinoma espinocelular, melanoma (en las variantes pigmentadas de QFI): la histopatología ayuda a descartar estos diagnósticos[3, 7].
- colesteatoma del CAE: patología infrecuente también, es un tumor de queratina que erosiona el CAE, lo que provoca necrosis y lisis ósea[11]. La TAC de nuestro paciente sólo mostró tejido isodenso a nivel de conducto auditivo externo, sin compromiso óseo ni de la membrana timpánica.
- queratosis obturans: también conocida como tapón epidérmico, es un tapón de queratina que se acumula en el CAE y produce su ensanchamiento circunferencial, otorrea a repetición, dolor e hipoacusia, pero no lisis ósea[11].
- osteoma: es un tumor óseo benigno, en la mayoría de los casos solitario y voluminoso, poco frecuente, redondeado, pediculado y asintomático si no obstruye el CAE. El tratamiento es quirúrgico[12].
- exostosis: es un tumor óseo benigno muy frecuente. Los factores de riesgo son la irritación mecánica y térmica por el agua. Se presenta como una o varias excrecencias óseas mamelonadas, que se desarrollan esencialmente en la parte media y medial del conducto óseo, en la mayoría de los casos asintomáticas. La aparición de una hipoa-

cusia de transmisión por estenosis es poco frecuente. El tratamiento es quirúrgico[12].

- adenoma ceruminoso y adenoma pleomorfo: son patologías muy infrecuentes. Estos dos tumores tienen en común que: se desarrollan a partir de las glándulas ceruminosas; se mantienen asintomáticos durante mucho tiempo y que se manifiestan cuando se vuelven obstructivos, como una hipoacusia de transmisión o infecciones. El diagnóstico se confirma por el análisis histológico de la pieza[12].

- hiperplasia angiolinfoide con eosinofilia (HALE): también conocido como hemangioma epitelioide o hemangioma histiocitoide, es un tipo de tumor vascular de etiología desconocida que se localiza con más frecuencia a nivel de cabeza y cuello, en las zonas preauricular y auricular. Clínicamente suelen presentarse como pequeñas tumoraciones violáceas[13].

El tratamiento de elección de la QFI es la extirpación completa de la lesión, con excelente pronóstico y casi sin recurrencias. Dada su naturaleza benigna no se recomiendan cirugías más extensas[1-4, 7]. El imiquimod 5% crema, droga inmunomoduladora con actividad antitumoral y antiviral, representa una alternativa terapéutica no invasiva. Karadag y col.[13] comunicaron en 2016 el caso de un paciente tratado con imiquimod 5% crema 3 veces por semana durante dos meses, con buena respuesta (regresión casi completa de la lesión).

En el caso de nuestro paciente, dado el tamaño, la localización y los síntomas que la lesión le ocasionaba decidimos, en conjunto con el Servicio de Otorrinolaringología, realizar la extipación completa de la lesión. Por el antecedente de dos recidivas en procedimientos anteriores y con la intención de evitar un nuevo episodio, luego de la resección topicamos con TCA 50% todo el epitelio del CAE. A más de 6 meses de seguimiento el paciente se encuentra asintomático y sin evidencia de nuevas lesiones.

CONCLUSIONES

La QFI es un tumor anexial benigno poco frecuente que se origina en la vaina radicular externa del epitelio del infundíbulo piloso. 90% de las lesiones se localizan en cabeza y cuello de adultos mayores, con predilección por mejillas y labio superior. La localización en CAE es sumamente infrecuente y obliga a considerar numerosos diagnósticos diferenciales. La extirpación completa es la terapéutica de elección para esta entidad. Nuestro paciente representa el cuarto caso de QFI del CAE comunicado en la literatura.

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RESUMEN

La queratosis folicular invertida es una neoplasia benigna poco frecuente originada en el epitelio folicular a nivel del infundíbulo. Si bien no posee una clínica característica ni patognomónica, suele presentarse como una lesión solitaria, asintomática, de crecimiento lento, en la cara. El diagnóstico de certeza se realiza con el estudio histopatológico, que evidencia una proliferación de células basaloides y de células escamosas queratósicas con remolinos escamosos, que se extienden a la dermis. El tratamiento de elección es la extirpación quirúrgica, con buen pronóstico y escasa tendencia a la recurrencia. La localización en conducto auditivo externo es extremadamente infrecuente (en la literatura revisada hemos encontrado sólo tres casos comunicados) y representa un desafío tanto diagnóstico como terapéutico.

Se presenta un paciente con una queratosis folicular invertida localizada en conducto auditivo externo izquierdo de 5 años de evolución que comprometía significativamente su calidad de vida por la sintomatología (dolor, otorrea, hipoacusia) y que evolucionó satisfactoriamente con el tratamiento instaurado (extirpación quirúrgica y posterior topicación de la zona con ácido tricloroacético 50%).

Palabras clave: queratosis folicular invertida, conducto auditivo externo.

Sífilis, la gran simuladora

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ABSTRACT

Syphilis has defied humanity for centuries, is transmitted sexually and vertically during pregnancy. This disease became known in Europe at the end of the 15th century,[13] and its rapid spread throughout the continent transformed it into one of the main world plagues. The growth of the syphilitic endemic in the 19th century was worrisome.[13.1] In contrast, medicine developed, and the synthesis of the first drugs became a reality. Perhaps the biggest impact was the introduction of penicillin in 1946, which, due to its effectiveness, led many to believe that the disease was controlled. In a literature review study, it is said that following the introduction of penicillin the incidence of syphilis (and syphilitic uveitis) was steadily decreasing until the end of the 90s.[3] resultando in the decrease of interest in its study and control. With the onset of acquired immunodeficiency syndrome (AIDS). the evolution of this disease increased dramatically. In the ophthalmological literature, more and more cases have been documented and there has even been talk of the "new epidemic of ocular syphilis".[1.1] It is estimated that some 357 million people each year get one of the four sexually transmitted infections (STIs) following: chlamydia, gonorrhea, syphilis or trichomonas's. In the world there is an annual incidence of approximately 12 million patients with syphilis 90% occurs in developing countries (WHO).[7]

MICROBIOLOGÍA

El germen causante de la sífilis es la espiroqueta *Treponema pallidum* y fue aislado por primera vez en 1905 por Schaudin y Hoffman.[12] La sífilis es una enfermedad infecciosa crónica, provocada por la espiroqueta *Treponema pallidum* del género *Treponema*[11]. Se habla de tres subespecies: el *T. pallidum pallidum* (el responsable de la sífilis), el *T. pallidum pertenue* causante de la frambesía o pian) y el *T. pallidum endemicum* (etiología de la sífilis endémica o bejel). Antes de la descripción de estas variedades, hubo mucha confusión que afectó al debate en torno al origen de la enfermedad.[4]

INTRODUCCION

La uveítis sifilítica sigue siendo un problema clínico importante en oftalmología. Amenaza la visión de inmediato y se asocia con el riesgo de complicaciones sistémicas y oculares a largo plazo que pueden resultar en una pérdida severa de la visión.[2] La sífilis ocular es una afección poco frecuente; corresponde al 1-2 % de las uveítis; [1.1] Así, ante cualquier tipo de uveítis la sífilis es una causa que siempre tiene que estar presente en nuestro diagnóstico diferencial[3].

También se considera a la sífilis ocular una de las grandes simuladoras, ya que tiene una gran variedad de presentaciones clínicas que asemejan otras causas de uveítis.[3.1] La sífilis cursa con episodios de actividad y latencia, y da lugar a un amplio abanico de manifestaciones clínicas[3.2], tanto en su forma congénita como adquirida. Así mismo la sífilis congénita es una enfermedad grave cuya atención adecuada se basa en el diagnóstico precoz de la enfermedad en la embarazada y su tratamiento oportuno[11]. La enfermedad ocular puede debutar en el nacimiento o varias décadas después, en forma de uveítis, queratitis intersticial, neuritis óptica, glaucoma y cataratas, las manifestaciones uveíticas más frecuentes de la infección congénita temprana son: la coriorretinitis y la vasculitis retiniana, que pueden dar lugar al característico fondo en sal y pimienta en la retina periférica o en el polo posterior.[5]

Por otra parte, la sífilis adquirida se manifiesta tres a seis meses después de la infección primaria, la diseminación puede asociarse a síntomas y signos propios de la sífilis secundaria, tales como fiebre, malestar general, linfadenopatías, pérdida visual y con menor frecuencia meningitis, hepati-

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tis, síndrome nefrótico y periostitis de huesos largos.[5.1]

A nivel de la retina y coroides, la manifestación más común de la sífilis adquirida es una neurorretinitis difusa irregular, que puede acompañarse de hemorragias intra y subrretinianas, La neurorretinitis cursa con pérdida de visión y con contracción irregular y progresiva (semanas o meses) de los campos visuales.[5.2] Sin embargo, la uveítis anterior es la forma de presentación más habitual, pudiendo ser granulomatosa o no granulomatosa, y bilateral en la mitad de los casos.[7,] La enfermedad tiene tres estadios: la sífilis primaria, caracterizada por la presencia del chancro, el cual se inicia en el sitio de la inoculación, usualmente en la región genital.[5.3] Si la enfermedad no es tratada progresa a sífilis secundaria, con un rash maculopapular generalizado, más pronunciado en las palmas de las manos, las plantas de los pies[1.2] y linfadenopatía; ocurre entre la cuarta a la décima semanas de la manifestación inicial de la enfermedad.[11.1] El cuadro clínico particular es: fiebre, debilidad, cefalea, náuseas, anorexia y dolor articular; otros órganos que se afectan en este estadío, son riñones, hígado, tracto gastrointestinal[13]. Los ojos se afectan en aproximadamente un 10% de los casos.[13.1] El periodo siguiente llamado «estadío tardío» se divide en una fase latente temprana (un año después de la infección inicial) y la fase latente tardía (años después)[3.4]. Aproximadamente, un tercio de los pacientes progresa a la sífilis terciaria. La sífilis terciaria puede ser definida en tres grandes grupos: 1) sífilis terciaria benigna, 2) sífilis cardiovascular y 3) neurosífilis[7.1]. La lesión característica en este estadio es el «goma» que histológicamente es un granuloma; se encuentra en la piel y membranas mucosas, incluyendo la coroides y el iris. 13.2

La sífilis cardiovascular puede afectar las arterias coronarias o la aorta; la neurosífilis llega al sistema nervioso central vía vascular o daña directamente el parénquima.[13.3] Por falta de tamizaje en el recién nacido, la sífilis congénita se detecta con las alteraciones por sífilis después de los dos años de edad con hepatoesplenomegalia, cambios radiológicos en huesos largos, rash descamativo, en la piel, baja de peso, neumonía y anemia intensa.[6.2]Con referencia al pronóstico de la sífilis ocular es favorable cuando afecta a las capas de origen mesodérmico (coroides, esclerótica, iris y córnea), pues sus lesiones son productivas y responden bien al tratamiento. En cambio, es desfavorable en relación con las secuelas visuales, cuando se ven afectados los derivados ectodérmicos (ectodermo neural, retina, nervio óptico y cristalino), ya que se trata de lesiones degenerativas irreversibles.[12]

Objetivo: describir un caso clínico de una paciente de 19 años diagnosticada con uveítis sifilítica.

Metodología: Estudio observacional, descriptivo y retrospectivo. Se presentan los antecedentes de riesgo, diagnostico, cuadro clínico general y oftalmológico, datos de laboratorios.

Presentación del Caso Clínico de Uveítis Sifilítica: Femenina de 19 años acude a guardia del hospital oftalmológico Juan domingo Perón de José C. Paz, provincia de Buenos Aires, por presentar disminución de la agudeza visual y ojo rojo, progresivo, indoloro, de cuatro días de evolución. Asociado a dolor osteomuscular de predominio lumbosacro, brote eritematoso en tronco y extremidades de larga data. (Figura 1).

Figura 1. Rash cutáneo en tronco y extremidades (elaboración propia)

A la observación y anamnesis se hacen evidentes conductas de riesgo para enfermedades de transmisión sexual.

A la valoración oftalmológica encontramos agudeza visual (AV) mejor corregida: OD: 20/50 OI: 20/20.

A la biomicroscopía (BMC): OD: Inyección ciliar intensa 360° con cámara anterior (CA) con célularidad (Tyndall) ++++. Precipitados

retroqueraticos (PQ) +++ finos, difusos, con pupila reactiva, sin presencia de sinequias.

OI: conjuntiva con Inyección ciliar leve, cámara anterior CA con trazas de precipitados retroqueraticos (PQ) + finos, y pupila reactiva sin sinequias. (Figura 2).
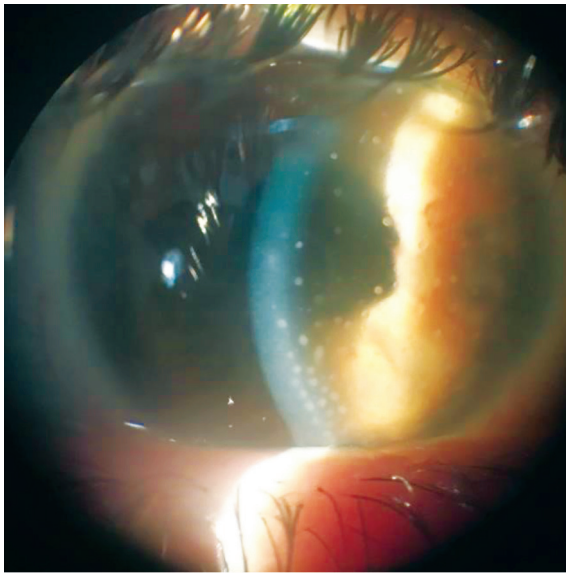
Figura 2. Presencia de Tyndall y precipitados. Retroquerático en cámara anterior. (E. P.).

La paciente presentó presión intraocular (PIO) 13 mm Hg A.O. Al fondo de ojos (FO): papila de bordes netos con excavación fisiológica macula sin edema ni exudados. Envainamiento vascular en arcada temporal y nasal superior de predominio en ojo derecho.

Se realizaron laboratorios de muestras de sangres como hemograma y eritrosedimenta-

cion (figura 3.) Y pruebas treponemicas y no treponemicas, las cuales reportaron positivas. VDRL 128 DLS y TP-PA positiva. También los anticuerpos como el factor reumatoide positivo, anti streptolisina O positivo, auto anticuerpos por LIA Anti RO y el Anti LA, positivos ambos. (Figura 4).

Tratamiento inicial se decide aplicar Inyección parabulbar de Betametasona 6mg, Prednisolona acetato colirio una gota cada hora, Atropina colirio una gota cada 8 horas. Se deriva la paciente con orden de solicitud de retinografías con fluoresceína e internación para un tratamiento intrahospitalario, interdisciplinario con infectología y reumatología La paciente regresa 40 días posterior a la primera consulta. Informa que fue valorada por infectólogo y reumatólogo quienes decidieron tratamiento ambulatorio con Penicilina intramuscular dosis única. A la exploración física de control, presentó agudeza visual AV: 20/20 en A.O. Sin reacción inflamatoria en cámara anterior, fondo de ojos (F.O) sin signos de vasculitis. La retinografía con fluoresceína (RFG) de control no presentaba alteraciones. (figura. 4)

Se actualizo laboratorios serológicos reportando negativización de VDRL y persistencia de factor reumatoide positivo.

DISCUSIÓN

La sífilis, considerada como una gran imitadora de otras enfermedades infecciosas y de algunas autoinmunes, logra dificultar el diagnostico óp-

Figura 3. Hemograma completo inicial.
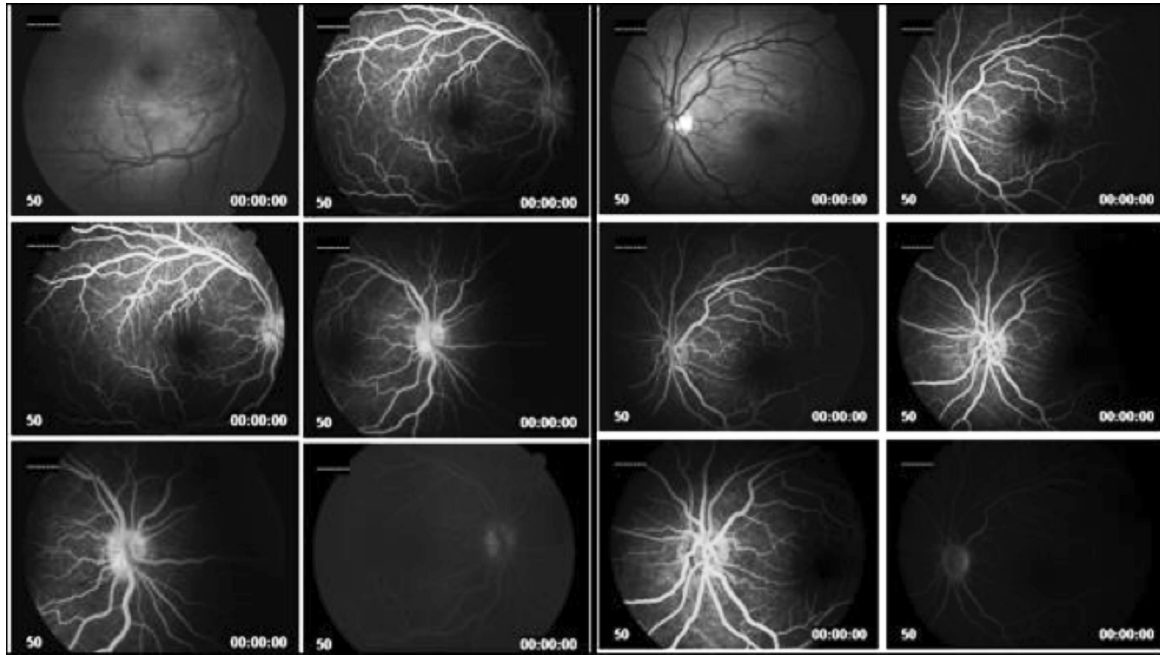
Figura 4. RFG de AO realizado a 40 días de evolución, sin signos de vasculitis. (E.P)

timo y oportuno. La uveítis sifilítica se presenta con mayor frecuencia en el estadío de Sífilis secundaria. Para un Diagnóstico correcto, es importante realizar un interrogatorio profundo, pesquisando conductas de riesgo para contraer enfermedades de transmisión sexual, y la adecuada identificación de los signos y síntomas característicos de los estadios iniciales de la enfermedad; a saber: artralgias y mialgias, y rash cutáneo indoloro que compromete tronco y extremidades de predominio en plantas y palmas, las cuales presento la paciente. Así mismo, de igual valor es la evolución y tratamiento interdisciplinario donde interactúen de manera mancomunada el oftalmólogo, infectólogo y reumatólogo para dar una óptima y oportuna terapéutica.

Es transcendental realizar siempre, tanto las pruebas no treponémicas (VDRL y
RPR) como las treponémicas (FTA-ABS y TP-PA) para confirmar el diagnóstico.[9.1]

El VDRL puede ser positivo en algunas colagenosis entre ella la artritis reumatoide en los pacientes que padecen de sífilis por esa razón se realizan pruebas confirmatorias como son la FTA- ABS, TP-PA, TPHA. ELISA y IgM para descartar la posibilidad de que el paciente este padeciendo algunas de estas patologías inmunológicas.[9.2] Teniendo en cuenta que la paciente presentó resultados positivos en las di-

ferentes pruebas reumatológicas como el factor reumatoide positivo, anticuerpos por LIA Anti RO y el Anti LA, positivos ambos, que confirman la presencia de colagenosis lupus eritematoso sistémico (LES) se le realizó valoración y seguimiento por parte de reumatología. las pruebas no treponémicas pueden ser tituladas y por eso son importantes en el control de la curación. La Sífilis Ocular se trata de la misma forma que la neurosífilis: 18-24 millones de unidades de Penicilina G UI/día, durante 10 a 14 días y continuar Penicilina Benzatínica 2,4 millones UI semanal IM durante tres semanas, La Ceftriaxona es la droga de elección en los pacientes alérgicos a la Penicilina. El tratamiento de forma oportuna suele significar la cura en la mayoría de los pacientes. por el contrario, el tratamiento inadecuado de penicilina intramuscular de manera ambulatoria puede conllevar a la morbi-mortalidad asociada a los estadíos más avanzados de la enfermedad.

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RESUMEN

La sífilis desde hace siglos desafía a la humanidad, es transmitida por vía sexual y verticalmente durante la gestación.[9] Esta enfermedad se hizo conocida en Europa a finales del siglo XV,[13] y su rápida propagación por todo el continente la transformó en una de las principales plagas mundiales. Era preocupante el crecimiento de la endemia sifilítica en el siglo XIX[13.1]. En contrapartida la medicina se desarrollaba, y la síntesis de las primeras drogas se hacía realidad. El mayor impacto tal vez fue la introducción de la penicilina en 1946, la cual por su eficacia hizo a muchos pensar que la enfermedad estaba controlada. En un estudio de revisión de literatura se dice que a raíz de la introducción de la penicilina la incidencia de sífilis (y de uveítis sifilítica) fue disminuyendo constantemente hasta finales de los años 90.[3] resultando en la disminución del interés por su estudio y control. Con la aparición del síndrome de inmunodeficiencia adquirida (SIDA).[2] se incrementó dramáticamente la evolución de esta enfermedad. En la literatura oftalmológica se comenzaron a documentar cada vez más casos e incluso se ha llegado a hablar de la «nueva epidemia de sífilis ocular»[1] Se estima que, anualmente, unos 357 millones de personas contraen alguna de las cuatro infecciones de transmisión sexual (ITS) siguientes: clamidias, gonorrea, sífilis o tricomoniasis.[7] En el mundo hay una incidencia anual de aproximadamente 12 millones de pacientes con sífilis el 90% ocurre en países en desarrollo (OMS).[7.1]

Palabras claves: sífilis, enfermedad ocular, congénita, adquirida, diagnóstico, infección, tratamiento.

Cociente ganglionar como factor pronóstico de sobrevida. Recurrencia en carcinoma escamoso de cavidad oral

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SUMMARY

Background: Oral cavity squamous cell carcinoma is one of the most common pathologies in head and neck surgery. Surgery is the treatment of choice with high rates of locoregional control. However, recurrences are seen even in early stages of the disease. This explains the need of new prognostic factors to provide a better staging, treatment and follow up. Nodal Ratio has been validated as a prognostic tool in other tumors and in oral cavity in a multicentric study by Memorial Sloan Kettering Center of Nueva York.

Objective: To validate the Nodal Ratio as a prognostic factor in terms of survival and recurrence in oral cavity carcinoma in our medium.

Design: Institutional retrospective cohort.

Setting: Tertiary Public Hospital for treatment of tumours.

Population and Methods: We retrospectively review 92 patient´s information from clinic histories, surgical protocols and pathologic informs. Inclusion criteria were squamous cell histopathology, T1-4 and pN0-N+ (pN1-2) stage. We calculate survival with Kaplan-Meier method and multivariate analysis was done to determinate the independence value.

Results: A Nodal Ratio higher than 5% was statistically significant as a prognostic factor of survival [HR 5,22(IC95% 1,86; 14;62) (p 0.002)] and recurrence [HR 13.33 (IC95% 3.85; 46.16) There was no prognostic difference between patients with pN0 dissections and those with pN+ dissections plus NR less than 5%. pN1 patients with nodal yields of 20 (1/20) or more could have the same forecast as a pN0.

Conclusions: We were able to validate NR as a prognostic factor. We postulate the potential use in the indication of adjuvant therapy.

Keywords: Nodal Ratio, Oral squamous cell carci>noma, Neck dissection.

Introducción

El carcinoma de cavidad oral es el 6to cáncer más común en el mundo correspondiendo al 2-3% de todas las malignidades[8]. Se estima que cada año en el mundo se diagnostican alrededor de 3.000 casos de carcinoma escamoso de cavidad oral y alrededor de 127000 pacientes mueren de la enfermedad [19]. Constituye el 8vo cáncer más frecuente en hombres. Aunque la incidencia de carcinoma oral ha disminuido en países desarrollados en las últimas décadas, continúa siendo un cáncer frecuente tanto en hombres como en mujeres del sur de Asia, Europa central y del este [19].

La identificación de factores de riesgo de recurrencia es de extrema importancia para optimizar el diagnóstico y el tratamiento. En la práctica diaria el tamaño tumoral, la invasión perineural, linfovascular y los márgenes de resección se utilizan comunmente para evaluar el riesgo de recurrencia locoregional [11]. Dentro de los factores pronósticos relacionados a ganglios linfáticos se destacan el número de ganglios positivos, la extensión extracapsular, metástasis contralaterales, metástasis bajas (IV-V) y el número de ganglios disecados [15].

El sistema TNM se basa en el concepto simplista de una progresión ordenada del tumor y los tejidos circundantes para posteriormente transgredir las barreras linfáticas y vasculares[20]. En este sistema la presencia o ausencia de adenopatías constituye el principal factor pronóstico, sin embargo, estudios recientes sugieren que la estadificación nodal por sí sola no es un pronosticador consistente y confiable [18].

Existe evidencia suficiente para determinar que el sistema es más complejo y en la evolución del proceso se requiere de la investigación de otros factores pronósticos [20]. Existe evidencia consistente que de-

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muestra que el *Cociente Ganglionar(CG)*es superior que la estadificación tradicional en la estimación de sobrevida [18,11].

Múltiples estudios demostraron que el *CG* definido como la relación entre los ganglios patológicamente positivos y el número total de ganglios resecados es fuerte predictor pronóstico independiente en carcinoma de cavidad oral [9,12,14,21,22.24]. El *CG* es utilizado como factor pronóstico en otras localizaciones como cáncer vesical, esofágico y cervical [7].

En estudios previos ciertos autores plantearon modificar la estadificación tradicional [19,3] resultando atractivo considerar el *CG* como parte del algoritmo terapéutico en lo que respecta a la indicación de tratamiento adyuvante. Urban [27] demostró la importancia de éste parámetro para estimar el beneficio de la radioterapia en carcinoma oral. Cabe destacar, que no existen trabajos que evalúen este factor como pronóstico de sobrevida y recurrencia en nuestro país.

El objetivo de este estudio es determinar la capacidad pronóstica, en términos de sobrevida y recurrencia del *CG*, en pacientes con carcinoma escamoso de cavidad oral sometidos a vaciamiento como parte del tratamiento quirúrgico integral. La hipótesis de superioridad de este factor pronóstico se debe a que tiene en cuenta 3 factores:1) número de ganglios positivos 2) número de ganglios resecados y el procedimiento quirúrgico en relación al cirujano y al patólogo.

Materiales y métodos

Se evaluaron 92 historias clínicas de pacientes diagnosticados de carcinoma escamoso de cavidad oral, operados entre enero de 2010 y diciembre de 2017. Los datos clinicopatológicos fueron recolectados de historias clínicas, partes quirúrgicos e informes de anatomía patológica.

Se analizaron todos los pacientes de cualquier edad y sexo con carcinomas localizados en la cavidad oral y su contenido confirmado por histología y vírgenes de tratamiento. Se incluyeron los subsitios: lengua, piso de boca, mucosa yugal, reborde alveolar y paladar duro y se estadificaron según la AJCC 8°Ed.[5] T1-T4a; N0-2; M0 pasibles de cirugía con criterio curativo y con posibilidad resectiva.

Se excluyeron pacientes con histologías diferentes a carcinoma escamoso, patología localizada en labio, enfermedad regional N3, pacientes sometidos a quimioterapia, con muerte perioperatoria y seguimiento menor a 3 meses, quedando para el análisis 92 pacientes evaluables con un seguimiento medio de 44.4 meses, la edad media fue de 60.9 (IC95% 58.4;63,5) perteneciendo al sexo masculino el 54,3.En 54.3% enfermos se registró el hábito de fumar, en el 22,8% de bebida alcohólica, el 12% diabéticos y el 2.5 con un traumatismo crónico previo. Todos los pacientes fueron evaluados de manera preoperatoria incluyendo: historia clínica, examen físico completo, rinofibrolaringoscopía flexible, laboratorio tomografía y/o resonancia magnética de cabeza y cuello y radiografía de tórax,

Los tumores primarios fueron resecados con un margen periférico y profundo de 1 cm mínimo, evaluados por congelación intraoperatoria para corroborar que los mismos estuviesen libres de lesión, en el caso de ser positivos se realizaron retomas del mismo. Los defectos fueron reparados en el mismo acto quirúrgico mediante cierre simple, colgajos locales, regionales o libres. Pacientes estadificados clínicamente como N0 (cN0) con invasión en profundidad >4mm y/o conT2 fueron sometidos a vaciamiento selectivo de niveles I-II-III. Pacientes con adenopatías clínicas positivas (cN1) fueron tratados con vaciamiento cervical radical clásico o modificado de los niveles I al V acorde a lo descripto por la American Head and Neck Society. En caso de adenopatías bilaterales o tumores que transgredían la línea media se realizó vaciamiento bilateral.

La terapia adyuvante postoperatoria se indicó en pacientes con factores de alto riesgo de recurrencia tales como márgenes positivos, extensión extracapsular y con buen estatus funcional. El mismo se basó en radioterapia externa normofraccionada y quimioterapia concurrente con Cisplatino 100 mg/m2 en días 1-22-43. Aquellos pacientes con factores de riesgo intermedios (pT3-4, pN2-3, márgenes <5mm, alto grado histológico, invasión vascular o angiolinfática, fueron tratados solo con radioterapia adyuvante. La identificación de los volúmenes de radiación y dosis se basaron en la información clínica, radiológica, quirúrgica e histopatológica.

El análisis histopatológico de los ganglios linfáticos fue realizado en el Servicio de Patología luego de fijación con formaldehído al 5%.

Los especímenes fueron procesados y examinados por patólogos certificados con especial interés en patología de cabeza y cuello. La preparación, disección, muestreo de tejido, fijación, corte y análisis microscópico del tumor primario y de las piezas de vaciamiento cervical, fueron realizadas de similar manera en cada uno de los casos, tal como es sugerido en las guías internacionales de estudio histopatológico.

Los ganglios linfáticos fueron definidos como agregados encapsulados de tejido linfoide de cualquier tamaño y con seno periférico. La extensión extracapsular se definió como la extensión tumoral por fuera de la cápsula con reacción desmoplásica estromal. El *CG* se definió como el resultado entre el número de ganglios positivos y el número total de ganglios resecados.

Los subsectores de cavidad oral resultaron: lengua 54.5%, piso de boca 7,6%, reborde alveolar 4.3%, trígono retromolar 17.4%, mucosa yugal 9.8% y paladar 4.3%.

Respecto al estadío, al momento de la presentación el tamaño tumoral fue: T1 17.4%; T2 39.1%, T3 19.6%; T4 23.9%. El estadío ganglionar resultó: cN0 70.7%, cN1 20.7%, cN2 8.7%. El 72% de los pacientes fueron sometidos a vaciamiento electivo (I-II-III) y 28% a vaciamiento radical (I-V).

El 50% de los pacientes fueron sometidos a radioterapia RT adyuvante mientras que 32,6% recibieron quimioterapia de manera concurrente postoperatoria.

Todos los pacientes realizaron los controles postoperatorios, de acuerdo a las recomendaciones de la NCCN. El seguimiento fue conjunto con el servicio de oncología clínica, nutrición, fonoaudiología, psicopatología y cuidados paliativos. Los controles consistieron en evaluaciones clínicas y por riniofibrolaringoscopía mensuales durante el primer año, bimensuales durante el segundo, trimestrales durante el tercero, semestrales del 3er al 5to año y posteriormente anuales. Concomitantemente se realizó estudio por imágenes (TAC y/o RMN) basal a las 12 semanas del postoperatorio y posteriormente cada 6 meses durante el primer año y anualmente en los subsiguien-

tes. Se realizaron biopsias directas en aquellos casos con sospecha de recaída local y punción aspiración con aguja fina de las adenopatías sospechosas, en aquellos casos en los cuales no se obtuvo resultado concluyente se realizaron biopsias ambulatorias o bajo anestesia general.

Del análisis histopatológico se registró: diferenciación, márgenes, invasión vasculolinfática y perineural, número total de ganglios resecados y positivos, y extensión extracapsular.

La sobrevida se calculó desde la fecha de cirugía a la fecha de muerte de cualquier causa o última consulta. La sobrevida libre de enfermedad se calculó desde la fecha de la cirugía a la fecha de aparición de recurrencia local, regional o a distancia.

La recurrencia local se definió como la aparición del tumor de similar histología luego de 6 semanas del tratamiento y hasta 3 años desde el inicio de la terapia. Se definió recurrencia regional a aquellas ocurridas en los ganglios linfáticos y recurrencia a distancia, a aquellas ocurridas fuera del territorio de cabeza y cuello.

Respecto a los hallazgos histopatológicos, 45.7% correspondieron a tumores bien diferenciados, 53.3% a tumores moderadamente diferenciados y 1.1% a tumores pobremente diferenciados. Respecto a la invasión vasculolinfática, ésta se presentó en el 13% de los pacientes y 21,7% presentaron invasión perineural.

Fueron resecados un total de 1545 ganglios linfáticos resultando un mínimo satisfactorio 20 ganglios obtenidos, la media por paciente fue de 14.6 (IC 95% 12,3; 16,6). La densidad ganglionar para vaciamientos electivos fue de 15.6 (IC 95% 13.7 -17.5) y, para vaciamientos terapéuticos, de 15.7 (IC 95% 12.5-19.1). En total 45 pacientes resultaron pN0, mientras que 40 de los mismos fueron pN+. Se constataron un total de 4 vaciamientos pN+ con estadificación inicial cN0. La extensión extracapsular se presentó en el 30,4% de los pacientes.

La información recolectada fue analizada utilizando el software SPSS versión 23.0 (SPSS Inc., Chicago, IL). Para cada variable se realizó el análisis estadístico descriptivo correspondiente. La estadística descriptiva fue sumariada como frecuencias, porcentajes, medias, medianas, rangos y desviaciones standard. Los valores de control local a 3 años, control regional

Cociente ganglionar como factor pronostico de sobrevida y recurrencia en carcinoma escamoso de cavidad oral

y a distancia, sobrevida libre de enfermedad y sobrevida global fueron determinados informaticamente utilizando el método de Kaplan-Meier y (log-Rank test). Se utilizó análisis uni y multivariado para identificar predictores independientes de control y sobrevida. Todos los factores identificados en el análisis univariado fueron utilizados para el análisis multivariado. Los factores pronósticos independientes fueron determinados utilizando el análisis de regresión de Cox. Valores de p<0,05 fueron considerados estadísticamente significativos.

Resultados

La sobrevida global media fue de 65,75 meses (IC 95% 55,84; 75,63). La mortalidad global de la muestra fue del 33,7%. (gráfico 1)
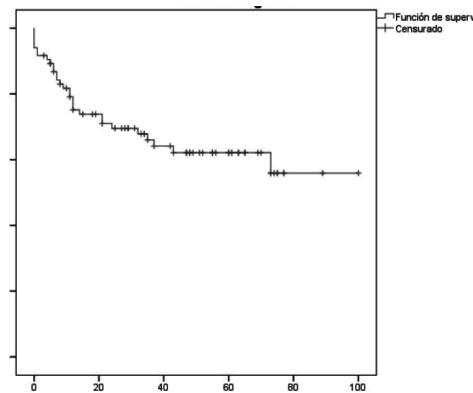
a) Análisis Univariado

Se identificaron como factores de riesgo a la edad mayor a 65 años [HR 3.08 (IC95% 1.36; 6.96) (p 0.007)], invasión vasculolinfática [HR 3,46 (IC 95% 1,38; 8,65) (p 0.008)], invasión perineural [HR 7,64 (IC 95% 3,49; 16,73) (p< 0.0001)] y extensión extracapsular [HR 4,57 (IC 95% 2,17; 9,6) (p< 0.0001)].

La estadificación nodal inicial N0 [HR 0.42 (IC 95% 0.20; 0.88) (p 0.021)] y la diferenciación histológica siendo la diferencia entre bien y moderada [HR 0,29 (IC95% 0,12;0,68) (p 0.005), resultaron factores protectores.

El CG. como variable contínua resulta estadísticamente significativo como predictor de la sobrevida global con un HR de 1,024 IC 95% (1,01; 1,04) (p 0,014). De esto se deduce que por cada punto que aumente el CG. el riesgo de muerte aumenta en 1,024 veces. A fin de que esta medida resulte válida y aplicable para la toma de decisiones se plantearon diferentes puntos de corte resultando significativo luego del análisis multivariado un valor de 0.05.

b) Análisis Multivariado

Se analizaron todas las variables que resultaron significativas en el análisis univariado y se descartaron las restantes hasta ajustar el modelo, obteniéndose tres factores de predictivos independientes de sobrevida global: edad mayor a 65 años [HR 4,01 (IC95% 1,62; 9,89) (p 0.03)], invasión perineural [HR 9,43 (IC95% 3,47; 25, 61) (p 0.00) y CG>5% [HR 5,22(IC95% 1,86; 14;62) p (0.002)].

Análisis de sesgos en los factores pronósticos de la sobrevida global

Se compararon los modelos ajustados para un valor menor a 5% y pN0 evidenciando un mejor coeficiente de ajuste [(-2log de la verosimilitud: 130,240) (p<0.000)] para el primero, por lo cual se interpreta que dentro de la población de pacientes operados de Carcinoma Escamoso de Cavidad Oral un CG. menor a 5% presenta igual pronóstico que pN0. (gráfico 2)
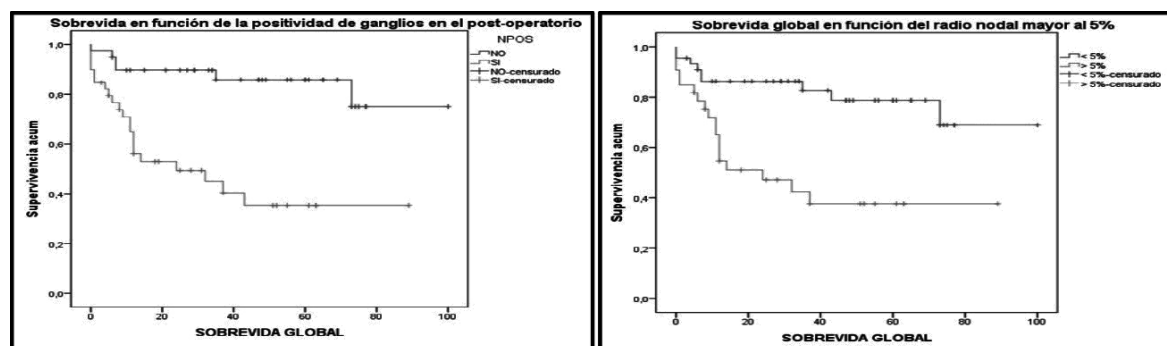
Gráfico 2. Sobrevidas en función de positividad de ganglios y CG.mayor a 5%.

Sensibilidad y especificidad:

Al evaluar el CG. en el grupo pN+ el mismo no resulta significativo.

Se puede observar en el gráfico 3 que el CG. se comporta como buen factor pronóstico, con una sensibilidad para el valor de corte identificado de 70% y una especificidad del 70%.
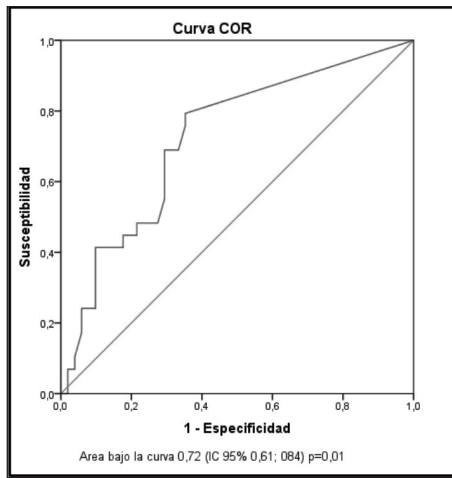
Gráfico 3. Sensibilidad y especificidad

RECURRENCIA

De los pacientes analizados 23.9% presentaros recaída de la enfermedad que se manifestó como local en 9.8%, regional en 9.8% y a distancia en 2.2%.

La sobrevida libre de enfermedad en meses fue de 70,51 (IC 95% 60,37; 80,65). (gráfico 4)
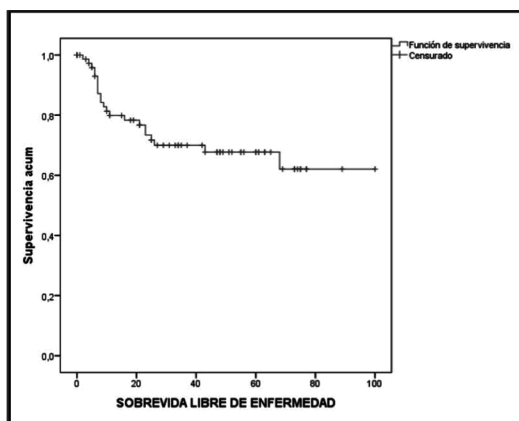
Gráfico 4. Sobrevida Libre de Enfermedad.

ANALISIS UNIVARIADO

Se analizaron cada una de las variables identificando como factores de riesgo de recurrencia: a la edad como variable contínua [HR 1.047 (IC95% 1.002; 1.09) (p 0.04)] y categórica (>65) [HR 3.53 (IC95% 1.44; 8.63) (p 0.006)]; localización en lengua [HR 0.34 (IC95% 0.14; 0.85) (p 0.02)]; tratamiento con vaciamiento terapéutico [HR 2.95 (IC95% 1.27; 6.85) (p 0.012)]; invasión vasculolinfática [HR 5.18 (IC95% 1.88; 14.29) (p 0.001)]; invasión perineural [HR 5.71 (IC95% 2.29; 14.21) (p 0.000)]; extensión extracapsular [HR 3.15 (IC95% 1.33; 7.42) (p 0.009)]; y el CG. como variable categórica (>5%) [HR 5.42 (IC95% 2.08; 14.16) (p 0.001)].Como variable protectora resultó significativo el status pN0 [HR 0.38 (IC95% 0.16; 0.88) (p 0.025)].

ANALISIS MULTIVARIADO

Se evaluaron todas las variables significativas del análisis univariado y se descartaron las que no resultaron significativas hasta ajustar el modelo, obteniéndose tres factores predictivos independientes de recurrencia: edad mayor a 65 años [HR 9.90 (IC95% 2.96; 33.09) (p 0.00)]; invasión perineural [HR 14.43 (IC95% 3.97; 52.40) (p 0.00)]; y CG>5% [HR 13.33 (IC95% 3.85; 46.16) (p 0.00)].

Sensibilidad y especificidad:
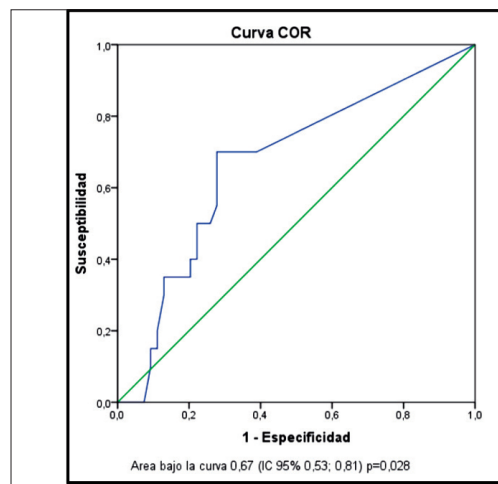
Gráfico 5. Sensibilidad y especificidad

De manera similar a la evaluación en términos de sobrevida, el CG como factor pronóstico de recurrencia presenta una Sensibilidad y Especificidad del 70% gráfico 5.

DISCUSIÓN

El tratamiento standard para el carcinoma de cavidad oral es la resección quirúrgica del tumor primario con tratamiento del cuello de manera electiva o terapéutica [19].

Uno de los factores pronóstico más significativos en cavidad oral, así como también en otros sectores del tracto aerodigestivo, es la presencia de metástasis ganglionares.

El riesgo de muerte en pacientes con carcinoma escamoso de cabeza y cuello se relaciona con la posibilidad de desarrollar metástasis a distancia. A su vez, existen múltiples factores de riesgo para estimar esta posibilidad: estadío avanzado, metástasis linfáticas y extensión extracapsular [28]. Un número reciente de estudios demostraron que el sistema de estadificación tradicional no sería confiable en la predicción del pronóstico o en la dirección del tratamiento, sugiriendo la necesidad de un sistema alternativo de estadificación [10].

El factor más importante en la estadificación ganglionar es el procedimiento de sampleo[19]. En el estudio de Mamalle[16], el número de linfáticos positivos fue factor predictor de resultados en pacientes con carcinoma de cabeza y cuello. A su vez, estudios previos demostraron que la posibilidad de encontrar adenopatías positivas se asoció a la disección de un alto número de ganglios resecados[1,2]. Los trabajos de Gil[12], Shrime[24,25] y Suslo[26] son las primeras publicaciones sobre el uso del CG como factor pronóstico en carcinoma escamoso de cavidad oral. Sin embargo, son los trabajos de Liao[15] y Urban[27] que incorporan a un mayor número de pacientes, validan a este factor como variable pronóstica.

Finalmente, la validación definitiva como herramienta pronóstica, proviene de un estudio multicéntrico publicado por Patel[19] del Memorial Sloan Kettering Cancer Center de New York.

El CG es el resultado entre el número de adenopatías positivas y el número total de ganglios resecados. Debido a que una disección ganglionar limitada puede resultar en una subestadificación patológica, el CG tiende a compensar este factor, al recapitular 2 piezas de información: la extensión del tumor en el cuello (número de ganglios positivos) y la extensión del clearence ganglionar durante el vaciamiento (número total de ganglios)[12].

Se considera que aquellos pacientes con CG elevado presentan peores resultados: sobreviven menos, tienen menor control local y una mayor cantidad de recurrencias. De manera detallada, la hipótesis es que el CG podría ser aplicado en el pronóstico, estadificación, terapéutica y seguimiento del carcinoma escamoso de cavidad oral.

Si se aborda directamente la ecuación de CG, aquellos cuellos pN0 van a obtener un valor de 0. Sin embargo, las metástasis ocultas son el principal factor pronóstico en cuellos pN0[24] y explican las recurrencias regionales en estos pacientes[28]. En los mismos, el recuento ganglionar total o Nodal Yield (denominador del Nodal Ratio), es la variable que modifica la ecuación y, es la que tendría que tomarse en cuenta como factor pronóstico y posible herramienta en la terapéutica adyuvante. Un estudio multicéntrico determinó 18 ganglios como corte[8], teniendo como extremos 10 y 20 ganglios resecados [1,4,5,7,13,23] mientras en el H. María Curie el corte es de 20 ganglios, la media fue de 15,6. El número total de ganglios resecados es un marcador de calidad de los vaciamientos cervicales. La posibilidad de un análisis histopatológico más completo, con un mayor número ganglionar analizado, reduce la posibilidad de subestadificar la enfermedad, especialmente en aquellos casos en los cuales el vaciamiento es electivo[8].

En el presente trabajo el CG se asoció a sobrevida global [HR 1,024 (IC 95% 1,01; 1,04) (p 0,014)], deduciendo que por cada punto que aumente el CG, el riesgo de muerte aumenta en 1,024 veces. Por otro lado, al categorizar la variable con un punto de corte de 0.05(5%) se determinó que, un valor mayor a éste, constituye un factor de riesgo independiente de sobrevida global [HR 5,22 (IC95% 1,86; 14;62) (p 0.002)]. Por el contrario, un valor menor a 5% resulta ser un factor protector [HR 0,19 (IC 95% 0,68; 0,53)].

Debido a que no existen diferencias estadísticas, en términos de sobrevida global, entre pacientes pN0 y pN+ con CG <5%, se puede inferir que, para obtener el mismo pronóstico ante el hallazgo de un ganglio metastásico, el cirujano tiene que haber obtenido un mínimo de 20 ganglios (1/20=0.05) durante el vaciamiento.

Cuando se evalúa el CG como variable contínua no resulta significativa en término de recurrencia. Sin embargo, un valor del mismo mayor al punto de corte (0.05 o 5%) resulta un factor de riesgo independiente de recurrencia [HR 13.33 (IC95% 3.85; 46.16) (p 0.00)]. Se sugiere considerar al Nodal Yield, y no el CG, como posible factor pronóstico a considerar en vaciamientos pN0.

El hecho de que valores más bajos de CG se correlacionen con un mejor pronóstico sugiere por sí mismo qué, linfadenectomías más extensas podrían mejorar la sobrevida. La subestimación de la enfermedad al evaluar un número menor de ganglios linfáticos genera una falla en la identificación de aquellos pacientes que se beneficiarían con un tratamiento adyuvante y adicionalmente, tienen mayor riesgo de enfermedad residual en el cuello al no realizar un clearence ganglionar adecuado[9].

A lo largo de la bibliografía los dos puntos de inflexión en los que podría inferir el CG se encuentran en la terapia adyuvante y en la posibilidad de extensión del vaciamiento. A partir de estudios previos[6], se demostró un beneficio en la sobrevida de pacientes tratados con radioquimioterapia concurrente comparado a radioterapia sola, sin embargo, la morbilidad asociada a la intensificación del tratamiento no es despreciable[19] existiendo controversia aún, respecto a su utilización. Este trabajo intenta predecir la necesidad de tratamiento adyuvante o incrementar la intensidad del mismo, aunque otras variables como: tamaño, volumen, nivel ganglionar, presencia de micrometástasis ocultas y presencia y extensión de la ruptura capsular, son igualmente predictores de resultados.

La discusión puede plantearse desde 2 puntos de vista: la posibilidad de indicar radioterapia postoperatoria (RT) a través del CG, o la posibilidad de evitar el uso innecesario de la misma. La indicación de RT se basa en opinión de expertos y estudios de cohortes que establecieron los factores de riesgo de recurrencia locoregional y a distancia[27]. Dos de estos factores: extensión extracapsular y márgenes positivos fueron determinantes de tratamiento con quimioterapia concurrente en dos trials fase III[6]. Sin embargo, la indicación de RT, sin la adición de quimioterapia carece de evidencia Nivel I[27]. Más aún, un estudio no demostró beneficio de la RT adyuvante en enfermedad pN1 poniendo en evidencia la necesidad de criterios claros para su indicación[9]. Las guías de la NCCN[17] determinan que en tumores pT1-T4 con 1 sola adenopatía positiva en el espécimen (pN1-pN2a) la RT adyuvante es opcional en ausencia de factores de riesgo adicionales como extensión extracapsular, márgenes positivos, invasión perineural o angiolinfática. Esto es tema de debate común en los ateneos interdisciplinarios[14].

Bajo criterios estrictos un paciente con 20 ganglios resecados y 1 positivo sería candidato a RT adyuvante al igual que otro paciente con 10 resecados y 1 positivo. Sin embargo, el riesgo no es similar en ambos. Es muy probable que el segundo paciente tenga: mayor posibilidad de subestadificación de la enfermedad, enfermedad ganglionar residual con recurrencia regional, a distancia y muerte. Por otro lado, la indicación de RT en el primer paciente no solo podría no ser necesaria desde el punto de vista del control local, debido a que no le va a otorgar mejores resultados, sino que lo va a exponer a la toxicidad del tratamiento nada despreciable y a condicionar el tratamiento de un posible segundo primario, especialmente si se trata de un paciente joven.

Sugerencias: Pacientes pN1 sin otros factores de riesgo podrían ser excluídos de la indicación de RT adyuvante con CG <5% debido a que no existen, en este trabajo, diferencias pronósticas entre un cuello pN0 y otro pN+ con CG <5% en términos de sobrevida y recurrencia.

Pacientes pN1 sin otros factores de riesgo se beneficiarían del tratamiento adyuvante con valores >5% de CG debido a que, en el presente estudio, resultó ser el factor de riesgo con mayor Hazard Ratio luego de la invasión perineural [HR 13.33 (IC95% 3.85; 46.16) (p 0.00)].

En el trabajo de Urban[27] se determinó que solo aquellos pacientes con adenopatías positivas en el análisis histopatológico, pero con CG >12.5%, se beneficiarían en términos de sobrevida de la RT postoperatoria. Según concluye, en pacientes con vaciamientos insuficientes por experiencia o volumen del cirujano o enfermedad extensiva en cuello, la mayor posibilidad de tumor residual podría compensarse con uso de RT adyuvante.

Limitaciones del trabajo

El presente es un estudio retrospectivo institucional lo que limita la generalización de los resultados.

Se incluyeron todos los subsitios de cavidad oral. Esto es considerado como probable fuente de factores confundidores debido a que cada subsitio tiene diferentes relaciones anatómicas como proximidad a hueso, densidad linfática regional y accesibilidad a la inspección y diagnóstico ([18]).

No se diferenció entre aquellos pacientes que fueron sometidos a vaciamiento electivo/selectivo de los que fueron tratados con vaciamiento terapéutico/radical. Es probable que diferentes puntos de corte de CG sean necesarios de acuerdo al tipo de vaciamiento realizado[15]. En muchos de los estudios evaluados se plantea la hipótesis de que el CG puede quedar sujeto a sesgo al incluir un número elevado de pacientes pN0. Para mitigar este efecto Ong[18] incluye a los pacientes pN0 en un grupo separado.

Un sesgo potencial del CG es su dependencia de la técnica quirúrgica y el procesamiento de las muestras por el patólogo. En el presente trabajo los cirujanos actuantes y patólogos no fueron los mismos a lo largo del estudio.

Se incluyeron pacientes con o sin tratamiento adyuvante y a su vez el tratamiento adyuvante varió entre los mismos. Ebrahimi[8] en su estudio multicéntrico menciona que el pronóstico del número total de ganglios resecados puede ser modificado por la RT adyuvante. Esto se genera al disminuir el riesgo asociado a un valor bajo en la densidad ganglionar (Nodal yield), lo que se extrapola al CG por estar este factor asociado a la modificación del numerador de la ecuación. Por tal causa Liao[15] en su

trabajo evalúa el CG en carcinomas de cavidad oral con cuello positivo y sometidos, en su totalidad, a radioterapia adyuvante lo que permitió una muestra más homogénea.

Conclusiones

Por medio de este trabajo, el primero en nuestro medio, se intenta validar los resultados obtenidos en múltiples estudios retrospectivos institucionales y multicéntricos. Se determina que el CG constituye un factor pronóstico, adicional e independiente, de sobrevida global y recurrencia en la evaluación de pacientes con carcinoma escamoso de cavidad oral.

No fue posible independizar al CG del status ganglionar (pN) en pacientes pN+, por lo que se requiere un mayor número de éstos para determinar la superioridad pronóstica de este parámetro respecto al sistema tradicional TNM.

Por último, existe un potencial uso del CG en la indicación del tratamiento adyuvante en pacientes con adenopatía única (pN1-pN2). Debido a los hallazgos en el presente trabajo, se sugiere se tenga en cuenta al momento de no indicarlo en pacientes pN1 sin otros factores de riesgo, así como también, al momento de indicar la RT adyuvante en pacientes sin factores de riesgo con adenopatía metastásica única. Se requieren de estudios prospectivos y ensayos clínicos para determinar la potencial modificación del esquema terapéutico por medio del CG.

Se necesitan cohortes con mayor número de pacientes para evaluar y corroborar nuestros hallazgos y mejorar la comprensión de la influencia del NR en carcinoma de cavidad oral.

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RESUMEN

Antecedentes: El carcinoma escamoso de cavidad oral constituye una de las patologías de mayor frecuencia en cabeza y cuello. El tratamiento de elección es quirúrgico con altas tasas de control local. Sin embrago, se evidencian recidivas aún en etapas tempranas de la enfermedad, lo que lleva a la necesidad de identificar factores pronósticos confiables para mejorar la estadificación, tratamiento y seguimiento. El Cociente Ganglionar fue ratificado como herramienta pronóstica en otros tumores y validado en cavidad oral en un estudio multicéntrico dirigido por el Memorial Sloan Kettering Center de Nueva York.

Objetivo: Validar en nuestro medio al Cociente ganglionar como factor pronóstico de sobrevida y recurrencia en carcinoma de cavidad oral.

Lugar de aplicación: Hospital público de atención terciaria de tumores.

Diseño: Cohorte Retrospectivo Institucional.

Material y Métodos: Se recabó de manera retrospectiva información de historias clínicas, partes quirúrgicos e informes de anatomía patológica de un total de 92 pacientes. Se incluyeron aquellos con carcinoma escamoso de cavidad oral T 1-4 pN0-pN+ (pN1-2). Se calculó la sobrevida utilizando el método Kaplan-Meier y se realizó el análisis multivariado.

Resultados: Un Cociente Ganglionar (CG.) mayor a 5% resultó estadísticamente significativo como factor pronóstico de sobrevida [HR 5,22(IC95% 1,86; 14;62) (p 0.002)] y recurrencia [HR 13.33 (IC95% 3.85; 46.16) No se evidenció diferencia pronóstica entre aquellos pacientes con vaciamientos pN0 y pN+ asociado a CG. menor a 5%. Pacientes pN1 y vaciamientos con recuento ganglionar total de 20 (1/20) podrían obtener un similar pronóstico al de pN0.

Conclusiones: Fue posible ratificar al CG. como factor pronóstico y se plantea la posible utilidad del mismo en la indicación del tratamiento adyuvante.

Palabras Clave: Cociente Ganglionar, Carcinoma escamoso de cavidad oral, Vaciamiento cervical

Renovación de las Pensiones Asistenciales No Contributivas en el año 2019

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ABSTRACT

Descriptive Study. Analysis and comparison of the population´s characteristics who concur to the Legal Medicine Service at the National Hospital A. Posadas, to renovate their welfare pensions through the confection of the Digital Official Medical Certificate, year 2019, and comparing them with the national renovations which happened in 2014.

Keywords: Official Medical Certicate. Welfare Pensions. National Hospital Alejandro Posadas

Introducción

El Sistema Previsional Argentino (SIPA) otorga dos tipos de pensiones. Por un lado, las Pensiones Contributivas comprendidas en el Sistema Integrado Previsional Argentino a cargo de la Administración Nacional de la Seguridad Social (ANSES) regidos por la ley 24241/93 y por el otro, las Pensiones No Contributivas, que si bien son sostenidas con fondos del ANSES se tramitan a través del Ministerio de Desarrollo Social de la Nación (1), las cuales se extinguen con el fallecimiento del titular.

Las pensiones no contributivas representan aproximadamente el 3,34 % de la población general -según datos del ANSES a Marzo del 2018 (2)-, y se dividen en varios grupos:

Pensiones Asistenciales, representan la gran mayoría de las pensiones no contributivas (93,37 %) destinadas a personas con necesidades básicas insatisfechas, actualmente son renovables y están comprendidas en tres categorías **1)** Por Edad Avanzada, mayores de 70 años (Ley 13478 reglamentada por el Decreto 582/03) y que representan el 0,2 % de las pensiones asistenciales; **2)** Madres con siete o más hijo (Ley 23746 Decreto 2360/90) y representan el 22,4 % y, **3)** Pensión por Invalidez, la más frecuente y representan el 77,4 % restante; para obtener éste beneficio, la incapacidad debe ser igual o mayor al 76% de la Total Obrera (T.O.), según la Ley 18910 Decreto 432/97.

Pensiones Graciables, son aquellas otorga-das por Diputados y Senadores Nacionales (Ley 13337) cuya duración es de 10 años, con posibilidad de prórroga por 10 años más, que requiere una incapacidad laboral igual o mayor al 66 % de la T.O. y, representan el 4,57 % de las pensiones No Contributivas.

Otras, como las Pensiones otorgadas a Ex - Combatientes de Malvinas o por leyes especiales, representan el 2 % de las Pensiones No Contributivas.

El proceso de otorgamiento de las pensiones no contributivas comienza con el Certificado Médico Oficial (CMO), documento donde se consigna el porcentaje de incapacidad que presenta el individuo basado en el Baremo Previsional Nacional del Decreto 478/98 que reglamenta la Ley 24241 o por el Baremo de las Aseguradoras de Riesgo de Trabajo (ART) de incapacidades laborales, Decreto 656/96.

Una vez emitido el documento, la adjudicación o no de la pensión, dependerá de varios factores; de una evaluación final por una junta médica y del estudio socio-ambiental llevado a cabo por personal del Ministerio de Desarrollo Social de la Nación, que tendrá en cuenta que la persona no posea bienes, ingresos o recursos que permitan su subsistencia y que tampoco tenga parientes obligados legalmente a proporcionarles alimentos o que, teniéndolos, se encuentren impedidos para hacerlo.

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El Certificado Médico Oficial (CMO) es el documento médico inicial para tramitar las pensiones no contributivas. Es un formulario oficial que debe ser confeccionado o completado por médicos matriculados en cualquier especialidad, no solo por médicos legistas.

En este documento se hace constar todas las enfermedades y las secuelas que presenta el solicitante y el tipo de incapacidad que le producen: total o parcial; temporaria o permanente, y el valor expresado en un porcentaje.

Para ser un beneficiario de la Pensión Graciable, la incapacidad debe ser total; que, en términos de porcentaje, éste debe ser más igual o mayor al 66% de la T.O.; y en las Pensiones Asistenciales por Invalidez, el porcentaje deberá ser igual o mayor al 76 %, también deberán ser permanentes, es decir que no pueda revertirse con un tratamiento adecuado o, con el paso del tiempo.

Para determinar el porcentaje, como se dijo previamente, se utiliza el Baremo Previsional Nacional decreto 478/98 de la ley 24241, normas para la evaluación, calificación y cuantificación del grado de invalidez de los trabajadores (3)(4).

Cabe señalar que, las secuelas que se evalúan para determinar el porcentaje de incapacidad deben ser lesiones objetivables a partir de lesiones anatómicas evidentes, trastornos funcionales medibles por diferentes métodos o alteraciones psíquicas evaluables. Aquellas patologías que sólo pueden manifestarse por síntomas, no son medicamente determinables en término de incapacidad.

Ejemplo de esto es el dolor de tipo crónico, uno de los síntomas más frecuentes en la consulta médica (5). Todas las mediciones que puedan hacerse del dolor, poseen un componente subjetivo muy importante, lo cual hace difícil cuantificarlo objetivamente; por lo cual, aunque genere a veces en la persona una severa discapacidad para realizar sus tareas habituales, agrega un porcentaje bajo de incapacidad y no se lo encuentra como patología inculpable en los baremos de uso habitual.

Al Servicio de Medicina Legal del Hospital Nacional A. Posadas, concurren aquellas personas que solicitan el beneficio de una Pensión Asistencial o quienes deben renovarla. Una de las actividades del Servicio, es realizar la valoración del porcentaje de incapacidad en estos pacientes, basados en las secuelas de sus procesos mórbidos certificados por sus médicos tratantes con los estudios actualizados y de esta manera, poder completar los formularios del CMO.

A partir del mes de Febrero de 2019, el Ministerio de Modernización de la Nación junto con la Agencia Nacional de Discapacidad (ANDIS), decidieron mediante la Resolución Administrativa 39/19, proceder a digitalizar el trámite administrativo.

Todas aquellas personas pensionadas a nivel nacional, recibieron una notificación mediante la emisión de una Carta Documento intimándolos a concurrir a un Hospital Público autorizado para completar el formulario del CMO digital, a través de internet. El trámite es realizado por un médico capacitado, además, en informática y con los recursos técnicos necesarios para el mismo.

Las dificultades con las que ocasionalmente nos encontramos fueron: la falta de una internet adecuada, problemas de conexión con el sitio oficial asignado, pero el hecho más importante que complicó la tarea de la renovación se debió a la falta en recursos con esa tecnología (teléfono de línea o celular; computadora personal, acceso a internet) en nuestra población asistida o evaluada requisitos obligatorios para poder completar el propio certificado. Por tal motivo en el mes de Enero del corriente año, fueron habilitados, nuevamente, aquellos centros periféricos llamados Centro de Atención Primaria de la salud (CAPs) zonales, en donde se confecciona el CMO en modo papel, quedando entonces, en la actualidad ambas formatos vigentes (6).

OBJETIVO

Realizar un análisis de los CMO digitales completados en este servicio durante el año 2019, en donde evaluamos y comparamos las características de las pensiones que se renovaron con las estadísticas realizadas en el año 2014; año en el que se realizó la revisión anterior de las pensiones asistenciales a nivel nacional.

Además, comparar los CMO emitidos por este Servicio por primera vez en los años arriba citados como así también, definir las enfermedades más frecuentes en nuestra población.

Método

El estudio realizado es de tipo observacional, descriptivo y retrospectivo, cuya población evaluada fueron aquellos pacientes que concurrieron al Servicio de Medicina Legal del Hospital Nacional Alejandro Posadas en el año 2019 para comenzar el trámite de una pensión asistencial digital, tanto por su renovación o por su primera vez para compararlos con los datos colectados de nuestra propia base de datos, del año 2014.

Las variables utilizadas fueron edad, género y enfermedades determinantes.

Para la comparación de las frecuencias relativas de aquellas enfermedades que más requirieron ser renovadas durante los años 2014 y 2019, hemos utilizado un trabajo previo, realizado en este Servicio, entre los años 2010 y 2015.

Resultados

En el año 2019 de un total de 2650 pacientes citados, se asistieron 2094 pacientes con un 21 % de ausentismo. Emitimos en total 1034 CMO (49,3 % del total de pacientes asistidos); de los cuales 548 fueron hombres y 486 mujeres; el 50.9 % (n=527) correspondió solo a renovaciones (hombres 237 y mujeres 290) y los CMO de primera vez fueron el 49 % restante (n=507), dividido en 310 hombres y 197 mujeres. (Gráficos 1 y 2)

CMO renovados por debajo del 66 % 164 pacientes (15 %).

Enfermedades más frecuentes: Infectológicas 14,4 %, Neurológicas 13,4 % y Reumatológicas 12,9 %.

En el 2014 de un total de 3377 pacientes citados, 2296 fueron asistidos, con un 31,9 % de ausentismo. Se emitieron un total de 896 CMO (39 % de los pacientes asistidos) con 451 hombres y 445 mujeres, de los cuales el 72,5 %

(n=650) correspondió a renovaciones (hombres 314 y mujeres 336) y los CMO de primera vez fueron el 26,5 % restante (n=246), divido en 137 hombres y 109 mujeres. (Gráficos 3 y 4)

CMO renovados por debajo de 66% un solo paciente.

Enfermedades más frecuentes: Neurológicas 19 %, Reumatológicas 14,6 % y Oncológicas 11,2 %.

Gráfico 1: Total de pacientes citados 2650 **(2019)**

- ☐ Asistidos 79%
- ☐ Ausentes 21%
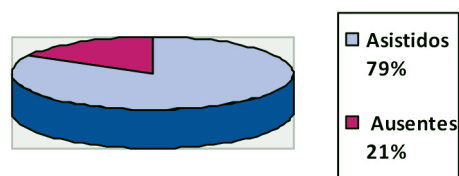
Gráfico 2: Total de CMO completados 1034 **(2019)**

- ☐ Renovaciones 51%
- ☐ 1° vez 49%
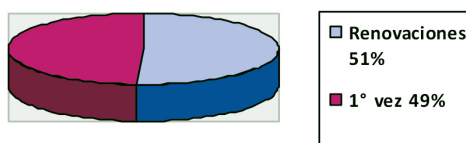
Gráfico 3: Total de pacientes citados 3377 **(2014)**

- ☐ Asistidos 68%
- ☐ Ausentes 32%
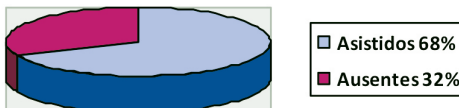
Gráfico 4: CMO completados 896 **(2014)**

- ☐ Renovaciones 73%
- ☐ 1° vez 27%
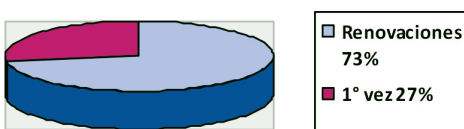
Al comparar el año 2019 con el año 2014, se observa un 21,5 % menos de pacientes citados, pero proporcionalmente concurrieron más pacientes, ya que hubo un 14 % más de pacientes asistidos y un 34 % menos de ausentismo, con un 20 % más de CMO completados, tal como puede observarse en la Tabla 1.

Tabla 1

	2014	2019	Diferencia
Pacientes citados	3377	2650	- 21,5%
Asistidos	2296 (68%)	2094 (79%)	+14%
Ausentes	1078 (32%)	556 (21%)	- 34%
CMO	896 (39%)	1034 (49%)	+ 20%

Dentro de la categoría de Enfermedades Neurológicas, fueron agrupados aquellos pacientes con accidentes cerebro vascular de toda etiología, oligofrenias, síndrome de Down, epilepsia, encefalopatías, hidrocefalias, enfermedad de Parkinson y tumores benignos del Sistema Nervioso Central. En la categoría de Enfermedades Reumatológicas están comprendidas, principalmente, la Artritis Reumatoidea y el Lupus Eritematoso Sistémico. En la categoría Oncológicos, los más frecuentes fueron los canceres de mama, colon, pulmón, útero y próstata. Por último, en la categoría de Enfermedades Infecciosas, se correspondieron en un 93 % a pacientes con serología positiva para VIH; de los cuales el 37 % eran estadios tempranos de enfermedad, los cuales no alcanzan el porcentaje de incapacidad requerido por la ley para el beneficio de la pensión (76 %). (Gráfico 14)

Los ciudadanos extranjeros, según la propia ley en Argentina tienen también derecho al beneficio de una pensión asistencial, siempre y cuando demuestren 20 años o más de radicación en el país, a partir de la fecha que consta en el Documento Único de Identidad. En el año 2019, hubo solo 12 ciudadanos de extranjeros (1,16 %) quienes renovaron su pensión. No hemos registrado el dato de ciudadanos extranjeros en el año 2014.

Cuando observamos la distribución de pacientes, según Edad y Género, nos permite apreciar una gráfica diferente según se evalúen los CMO realizados por primera vez o por la renovación. Según la edad, en el año 2019 al comparar los CMO de primera vez con los CMO renovados, se permite apreciar claramente que, los niños menores de 10 años, no han concurrido a renovar las pensiones asistenciales, lo cual nos hace pensar que o estos niños no fueron intimados a realizar el trámite digital de las pensiones o, no concurrieron por otras causales que no son pasibles de evaluación por este Servicio. (Gráficos 5 y 6)

En el año 2014, si bien hubo una mayor renovación de las pensiones en los niños, no fue de la misma magnitud que aquellos niños que acudieron a realizarlas por primera vez. (Gráficos 7 y 8)
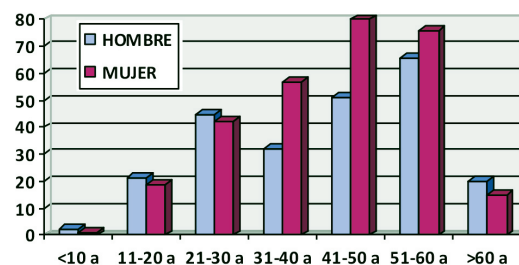
Gráfico 6: Distribución CMO primera vez por Edad y Género (Año 2019).
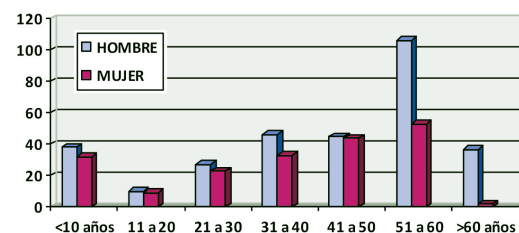
Gráfico 7: Distribución de CMO de Primera Vez, por Edad y Género (Año 2014).
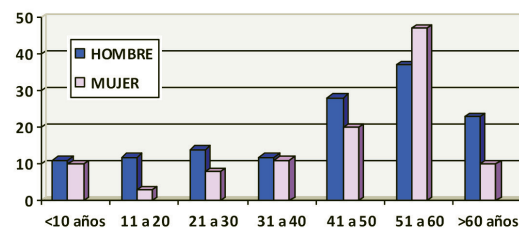
Gráfico 8: Distribución de CMO Renovados, por Edad y Género (Año 2014).
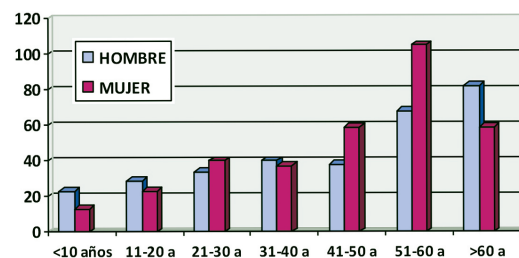
Cuando evaluamos la diferencia de Género en los CMO renovados observamos que, en el 2014 como en el año 2019, un mayor porcentaje de ellos fueron mujeres; cuando habitualmente en los CMO completados por primera vez, son mayoritariamente hombres, quienes lo solicitan. (Gráficos 5 y 8)

Gráfico 5: Distribución de CMO Renovados, por Edad y Género (Año 2019).

Al comparar la variable Edad en los CMO totales que fueron emitidos en ambos años, podemos describir y observar que en el año 2019 hubo un 20 a 30 % más de adultos jóvenes (entre los 30 y 50 años) y que, en el año 2014 hubo una diferencia muy significativa en adultos mayores de 60 años, alcanzándose el 63 %. (Gráfico 9).

Gráfico 9: Distribución de CMO totales, según la Edad, entre 2014 y 2019.
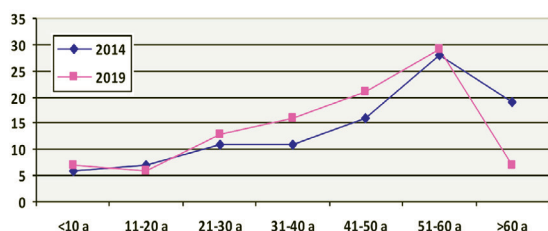
Gráfico 10: Comparación según Edad de los CMO de primera vez, entre los años 2014 y 2019.
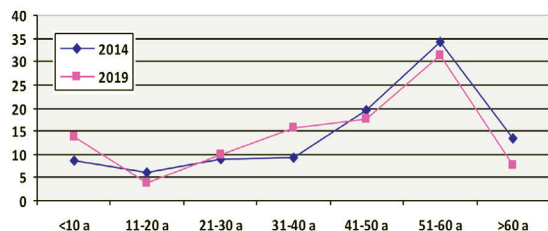
Gráfico 11: Comparación según Edad, en los CMO Renovados entre los años 2014 y 2019.
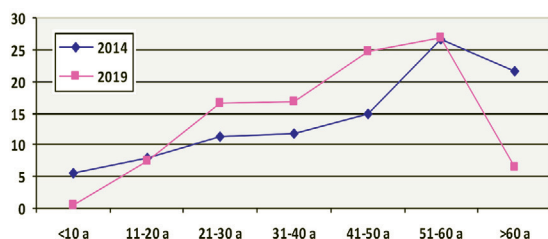
Las diferencias entre estos años, se pueden apreciar mejor, cuando separáramos los gráficos entre las renovaciones y los CMO de primera vez.

Con respecto a los CMO realizados por primera vez (Gráfico 10), el gráfico permite observar a un trazado con diferencias no muy significativas; salvo, entre los 30 y 40 años, donde se aprecia un incremento a favor del año 2019 (16 %) vs. el año 2014 (9 %), donde la amplitud representa un 40 % más de pacientes.

En el Gráfico 11, se representaron aquellos CMO que fueron renovados en nuestro Servicio de Medicina Legal, se pueden observar algunas diferencias muy significativas entre estos años, a saber: 1. En 2019: a) No renovación de los certificados en niños menores de 10 años; b) Mayor número de renovaciones en adultos jóvenes, entre los 30 y 50 años y, 2. En 2014, una mayor realización de CMO en pacientes mayores de 60 años de edad.

En el Gráfico 12, se aprecia que en el año 2019 a un mayor números de renovaciones en el grupo de pacientes definidos como adultos jóvenes; y que, en los CMO emitidos por primera vez, se asistieron más niños y adultos mayores (entre 50-60 años).

Gráfico 12: Comparación por Edad de CMO emitidos por primera vez con las renovaciones en el año 2019.
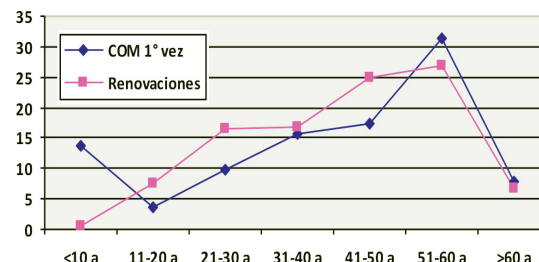
En el Gráfico 13 se observa que, tanto en las renovaciones como en aquellos certificados realizados por primera vez, en el año 2014, la población fue aún más añosa que en el año 2019, siendo ello más relevante, en las renovaciones en los sujetos mayores de 60 años.

Gráfico 13: Comparación por Edad de CMO emitidos por primera vez con las renovaciones en el año 2014.
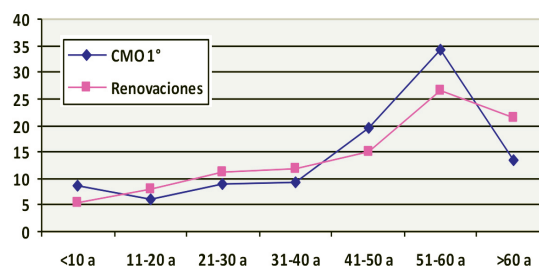
Con la implementación y obligatoriedad de confeccionar los CMO Digitales, los pacientes que ya recibían el beneficio de la Pensión Asistencial, a todos los citó a nuevamente evaluados para actualizar su condición física y/o psíquica, según la resolución emitida por el Ministerio de Modernización y el propio ANDIS. Es así que en nuestra propia población en estudio, hemos encontrado que en un 25,5 % de pacientes su evaluación se hallaba por debajo del 66 % de incapacidad, es decir con un porcentaje por debajo del requerido por ley para el beneficio de la pensión. Desconocemos el o los motivos por el cual estos pacientes fueron pensionados; ya que los CMO para estas pensiones no fueron confeccionados en este mismo Servicio, sino que quizás pudieron deberse a otras causas. Otra característica a destacar, es que la mayoría de estos fueron pacientes jóvenes, entre los 20 y 50 años de edad.

Como más arriba se expresó, las enfermedades más frecuentes fueron divididas en categorías, encontrándose un franco aumento de pacientes con patologías infecciosas, por encima de la distribución habitual para nuestro Servicio, en el año 2019.

En el trabajo previo, realizado entre los años 2010-2015, las categorías más frecuentes fueron: las Enfermedades Neurológicas, las Enfermedades Cardiovasculares y, en tercer lugar, las Enfermedades Reumatológicas. Las Enfermedades Oncológicas ocuparon el cuarto lugar y las Enfermedades Infecciosas, el séptimo (Tabla 2.)

Tabla 2. Evaluación de las patologías agrupadas, según las frecuencia alcanzadas.

Patologías	2010-2015	2014	2019
Neurológicas	594 (20,5%)	124 (19%) (1)	71 (13,4%) (2)
Cardiovasculares	343 (11,8%)	48 (7,4%)	19 (3,6%)
Reumatológicas	339 (11,7%)	95 (14,6%) (2)	68 (12,9%) (3)
Oncológicas	332 (11,5%)	73 (11,2%) (3)	26 (4,9%)
Diabetes	288 (10%)	65 (10%)	53 (10%)
Psiquiátricas	170 (5,8%)	29 (4,4%)	24 (4,5%)
Infecciosas	103 (3,5%	17 (2,6%)	76 (14,4%) (1)

En este nuevo trabajo, según comprobamos en el año 2014, las Enfermedades Neurológicas se mantuvieron como las más frecuentes, pero las enfermedades cardiovasculares pasaron a ocupar el quinto lugar, dejando a las Enfermedades Reumatológicas en un segundo y a las Enfermedades Oncológicas al tercer lugar; y las Enfermedades Infecciosas, permanecieron en el séptimo lugar.

En el año 2019, llamativamente las Enfermedades Infecciosas fueron las más frecuentes, seguidas por las Enfermedades Neurológicas y las Enfermedades Reumatológicas (Gráfico 14).

Gráfico 14: Enfermedades más frecuentes, comparación entre 2010-2015, 2014 y 2019 (N: neurológicas; CV: cardiovasculares; R: reumatológicas; O: oncológicas; I: infecciosas)
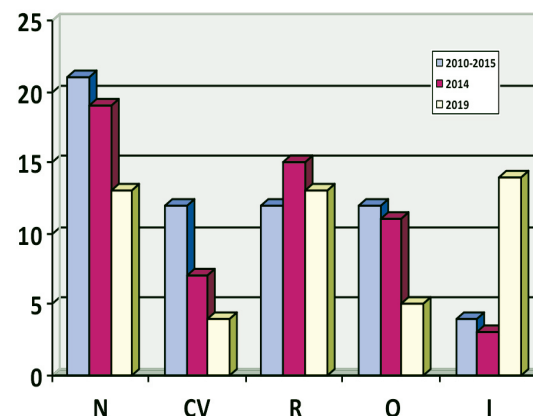
Conclusiones

Del estudio descriptivo y retrospectivo realizado sobre el total de los CMO completados por los médicos integrantes del Servicio de Medicina Legal del Hospital Nacional A. Posadas debido a la obligatoriedad de la digitalización del trámite impuesta a partir del año 2019, hemos realizado una comparación en los datos estadísticos de esta renovación de las pensiones con la renovación que fuera realizada en el año 2014, desde aquellos registros obtenidos de nuestra propia base de datos.

En el año 2019 observamos: una concurrencia mayor a este Servicio de Medicina Legal en el número de pacientes que fueron

asistidos; con un menor índice o tasa de ausentismo al compararlo con el año 2014, por lo que entonces fueron completados un mayor número de CMO Digitales. Además, hemos observado que según el Género tanto en el año 2014 como en el año 2019, fueron las mujeres quienes renovaron más CMO que los hombres.

En las renovaciones realizadas en el año 2019, se observó una disminución de las solicitudes para ello, en niños menores de 10 años, desconociéndose su causal; aunque podría deberse, a que los niños no fueron intimados por una carta documento a renovarla como el resto de los pensionados. Si, se pudo observar en aquellos casos que concurrieron a formular la renovación, que era una población más joven comparándose con las del año 2014; esto puede deberse, quizás, a una mayor frecuencia de jóvenes portadores de VIH y de adolescentes portadores de Enfermedad Celíaca, quienes han concurrido por su renovación en el año 2019.

Por el contrario, en el año 2014 la población que concurriere a renovar el CMO fue más añosa, y con mayor frecuencia en adultos mayores de 60 años de edad.

En el año 2019, del total de CMO Digitales confeccionados en este Servicio de Medicina Legal (n=1034), las renovaciones alcanzaron a 527 pacientes. De éstas, un 25 % (n=137), obtuvieron como resultante un porcentaje igual o inferior al 66 % de incapacidad de la T.O.; motivo por el cual, esto significa que los pacientes estaban por debajo del límite inferior requerido según Ley para obtener o continuar con el beneficio previsional. No es posible conocer o determinar el porqué estaba recibiendo ese beneficio esta población ya que estas no fueron previamente otorgadas por este Servicio. De ese 25 % entonces, la mayoría de los CMO Digitales fue destinado a pacientes jóvenes en donde, el 70 % en este grupo, se hallaba comprendido entre los 20 y 50 años de edad, destacándose los casos de pacientes con serología positiva por VIH en estadios tempranos de la enfermedad y pacientes con Enfermedad Célica, asintomáticos, ambos grupos pacientes solo portadores de anticuerpos.

Es importante decir, que este Servicio de Medicina Legal, no posee la potestad ni la habilidad para decidir en el acto administrativo de otorgar una Pensión de tipo Asistencial.

En el año 2014, según nuestra propia fuente, el dato que llama la atención, es haber confeccionado, en un solo paciente, la renovación de un CMO por debajo del 66 % de la T.O., luego de la evaluación médica actualizada.

Con respecto a aquellas enfermedades que frecuentemente fueron una causal mayoritaria y determinante de una incapacidad, hemos observado algunas diferencias en la comparación con aquellas enfermedades descriptas en el trabajo previamente realizado por este Servicio de Medicina Legal, durante los años 2010-2015.(7)

Es dable poder aclarar, que durante los primeros meses de funcionamiento de este sistema digital, el Hospital Nacional A. Posadas fue el único referente público donde se lo realizaba, con lo cual la población vista durante el año 2019, no fue solo la población que se asiste habitualmente en este hospital, sino que además, concurrieron pacientes provenientes de otras partes de la provincia y de la Ciudad Autónoma de Buenos Aires (CABA). Este hecho puede haber generado una tendencia en algunas de las diferencias encontradas, como ejemplo, en el Gráfico 14 se puede observar y recalcar la importante concurrencia de pacientes con serología de VIH positiva y Enfermedad Celíaca que concurrieron a renovar en 2019, y no así en años previos.

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RESUMEN

Estudio Descriptivo. Análisis y comparación de las características de la población que concurrió al Servicio de Medicina Legal del Hospital Nacional A. Posadas, a renovar sus pensiones asistenciales a través de la confección del Certificado Médico Oficial Digital, en el año 2019, y comparándolas con las renovaciones a nivel nacional ocurridas en el año 2014

Palabras claves. Certificado Médico Oficial. Pensiones Asistenciales. Hospital Nacional A. Posadas

Homeopathy and the Law of Infinitesimals

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ABSTRACT

This paper explains the second elementary law of homeopathy, the Law of the Infinitesimals, on the basis of a kinetic model. When a remedy occurs in the human cell of a healthy person and forms a reaction product (simillimum) that induces the finest medical symptoms of an ill person, then remedies entering the cell of the ill person will form identical simillimum molecules and re-establish the initial equilibrium of the healthy state and cure the ill person. However, this will also induce a molecular crowding in the cells of the ill person. For kinetic reasons this will aggravate the re-establishment of the initial equilibrium and consequently worsen or even interrupt the medical treatment. At a low remedy concentration, the molecular crowding becomes negligible while the formation of the simillimum and the re-establishment of the initial equilibrium will take place continuously and cure the person who is ill.
Kay words: Law of Infinitesimals, Law of Similars, homeopathy, simillimum, biochemical equilibrium, molecular crowding.

Introduction

Homeopathy is based on the idea of 'let like be cured by like' that is known as the "Law of Similars", which states that an illness can be cured by an ingredient that produces similar symptoms in healthy people. This is the primary law of homeopathy and, dates back to Hippocrates (460-377 BC), and even back to the time of mankind's early development. It was accepted very early on by the ancients and perhaps even before those times. This phenomenon, which has been part of human history from the very beginning, was positively experienced in a unoptimized mode in various periods of our development. After Samuel Hahnemann[1] in the late 18th century described the routine of homeopathy it continues in an optimized way to the present day. Since then, homeopathy has remained popular and in its present form it has been used all over the world for more than 200 years. Homeopathy is often disparaged because of it use of very dilute medicines. However, there is a significant body of clinical research, including randomized clinical trials and meta-analyses of such trials, which suggests that homeopathy has actions that are not simply placebo effects. There are several schools of homeopathy. Despite an extended history of scientific disagreement, homeopathy has remained strong and is now common in many parts of the world. There is a significant body of scientific evidence with positive results. Certainly, homeopathy is an anomaly that is worthy of further investigation, Fisher[2].

Today, there is a great deal of research focused on the scientific validation of homeopathy and on the technical issues involved in comparing this discipline with pharmacology. In particular, the physicochemical nature of homeopathic remedies obtained by the successive dilution method is often discussed and has been investigated many times.

Investigators have planned many explanations linked to the mechanism of homeopathy and its impact on treatment, depending on the concentration of low doses of the remedy in the human body. Between them, clarification of the "water memory" was very often the topic of a publication[3-11]. In addition to these valuations, several models have been published: quantum mechanical entanglement[12,13], quartz crystals and the structural concept of glass[14], electromagnetic activities[15], biological signaling[16], nonlinear dynamics of complex systems[17-19], model of stressor effect and hormesis[20,21], biopsychosocial model[22], and the thermodynamic model[23-25]. Recently, Bell and Koithan published an extensive article covering an overview of the results of key publications[26], and Vithoulkas published a book[27] covering scientific explanations and the practical application of homeopathy.

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Despite the desirable success of homeopathy, it is important to emphasize that similar homeopathy treatments were used before Hahnemann. These procedures worked less efficiently, but were based on the same principles of the "Law of Similars". Hahnemann has described in detail the work of previous authors and systematically examined the effect of remedies by testing them on healthy people[28]. On the other hand, when Hahnemann started to investigate the efficacy of the remedy in homeopathy treatment, he gradually reduced the concentration of the remedy and achieved an increase in the healing effects. The result of Hahnemann's optimization was an unusual finding that a lower remedy concentration has a more effective result on the course of medical treatment. This finding is linked to the "Law of Infinitesimals", which states that the lower the concentration of the remedy, the stronger the therapeutic effect it creates, which is the opposite of the dose-and-response effect considered by pharmacologists. This law is the second most important principle of homeopathy.

The role of the small remedy concentration in most exposed models

When Hahnemann's optimizations reached very low therapeutic concentrations, the researchers believed that they have pure water as a drug-free solvent, and suggested different models that would clarify the treatment process under these conditions.

During the development of homeopathic remedies, the preparation process involves very strong mechanical mixing (succession) with lactose and serial dilution in an ethanol-water solution, usually in glass containers. The concrete significance of this process is to break the physical bonds of the molecular aggregates and to chemically activate them. At high dilutions, these molecules will not be able to re-associate, but will physically associate with the sugar-lactose molecules, which were in large excess. The sugar molecules will be digested in the human body and the chemically activated remedy molecules will remain suitable for targeted

biochemical reactions in the healing process, i.e., the formation of simillimum and re-establishing the initial equilibrium associated with a healthy state.

When Hahnemann's optimizations reached very low therapeutic concentrations, the researchers believed that they had pure water as a remedy-free solvent, and suggested different models that would clarify the medical treatment under these conditions.

They suggested curing in the absence of healing substances in the frame of the model "memory effect of water", which was very popular in the 1980s[3-11]. In some cases, homeopaths were convinced that, after successive dilution, the solution/water no longer contained the active compound-remedy molecules; however, the effects were still observed. Based on such examples, the "memory effect of water" was proposed, according to which water "remembers" the properties of the substance originally contained and retains this healing effect of the solution even when it supposedly no longer contained the active substance. Recent studies that showed the substance molecules in extremely diluted medical preparations[29,30] have excluded this model[4].

One of the models addressed today by homeopaths concerns the use of the concept of the "Vital Force", as Hahnemann called it. According to this model, at very high dilutions combined with succession, we can no longer speak of "substances" in solution, but only about "fields of forces". In the same way we cannot talk about the organism that the remedy acts upon on a biochemical basis, but only about the energy part of the organism, i.e., the "Vital Force". So, we have an interaction of forces, not biochemical agitations. The "fields of forces" (electromagnetic wave interactions, hn) is an extensive property not dependent on the mass of the remedy. On the other hand, the amplitude is an intensive property and is dependent on the mass. So, a zero remedy concentration must exhibit zero amplitude and cannot upgrade anything concerning common electromagnetic effects: resonance, harmony, reinforcement and interference and cannot take part in the interaction of the "fields of

forces" nor in the energy medicine[27]. On the basis of the above-mentioned facts, it is to be expected that homeopaths always deal with a tiny amount of substance.

The origin of the Law of Infinitesimals

Here, we will proceed from the thesis that if it is possible to establish a medicinal fact in which the success of a therapeutic treatment is aggravated by increasing the content of added medicine (remedy), then the most probable explanation for this kind of treatment (in today's understanding of the matter) is that this phenomenon is related to the kinetics of the biochemical reactions in the human cell.

The processes involving homeopathy occur at the molecular level, compartmentalized in a human cell where equilibrium governs the biochemical status of the cell in an ill person. In the human body, biochemical reactions are in human cells where a vast number of highly sensitive, finely tuned and arranged biochemical reactions are about at any one time in a single cell.

To begin the curing process, the remedies must flow into the cells of the ill person. After increasing the initial remedy concentration at the entrance to the cell, the remedy molecules will use passive diffusion and cross the cell membrane in the direction of the concentration gradient. The remedy in a healthy person's cells produces the reaction product, simillimum, which will produce the symptom complex most nearly approaching that of the disease in question. This molecule is already present in the ill person's cells and causes the disease in question. When remedies go in the cell of the ill person, they will form identical reaction product, i.e., simillimum molecules, as in the cell of a healthy person and increase the concentration of simillimum molecules in the cell of the ill person. Subsequently, when simillimum enters the equilibrium of the ill person's cells, it will re-establish the initial equilibrium of the healthy state and heal the ill person in agreement with the "Law of Similars" ref. 25 (see Supplement). The remedy as a molecule by itself is not the critical issue, but the reaction pattern developed by every single remedy molecule that enters the cell of the ill person and forms the reaction products during forming simillimum molecules, which enter and shift the chemical equilibrium and cure the ill person. However, the healing is associated with molecular crowding, due to the formation of reaction products which accompany the simillimum formation, what aggravates the re-establishing the initial equilibrium and consequently curing due to kinetic reasons, as will be considered below. The chemical reactions in human cells are in the steady state under the appropriate conditions and given sufficient time, individual biochemical reactions carried out in a test tube will eventually reach equilibrium. Within cells, however, many reactions are linked in pathways in which a product of one reaction serves as a reactant in another, or is pumped out of the cell. In this more complex situation, when the rate of formation of a substance (reaction products) is equal to the rate of its consumption, the concentration of the substance remains constant, and the system of linked reactions for producing and consuming that substance is said to be in a steady state. One consequence of such linked reactions is that they prevent the accumulation of excess intermediates, protecting cells from the harmful effects of intermediates that have the potential to be toxic at high concentrations[31,32].

The remedies that enter the cells of an ill person are strongminded to synthesize the reaction product, i.e., simillimum, after an identical procedure occurring in the cells of healthy people. So, there cannot be any linked reactions that might prevent the accumulation of excess intermediates formed during biochemical reactions in the cells of the ill person. Therefore, the remedy's influx will cause biochemical reaction products and induce molecular over-crowding. Due to that the kinetics of the biochemical reactions taking place after the entry of the remedy into the cells of an ill person will be heightened.

From the kinetic point of view[33], in a human cell, molecules move and collide and their bonds vibrate and rotate. When molecules collide, there is the possibility of a reaction taking

place, but only if the colliding molecules have enough energy and are aligned correctly. A collision in a liquid solution is regulated by diffusion instead of direct collisions, so the diffusion takes control of the collision frequency. A direct collision between the two targeting molecules no longer dominates since for any given molecule it has to collide with a lot of cytoplasm molecules, and other molecules before finding the proper molecule to react with. So, instead of a direct collision between targeting molecules, we must use the diffusion-controlled collision frequency[34]. A higher remedy inflow increases the concentration of the reaction products and consequently the molecular over-crowding occurs. Molecular crowding effects strongly decrease the intracellular diffusion and reaction rates. The cytoplasm network leads to a reduction in the mobility of molecules[35]. Namely, prior to a successful collision there will be a series of bumping (ineffective collisions) with other molecules, with the concentration increasing with every arrival of a remedy molecule. Each remedy molecule makes a number of reaction-product molecules, which can be treated as obstacles to effective reaction collisions and will hinder the reaction kinetics and aggravate the curing since the re-establishment of the initial equilibrium of the healthy will be strongly delayed.

To prevent such an over-crowding and enhance the healing process we must decrease the concentration of the remedy. To consider the "Law of Infinitesimals" at very low concentrations we must take into consideration two key statements:

i) it was shown that medical remedies that are diluted and hand-successed to 30 or even 200 C the potencies, retain source materials[30] and that the remedy constituent species in the solution declines asymptotically[31] during serial dilution, mainly due to the fact that the air–liquid phase boundary induced during succussion behaves as an impurity snare, keeping back the diluent molecular constituent species, so that we can expect a contamination of the solution with the remedy, whatever dilution protocol is planned and, secondly, ii) in the theory every single molecular constituent species (simillim-

um) being in excess while re-establishing the equilibrium is a necessary and sufficient condition to trigger the equilibrium restoration.

Taking into consideration the above facts when using extreme dilution, we are convinced that a typical homeopathic medicine is not a guarantee that therein (in the potentiated and diluted solution) are not any of the remedy molecules. A small number of remedy molecules might go through the process of "infinite dilution" and form a small number of simillimum molecules and re-establish the equilibrium and enable the healing of a patient, as is observed in clinical homeopathy.

In that case the status of the considered medicinal solutions can be taken as "infinity diluted" and as that will determine the lowest concentration level in the span of the concentration range covered by the "Law of Infinitesimals", i.e., "infinitesimal dose", that is the most characteristic feature of modern homeopathy. The increase of the curing efficiency with the strong decrease of the remedy concentration is to be expected and is also confirmed in clinical homeopathy.

On the other hand, as pointed out above, a relative high remedy concentration might even block the process of healing. In homeopathy we have two concentration extremes, i.e., a high and a low concentration, and in-between there remains a continuous increase in the curing efficiency.

In pharmacy the drug is a conventional solution of the active ingredient that heals and is consistent with dissolved and diluted bulk-form chemical medicine in a true solution that could act alone pharmacologically with a linear dose-response relation, which is diametrically different to homeopathy.

Conclusions

Today, there is a great deal of research focused on the scientific confirmation of homeopathy on the technical issues involved in comparing this discipline with pharmacology. On the basis of revising some key models we can conclude that homeopaths always deal with a tiny amount of substances in a solution. The

formation of simillimum and re-establishing the initial equilibrium in an ill person is essential to the healing process in homeopathy and depends on the remedy concentration and the kinetics of the chemical reactions in the human cells. Every remedy molecule that enters the cell of an ill person is encoded to synthesizes the simillimum and re-establishes the initial equilibrium; however, it will also induce molecular crowding, which will aggravate the healing of the patient. High remedy concentrations will strong aggravate or even block the existing biochemical procedures. To prevent such an overcrowding and enhance the healing process we must strongly decrease the remedy concentration. The exposed model governs the considered law from higher remedy concentrations inducing molecular crowding and aggravation of the medical treatment to a low and/or very low remedy concentrations when the molecular crowding is negligible and the medical treatment is not disturbed.

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Homeopathy and the Law of Infinitesimals

Segregated ligation of the superior thyroid artery minimize post-thyroidectomy injury to the external branch of superior laryngeal nerve, a novel practical approach

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ABSTRACT

External division of the superior laryngeal nerve supplies the crico-thyroid muscle to excite length and thickness of the vocal fold. Thus, increasing voice tone. The vicinity with the superior thyroid vessels sets the external branch of the superior laryngeal nerve in danger every time the superior end of the thyroid is dissected. Thus, the aim of present study is to assess the rate and complication of external branch of the superior laryngeal nerve injury post- thyroidectomy when segregated ligation of superior thyroid vessels closes to thyroid capsule without prior nerve identification and without nerve stimulator or intraoperative neuro-monitoring. The presented study is a prospective, non- randomized clinical study included 1450 patients who underwent thyroidectomy which either (total thyroidectomies, near total thyroidectomies or lobectomy and isthmectomy) in the Department of Surgery/AL-Diawania Teaching Hospital in Diawania City, Iraq, between January 2000 and February 2018. All patients underwent thyroidectomy through segregated ligation of superior thyroid artery very closely to thyroid capsule without prior nerve identification and without nerve stimulator or intraoperative neuromonitoring. Postoperative indirect laryngoscopy vocal cord examination with long term follow up through physical examination and clinical history to evaluate nerve integrity. In present study, the total cases with EBSLN injury were **38 (2.6%),** in which the transient EBSLN injury occurred in **28 (1.9%)** of patients and permanent injury occurred in **10 (0.7%)** of patients and majority of cases with EBSLN injury were occur in patients with large size goiter **29 (2%)** more than small size goiter **9 (0.6%)** And these differences were statistically significant differences, (P<0.005). In addition to, the majority of cases with EBSLN injury were occur in male {25(1.7%)} more than female patients {13(0.9%)} And these differences were statistically significant differences, (P<0.005). Segregated ligation of superior thyroid artery is a safe technical option, cost effective, time preserved and need surgical skills to minimized risk of injury to the external laryngeal nerve.

Keyword: -Thyroidectomy, Segregated ligation of superior thyroid artery, external laryngeal nerve injuries.

INTRODUCTION

The utmost common problems conversed with patients who experience thyroid surgery are hemorrhage, recurrent or external laryngeal nerve paralysis, hypocalcemia and hypoparathyroidism. However, injury of the external branch of superior laryngeal nerve (EBSLN) may happen through the ligation of the superior thyroid artery in about 58% of patients, and its revealing post operatively is hampered by the variable and restrained signs and alterations on post-operative laryngoscopy (1). EBSLN damage lead to prarlysis of the crico-thyroid muscle, which results in change essential occurrence of the voice, a worsening in voice presentation in making high-frequency sounds, and decrease vocal projection. This can be mainly important for those using their voice workwise, for example lawyers, teachers, are considerably at risk by the subtle alterations associated to its injury instead, the insight of an abnormal voice change the quality of life and decrease the overall health through many methods and patients cannt shout. From that viewpoint, EBSLN injury considers a threat to handicap all patients experiencing thyroid surgery. EBSLN damage can be tough to recognize intra-operatively and is hard to notice through post-operative laryngoscopy (2). The superior laryngeal nerve is one a branch of the vagus nerve (3). The superior laryngeal

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nerve (SLN) divides into an internal and external branch. The EBSLN run down dorsal to the carotid sheath, and then cross medially, spreading to the larynx. In its passageway, the EBSLN is usually positioned dorsal to the superior thyroid artery and superficial to the inferior pharyngeal constrictor muscle as it run down and crossing medially to innervate the crico-thyroid muscle on the anterolateral part of the lower portion of the cricoid cartilage of the larynx to endorse strength of the vocal fold, thus amassed voice pitch. The close correlation with the superior thyroid vessels (STV) puts the EBSLN in danger each time the superior end of the thyroid is dissected (2,3). Afterward the EBSLN journeys down the lateral surface of the larynx on the lower pharyngeal constrictor muscle, the EBSLN typically splitting into two divisions at the level of the cricoid, entering independently at the par's recta and pars obliqua of the crico-thyroid muscle bellies. The danger of damage to the nerve by transection, traction, entrapment, thermal harm or interrupted blood supply. The possibilities of surgical harm are also amplified by size and weight of the specimen, and little neck length. Cricothyroid muscle electroneuromyography (EMG) is the most exact tool to identify unusual EBSLN conductivity, but it is officially hard and not appropriate in routine preparation. The EBSLN is often about 0.8 mm wide, and its total length varies between 8 and 8.9 cm(4). The most largely known surgical cataloging of the EBSLN was planned in 1992 by Cernea *et al*. (5). In 1998, Kierner et al. put out a similar ordering to the Cernea system, using a fourth features of the EBSLN passing dorsally to the upper thyroid pedicle, which was detected in 13% of their dissection studies and was well-thought-out more difficult to visually identify (3). Numerous methods have been designated to reduce the likely risk of damage to the EBSLN during upper thyroid vessels dissection and ligation:

1. Save ligation of the single divisions of the superior thyroid vessels beneath direct vision on the thyroid capsule without visual identification of the nerve (6).

2. Visual identification of the nerve earlier to ligation of the superior thyroid pole vessels [7].

3. The use of intra operative nerve stimulator or neuro-monitoring for mapping and confirmation of the EBSLN identification (2,5,8, 9-11)

However, many surgeons do not apply this principle to the ESLN, and hence we conducted this study to assess the rate of ESLN injury during thyroidectomy when segregation of superior thyroid vessels and then ligated individually in branches very close to thyroid gland capsule without nerve stimulator or intraoperative neuro-monitoring.

Patients and methods

The present study is a prospective, non- randomized clinical study included 1450 patients underwent either (total thyroidectomies, near total thyroidectomies or lobectomy and isthmectomy) in the Surgical Department/AL-Diawaniyah Teaching Hospital in Diawaniyah City, between January 2000 and February 2018. Preoperative workup included full history taking, general and local clinical examination, and routine laboratory investigations in addition to thyroid function tests and serum calcium level, and neck ultrasonography. Fine needle aspiration cytology was performed on selected basis (suspicious lesions). Preoperative normalization of thyroid hormones and serum calcium levels. Exclusion criteria were previous neck irradiation, previous laryngeal or thyroid surgery or any other cervical exploration, vocal cord disorders, preoperative voice changes, and those with thyroid malignancy with extra thyroid extension were excluded from the study. All patients underwent a detailed voice evaluation was typically performed by fibroptic laryngoscopy for sub-clinical vocal cord paralysis, comprising faintness after use, incapability to reach high pitch or alteration in essential talking regularity. Pre-operatively, the sign of EBSLN damage on laryngoscopy were taken as rotation of posterior glottis near side of the

damaged and bowing and displacement of affected vocal cord.

Operative technique was constructed to evade surgical bias. Surgeries were done by training level of the senior surgeon only. In case of patient had a over-all thyroidectomy, all sideways of the organ was well-thought-out as single unit. Thyroid was uncovered after removing of platysma flap superiorly and inferiorly through a low transverse collar incision at the lower neck. The strap muscles were separated to expose the goiter. Subsequent to suturing of middle thyroid vein and entire medial rotation of lobe, superior end planning was begun. Care should be taken and stay near to capsule of organ and dissecting both laterally and at the fusion of upper thyroid pole with upper border of isthmus, the whole pole was encircled and taped. With gentle traction on this tape, additionally enabled by using of a hemostat at lateral edge of the pole. Watchful dissection in avascular crico-thyroid space was then initiated and care was taken to recognize nerve in this space. Capsular dissection, skeletonization and single ligation of superior polar vessels were then done. Keeping crico-thyroid muscles intact was emphasized during dissection. Integrity of strap muscles was restored if muscle cutting was performed. Closed negative suction drain was utilized routinely for every patient in the study.

All patients underwent follow-up for detailed voice evaluation was typically performed by fibroptic laryngoscopy for sub-clinical vocal cord paralysis on the 7th postoperative day. And long term follow-up of patients was carried out through physical examinations clinical history and conducted with each subsequent clinic visit. The transit EBSLN injury mean the voice retain to normal between a period of (3wk-3month).Outpatient and telephone visit follow-up examinations were performed for all patients ranging from 7 days, 1 month, 3month, 6 months, 9month, 1year to 17years after operation for the assessment of complications and the mean of 10 years interval. Therefore, telephone visits were particularly useful in the long-term. Patients with voice changes, suspected nerve injury, and those with hypocalcemia were followed up every month at any time during their postoperative course for the

detection of any improvement and better evaluation of their conditions. The patient were included in presented study, categorized in to the following as show in table (1).

Table (1): - characteristic of patients study preoperatively.

Patients characteristics (total n=1450)	Number/percentage
Sex of patients	
Male	160 (11%)
Female	1290 (89%)
Age of patients	
(range)	25y -70y
Mean ± SD	40±10
Type of operation	
Total thyroidectomy	376 (25.9%)
Near total thyroidectomy	472 (32.6%)
Lobectomy and isthmusectomy	602 (41.5%)
Thyroid disease	
Solitary nodule of multinodular goiter	465 (32%)
Multinodular goiter	900 (62.1%)
Diffused goiter	85 (5.9%)
Size of goiter	
Small goiter	930 (64.1%)
Large goiter	520 (35.9%)
Disease of thyroid	
Benign lesion	1130 (77.9%)
Malignant	320 (22.1%)
Follow up	At 7day,1mth, 3mth, 6mth, 9mth, 1-17year

All patients were included in presented study signed an informed consent form, for operation and well explain the possible complication and this approved by the Ethics Committee of the Medical Research institute.

Statistical analysis: -SPSS version 16 and Microsoft Office Excel 2007, Chi-square test and Fisher exact test all used to study association between any two nominal variables. P-value of less than or equal to 0.05 was considered significant.

Results

in the present study, post-operative data of the studied group are shown in **Table (2)**. The total

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number of patients **1450** underwent thyroidectomy, in which EBSLN injury **38 (2.6%)**,include **transient injury 28 (1.9%),** whereas **permanent injury** occurred in **10 (0.7%)** patients. The EBSLN injury in **22(1.5%)** in total thyroidectomy, **4(0.3%)** occur in near total thyroidectomy whereas **12 (0.8%)** occur in lobectomy and isthmusectomy. EBSLN injury occur **25(1.7%)** in male whereas in female **13(0.9%)**. Majority of EBSLN injury occur in large goiter **29(2%)** whereas in small goiter occur in **9(0.6%)**. in addition to these data EBSLN injury occur in teacher **3(0.2%),** in orator **2(0.1%)** whereas **33(2.3%)** work others job. As shown in table (2).

Table (2):- Postoperative ESLN assessment of studies patients (total number of cases =1450)

ESLN assessment	Number/percentage
EBSLN intact	1412 (97.4%)
EBSLN injury	38 (2.6%)
ESLN injury	
Transient injury	28 (1.9%)
Permanent injury	10 (0.7%)
Type of surgery	
Total thyroidectomy	22 (1.5%)
Near total thyroidectomy	4 (0.3%)
Lobectomy and isthmusectomy	12 (0.8%)
According to sex	
Male	25 (1.7%)
Female	13 (0.9%)
According to size of goiter	
Large goiter	29 (2%)
Small goiter	9 (0.6%)
According to job	
Teacher	3 (0.2%)
Orator	2 (0.1%)
Others	33 (2.3%)

The most common complication post-operative thyroidectomy which caused by EBSLN injury in study groups, loss of high pitch voice **11(0.7%),** Chocking **8(0.6%)** whereas both loss of high pitch voice and Chocking **19(1.3%)**. As shown in table (3).

Table (3):-Post-operative EBSLN injury in the study groups.

Complication of ESLN injury	
Loss of high pitch voice	11 (0.7%)
Chocking	8 (0.6%)
Both loss of high pitch voice and Chocking	19 (1.3%)

Further postoperative complication of thyroidectomy in studies patients **16(1.1%)** tension hematoma, and RLN **18 (1.2%),** in which transient injury of RLN **14(1%)** whereas permanent injury of RLN **3(0.2%)**. Transient hypocalcaemia **49(3.4%),** permanent hypocalcaemia **3(0.2%),** hypothyroidism, thyrotoxic crisis **3(0.2%)** whereas wound infection and stick granuloma occur in **32(2.2%).** As shown in table (4).

Table (4):- Further postoperative complication of thyroid surgery of studies patients (185 patients out 1450 of total cases).

Postoperative complication	Number/percentage 185/1450
Tension hematoma	16 (1.1%)
Recurrent laryngeal nerve injury (RLN)	18 (1.2%)
Transient injury of RLN	14 (1%)
Permanent injury of RLN	4 (0.2%)
Hypocalcaemia	52 (3.6%)
Transient hypocalcaemia	49 (3.4%)
Permanent hypocalcaemia	3 (0.2%)
Hypothyroidism	64 (4.4%)
Thyrotoxic crisis	3 (0.2%)
Wound infection and stick granuloma	32 (2.2%
Total number	185 (12.7%)

Correlation between EBSLN injuries with type of operation of patients

In present study the EBSLN injury in total thyroidectomy more than in near total thyroidectomy. Lobectomy and isthmusectomy more than near total thyroidectomy. And these dif-

ferences was statistically significant differences, (P<0.005). As shown in table 5.

Table (5): -EBSLN injury according to type of operation

Type of operation	N/%	P1	P2	P3
Total thyroidectomy	22(1.5%)			
Near total thyroidectomy	4(0.3%)	<0.001	0.003	<0.001
Lobectomy and isthmusectomy	12(0.8%)			

Correlation between EBSLN injuries with age of patient's study.

In present study there was no significant association between EBSLN injury and type of operation with age of patients, (P>0.005), as shown in the table (6).

Table (6):- Comparison between EBSLN injuries accords to type of operation with age of patients study.

Type of operation	Mean ±SD	P1	P2	P3
Total thyroidectomy	40±9.2			
Near total thyroidectomy	40±10	0.005	0.005	0.005
Lobectomy and isthmusectomy	40±9.5			

Correlation between EBSLN injury with sex of patient's study.

In present study the correlation of EBSLN injury and type of operation with male 25 **(1.7%)** more than in female patients **13 (0.9%)** and these differences was statistically significant differences, **(P<0.005),** as shown in the table (7).

Table (7). Comparison between EBSLN injuries accords to type of operation with sex of patients study.

Type of operation	Male	Female	P1	P2	P3
Total thyroidectomy	14(0.9%)	8			
Near total thyroidectomy	4(0.3%)	2	< 0.005	<0.005	< 0.005
Lobectomy and isthmusectomy	7(0.5%)	3			
Total cases	25(1.7%)	13(0.9%)			

Correlation between EBSLN injuries with size of goiter

In present study the EBSLN injury according to type of operation with large size goiter **29(2%)** more than in small size goiter **9 (0.6%)** and these differences was statically significant differences, **(P<0.005),** as shown in the table (8).

Table (8). Comparison between EBSLN injury according to type of operation with size of goiter

Type of operation	Large goiter	Small goiter	P1	P2	P3
Total thyroidectomy	154(1%)	4(0.3%)			
Near total thyroidectomy	6(4%)	2(0.1%)	0.005	0.005	0.005
Lobectomy and isthmusectomy	8(0.5%)	3(0.2%)			
Total cases	29(2%)	9((0.6%)			

Majority of cases with EBSLN injury were complain from both loss of high pitch voice and Chocking **19 (1.3%)** more than loss of high pitch voice **11 (0.7%)** and chocking **8(0.6%)** and these differences was statistically significant differences, **(P<0.005).**

Discussion

in present study, a large cohort project involving 1450 patients underwent thyroid surgery by using lateralization and segregated ligation of the superior thyroid vessels to minimize the EBSLN injury, without visual identification of EBSLN and without nerve stimulator or intraoperative neuromonitoring. In addition to, long-term follow-up patients which started from 7days after operation (1month, 3month, 6month,9month,1year until to 17 year) was carried out through clinical history and physical examination with each subsequent clinic visit and in addition to post-operative endoscopic examination of the vocal. The total cases with EBSLN injury **were 38 (2.6%),** in which the transient EBSLN injury occurred in **28 (1.9%)** of patients and permanent injury occurred in **10 (0.7%)** of patients. The outcome followed analyzing this large number of

cases is promising and reflect the experience of the surgeon.

Large multinodular goiter is one of the main indications for surgery and a risk factor for injury at the same time. In the present study the total cases with large goiter **520 (35.9%)** and the majority of cases with EBSLN injury were occur in patients with large size goiter **29 (2%)** more than small size goiter **9 (0.6%)** with statistically significant difference **(P <0.005)**. if the goiter is large, the superior pole occupies a higher position in the neck and is more closely related to the EBSLN in its descending course. Majority of cases with EBSLN injury were occur in male {25(1.7%)} more than female patients {13(0.9%)}, propose that could be the main major cause was the anatomy in male more difficult than the anatomy of female. In addition to these results there was no statistically significant correlation between age of patients and EBSLN injury. Thus, the age of patients can- not play any role in injury of EBSLN.

The results of present study were similar to other results reported by many authors, which used in their studies the same surgical protocol for management of the EBSLN during thyroidectomy as in the present study like Ahmed et al (12), whom reported that the transient EBSLN injury occurred in six (3%) patients, whereas permanent injury occurred in four (2%) patients.

Lekacos et al. (13) whom reported that, 0% EBSLN injury with distal ligation close to thyroid capsule and 5.6% injury with high ligation. Evaluation of EBSLN injury in their study was carried out using indirect laryngoscopy.

And the other studies were reported by, Likewise, Loré et al. (14) and Kierner et al. (3), whom reported that gentle mobilization of upper thyroid pole and individual ligation of superior thyroid vessels very close to thyroid capsule without systematic positive search for the EBSLN may avoid nerve injury.

Whereas other studies, that used the same surgical protocol for management of the EBSLN during thyroidectomy as in the present study, the rate of EBSLN injury was much higher from the presented study and these studies which reported by Aluffi et al. (15),

in which the results were 14% incidence for EBSLN injury despite their small study population (45 patients) and another study which reported by Teitelbaum and Wenig (1) in which the results were, a high permanent nerve injury (5%). The high rate of EBSLN injury in these two studies may be attributed to the use of both laryngoscopy and electromyography of cricothyroid muscles for the diagnosis of EBSLN injury, whereas, in the present study, we used indirect laryngoscopy post- operative and long-term follow-up through physical examination and clinical history.

On the other hand,the other studies used visual identification of nerve which additional step to their surgical operation protocol i.e (lateralization, visual identification of nerve and individual ligation of superior thyroid vessels) that studies employ comparative study between visual nerve identification group and non- visual nerve identification group, that found no benefit of systematic search for the EBSLN and these studies which reported by Page et al. (16), and another study which reported by the Bellantone et al. (6).

Whereas the study that reported by Hurtado-Lopez et al. (17) found that patients who underwent thyroidectomies without searching for the EBSLN had more voice changes compared with patients with thyroidectomies with intentional searching for the nerve (14 versus 8%, respectively) and give emphasis to the importance of intraoperative identification.

While study which reported by Patnaik et al. (18) whom not used comparative study but evaluate the visual identification rate of the EBSLN during 64 thyroidectomies. The nerve was identified and preserved in 83% of patients and could not be identified at all in the remaining patients (17%). None of these patients (17%) showed any symptoms and signs of ESLN paresis as their nerves were preserved using individual ligation of superior thyroid vessels close to thyroid capsule.

In present study, using lateralization and segregated ligation of the superior thyroid vessels to minimize the EBSLN injury, without visual identification of EBSLN and without nerve stimulator or intraoperative neuromonitoring because of some variant

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anatomy of EBSLN was buried under inferior constrictor muscle fibers, and later on it was impossible to identify the nerve in the field of thyroidectomy. They concluded that trial of nerve identification if it is buried under inferior constrictor muscle fibers would take a longer period of time with more injury to surrounding structures and without any benefit as the nerve could be preserved with individual ligation of superior thyroid vessels close to thyroid capsule. The long-term follow up patients postoperatively through physical examination and clinical history for integrity of vocal cord because of EBSLN injury can be difficult to identify intra-operatively and is difficult to detect during routine postoperative laryngoscopy.

However,other studies which reported by Barczyński et al. (8), Lifante JC (10) and Dionigi G (9), whom used more advance technique for visualization of EBSLN intra-operatively neuromonitoring (IONM) during thyroidectomy,these study concluded that the use of intraoperative neuromonitoring significantly improved intraoperative identification of the EBSLN and decreased early postoperative voice changes with no significant difference in delayed postoperative voice changes between IONM and surgical visualization. Improvement in the identification rate of the EBSLN by the use of IONM and thus limitation of the risk for nerve. We suggest that despite the benefit of IONM during thyroidectomy in these studies, it could not be used routinely our countries because of deficiency of incomes.

Conclusion

Segregated ligation of superior thyroid artery closely to thyroid capsule without nerve stimulator or intraoperative neuromonitoring is safe technique, cost effective with time preservation but need surgical skill to decrease risk of injury to the external branch of superior laryngeal nerve.

Conflict of Interest
The authors declare no conflict of interests.

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Preprocessing of the candidate antiviral drugs against COVID-19 in models of SARS cov2 targets

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ABSTRACT

Although many viral infections are self-limiting, other are real health challenges like COVID-19 since many viruses possess just few drug gable targets to be treated with small drug molecules. Corona virus genome encodes for up to 17 main proteins. Orf1ab encodes for polyprotein. COVID-19 structural proteins are the spike S, membrane M, envelope E and the nucleocapsid N protein while other are non-structural proteins designated as NSP1-13 for non-structural proteins. Among NSP the most important corona virus targets for developing antiviral drugs are the papain-like protease, PDB ID: 6m03 and RNA polymerase NSP12, PDB ID: 6nur. NCBI, NIH Genbank, Uniprot, PDB, Drug-Bank, ChemSpider databases and bioinformatics editor softwares like ICM Mol soft pro and Swiss Dock were used in addition to the in vitro lab model of viral protease were integrated to retrieve and analyze corona virus targets and to select the candidate ligands in an attempt to evaluate the inhibitory efficacy of different experimental and approved drugs which were further optimized and searched for the highly similar approved drug. This step aims to adopt drug repurposing to speed the development of antiviral drugs and recommend rational in vivo and clinical studies. After COVID-19 targets had been analyzed the drugs that shared > 70% similarity to the binding sites of those targets were reversin, pentagastrin, remdesivir, norfloxacin and nitazoxanide against COVID-19 papain-like protease whereas benzyl glutathione, lopinavir and hydroxymethylglutathione against RNA polymerase. The anti-resistance reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-protease binding energy with Mol soft pro analysis. The calculated inhibitory binding was -137.30 kJ/mol z > 1.9 as compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate antiprotease activity (>- 100 kJ/mol). Norfloxacin shoresults showed a slight consistency between in vitro and in silico models. Although benzyl glutathione is an experimental compound, however it had the highest RNA polymerase inhibiting efficacy with -129 kJ/mol binding energy which is even higher than lopinavir and Favinavir. From the overall results, reversin, oligopeptides, quinolones and antiviral drugs may widen the treatment options for COVID-19 if further evaluated in clinical studies.

Keywords: COVID-19, NSP, RDRP, papain-like protease, replicas and antiviral drugs

INTRODUCTION

Severe acute respiratory syndrome corona virus 2 (SARS cov2 or COVID-19) is a highly spreading viral infection caused by a novel type of Coronaviridae family called SARS nCov [1]. Similar to other viral infections, Covid-19 has unique virulence characteristics in that viruses possess just a few drug gable target proteins [2] like hemagglutinin, neuraminidase, polymerase, proteases, envelope and membrane proteins in addition to few polyproteins associated with their genome [3]. Viruses are semi-dormant units that are interactive with drugs only in their cycles of proliferation [4]. These factors diminish the ability to treat viral infections. However in concern to the COVID-19, the viral proteome is composed of 17 main proteins: four are structural proteins, envelope E, membrane glycoprotein M, surface glycoprotein spikes S, and nucleocapsid protein N for + RNA binding whereas hemagglutinin and other non-structural proteins NSP1-13 are encoded with different orf genes (PDB, Genbank, NCBI ID: 6lu7, 6lvn, 6lxt, 5r7y, 5r7z, 5r80, 5r81, 5r82, 5r83, 5r84) [5-7]. The following set of COVID-19 proteome contents is a reasonable pharmacological target for developing new or repurposed

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antiviral drugs. The orf1ab encoded polyprotein: COVID-19 replicas are a polyprotein that has multiple activities like -RNA transcription, RNA template, mRNAs and virion RNA. This polyprotein has also multiple proteinases activity to cleave this polyprotein into functional products. The non-structural protein 1 NSP1 (host translation inhibitor) blocks 40S of human ribosome so that it arrests host transcription and mediate mRNA lysis and spare COVID-19 mRNAs. Non-structural protein 2: through cell PHB and PHB2, NSP2 maintains cells mitochondria viable. Papain-like proteinases PLP: lyses the N-terminus of the replicas polyprotein and activates Lys-48 and Lys-63 linked polyubiquitin chains in cells. PLP mediates viral membrane assembly. In addition, PLP inhibits INF production and NFkB. Non-structural protein 4 NSP4: mediates viral membrane assembly. Proteinases 3CL-PRO: activates lysis C-terminus of replicas polyprotein. Non-structural protein 6 NSP6: Mediates endocytosis and prevents lysosomal fusion. Non-structural protein 7 NSP7: with NSP8 it acts as a primase. Non-structural protein 8 NSP8: synthesizes longer products than Oligonucleotide primers. Non-structural protein 9 NSP9: by ssRNA-binding protein it mediates viral replication. Non-structural protein 10 NSP10: mediates COVID-19 transcription by stimulating both nsp14 (exoribonuclease) and nsp16 (O-methyltransferase) for methylating viral mRNAs cap. RNA-directed RNA polymerase NSP12 RDRP: for replication and transcription of the viral RNA genome. Viral Helicase: a Mg-dependent and Zn-binding protein that unwinds RNA and DNA. Guanine-N7 methyltransferase: Have exoribonuclease activity (on both ssRNA and dsRNA) and a N7-guanine methyltransferase activity. Uridylate-specific endoribonuclease: Mn-dependent, uridyl enzyme. 2'-O-methyltransferase: for viral mRNA cap methylation at 2'-O-ribose site [8-9] in addition to the non-protein target against the viral membrane like terpenoids [10]. Another host factors that were investigated as reasonable targets to treat COVID-19 include angiotensin converting enzymes 2 ACE2 [11-13] furins and passive and active immunostimulants like INFs and ILs [14-15]. Many studies analyze viral protein targets virtually for docking sites [16-17] then the predicted compound with high energy of affinity to target site is optimized for predicting the 3D conformational isomers and analogues [18]. Such lead experimental or drug compounds are then rearranged in a rank according to their target binding energy in kj/mol with classifying types of ionic, vW, and hydrogen bonds by which they interact with target residues [19-20]. Many compounds and approved drugs like antiviral drugs are potential resources to be repurposed against different health challenges like corona virus infections. Of these potential drugs are the quinolones, small peptide molecules like reversin and pentagastrin, benzyl glutathione and antiviral drugs that inhibit viral replication like lopinavir, Favinavir in addition to the protease inhibitors like ritanovir, remdesivir and ribavirin while others are ant parasitic nitro-azole like nitazoxanide. In vitro viral model is a critical technical step for testing antiviral compounds [21] since that direct access of the researchers into the active viruses are forbidden according to the international regulation policy and the strict requisites for level 4 laboratories owning to their health risks [22] so that the artificial viral models including killed virion or its partial components are critical in conducting such researches [23]. In vitro testing is followed to determine pharmacokinetics and target binding dynamics. Although viruses have just limited number of proteins, however each protein like COVID-19 main protease and NSP12 has in average 3 docking sites for surveying many test compounds [24]. This means that developing a rapid medical treatment against the highly transmittable viral epidemics like COVID-19 is a possible approach. This study aimed to analyze all COVID-19 proteins and evaluate the highly predictive ligands for further optimization and to study ligand similar to the approved drugs to be re-evaluated and arranged for further clinical studies.

Models, Materials and Methods

This study had been accomplished in serial stages and originally designed to include both computer-based and laboratory assessment of candidate list of drugs against COVID-19.

1. The database retrieval and review study for COVID-19, SARS cov and Human corona virus Hcov OC43, NL63 whole genome, proteome, envelope and membrane analysis in addition to reviewing relevant host factors like furin and ACE2. NCBI, NIH, Uniprot, GenBank, Proteinpedia, PDB, NeXtProt, Genomix, Gene Card and Viral genome were the used database to specify the target genes, proteins in addition to analyze and optimize the control targets with BLAST analysis to determine synonymous and diverse segments. The whole genome FASTA format had been downloaded using different bioinformatics software NCBI (appendix IV). Ugene software was used to graph and conduct blast analysis between each corresponding proteins related to the SARS cov, SARS cov2 and Hcov, S, M, E, N, Orf1ab, orf3, orf6, orf7, orf8, orf9, orf10, HA and other NSP proteins that were deposited on softwares were assessed by multiple BLAST with NCBI to determine the exact synonym and structural variations and to be further confirmed by pdb superimposition with ICM. Viral protein complexes like S-ACE2 and N-RNA were also identified for their interacting sites. Host relevant proteins furin and ACE2 had been downloaded and assessed for their role in the pathogenesis of steps of viral infection. Those steps were conducted using pdb and ICM mol soft.

2. COVID-19 protein analysis was done using PDB, Uniprot, ICM mol soft pro, NCBI, NIH, Swiss Dock, BioXLab and Ugene software for determining viral protein 2D, 3D structures, similarity, biological roles, pathological effects, ligands, binding residues, surface map, protein health, strain points and target sites.

3. Ligand prediction. The target proteins were preprocessed for determination of the full fit ligand with highest binding energy, in addition to determining of the ligand trajectory and types of force field energy at the binding site.

4. Ligand optimization.

Using ICM, mol soft pro the ligand is optimized to best fit binding site after selecting a highly effective pocket among the determined table of protein binding sites and the final structure of the inhibitor is then edited and stored in several molecule formats like sdf, mol, mol2 for the next steps.

3. RCSB pdb, PDBe and PDBj were the main database softwares used to obtain pdb format of the viral and host proteins according to the following IDs: 6lu7, 6m3m, 6m03, 6lvn, 6lxt, 5r7y, 5r7z, 5r80, 5r81, 5r82, 5r83, 5gwy, 5r84.

Preprocessing of COVID-19 papain-like protease

With Mol soft pro, the target protein is loaded and converted to PDB format with water deletion. The protein is analyzed for health, surface electro potential and reviewing all domains and cofactors and then a project of table drug surveying is conducted by creating the project directory. Receptor mapping and surface map. The test drugs table is loaded too for later trajectory. Adjusting the cut-off distances and selecting the candidate drugs table. (Figure 1)

Preparing the table of candidate drugs for surveying for PL protease

The ligand based drug predictions were formulated by analyzing the ligand skeleton formula or functional group similarity in addition to the isomer and tautomer search were adopted, further optimization of the candidate to maximally fit the binding site in the target protein then retrograde surveying of the present drug to be tested for their binding energies to the candidate target COVID-19 proteins. These steps are analyzed with different editing softwares like Molsoft pro. (Figure 2)

Preparing COVID-19 nucleocapsid protein N for ligand prediction

The same steps were followed in preparing the previous targets. (Figure 3)

Preprocessing of COVID-19 NSP12 (RDRP), PDB ID: 6nur

Similar steps were followed in preparing the previous targets. (Figure 4)

Processing of COVID-19 NSP9 the replicase associated protein

Similar steps were followed in preparing the previous targets. (Figure 5, 6, 7, 8))

Figure (1): the main predicted binding scaffolds of PL protein at cut-off grid = 4.5
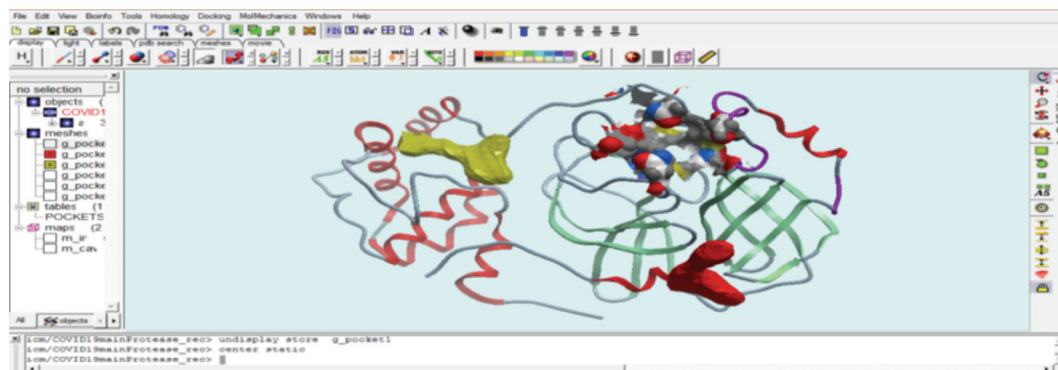
Figure (2): the drugs which were re-evaluated for their inhibitory efficacy against PL protease in Mol soft pro to analyze the trajectory, pockets, force field energy types and binding energy as indicators for the drug selectivity and affinity toward the binding site
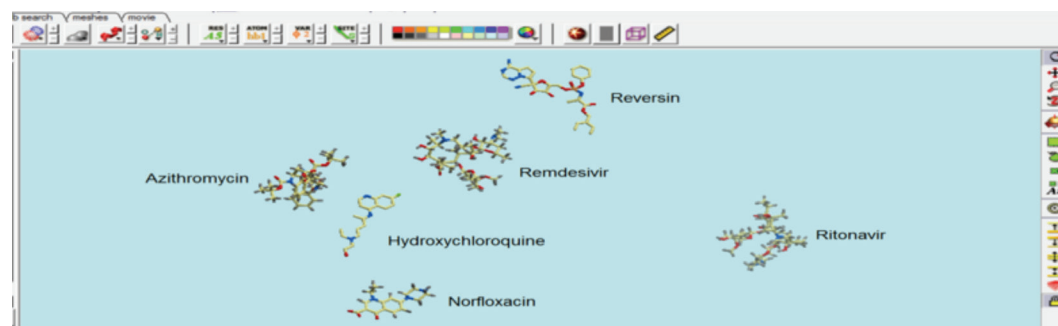
Figure (3): predicting and evaluating the main target sites in COVID-19 N protein for surveying inhibitor compounds and drugs.
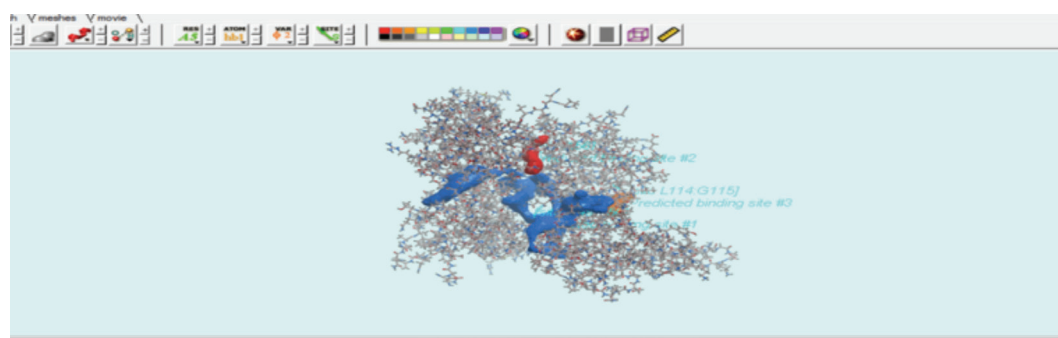
Figure (4): ribbon representation of COVID-19 NSP12 the main viral target is the RDRP before complexing with NSP7 and NSP8
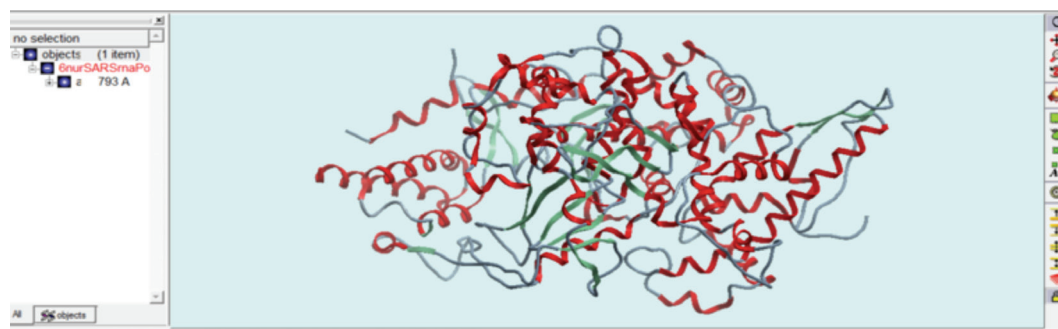
Figure (5): predicting and evaluating the target sites of RDRP
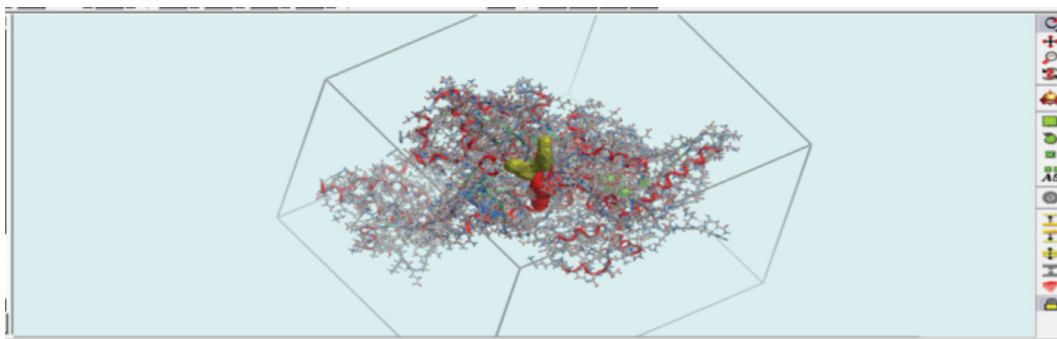
Figure (6): The complexes multidomain of COVID-19 NSP12 with NSP7, NSP8 and NSP9
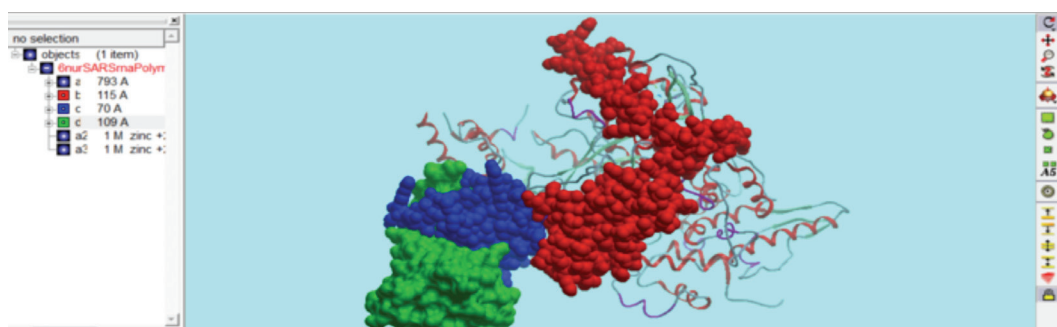
Figure (7): The FASTA format of the complexes multidomain of COVID-19 NSP12 with NSP7, NSP8 and NSP9
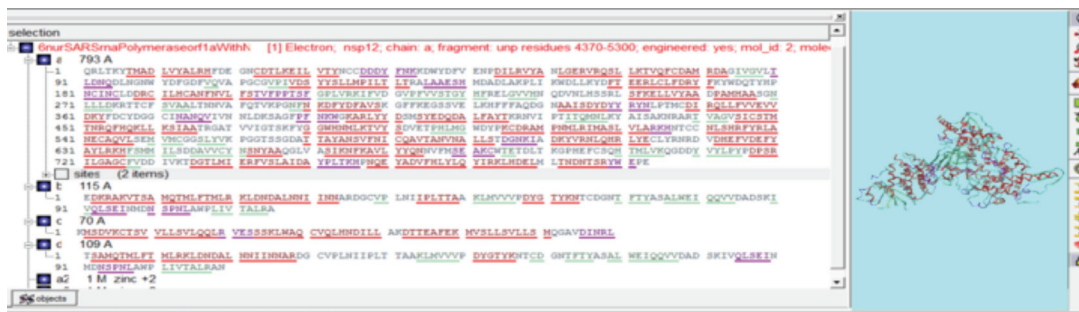
Figure (8): processing of the surface receptor of NSP12 complex
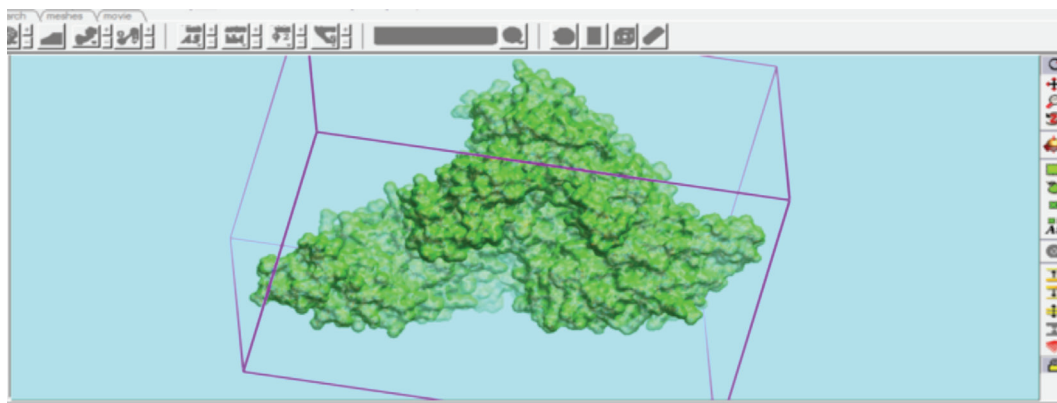
Processing of COVID-19 NSP9 the replicase associated protein

Similar steps were followed in preparing the previous targets. (Figure 9)

Processing of the viral spike S protein

Similar steps were followed in preparing the previous targets. (Figure 10, 11, 12)

Processing of the viral spike S protein

Similar steps were followed in preparing the previous targets.

The 3D representation of the structural (S, E, M, N) and non-structural viral proteins were processed and viewed with Mol soft pro and RasMol as shown below.

Processing and representing of the corona virus membrane M protein

Similar steps were followed in preparing the previous targets. (Figure 13)

Laboratory assessment of the test antiviral compounds

To review drugs physicochemical, pharmacokinetics and toxicity properties in order to select the candidate of choice if it shows highly safe profile otherwise it is submitted to the next step. Steps of in silico and in vitro study were conducted in University of Kufa/ College of Medicine/ the research lab of the department of Pharmacology and Therapeutics in addition to some steps conducted in private lab. Tis-phosphate buffer, alcohol 70%, norfloxacin pure powder, casein powder, papain-like protease powder, normal saline, distilled water, protease inhibitor powder were prepared. Simulink image intensity analyzer was used to measure casein opalescence in response to the test papain-like proease inhibiting drug, 1000X Olympus microscope, centrifuge; ultr-high speed mechanical stirrer, micropore filter and a mechanical shaker were used.

The artificial COVID-19 viral model

This model is aimed to test the bioavailability of the test drugs and their diffusion through the artificial viral membrane in addition to determining the inhibitory effects of those test drugs on papain-like protease by casein opalescence as-

say. The model is made of an artificial membrane which is obtained from human RBCs to formulate RBCs vesicles RV as it has been described in the appendix I procedure. After preparing the artificial viral membrane, papain-like protease is then incorporated into the RV to for the artificial virus AV. Casein in a pure form and was formulated as described in procedure in appendix II. Addition of 5 microg/ml of norfloxacin to the test well 1 in comparison to the control well and then the rate of casein opalescence change is measured with serial monitoring of the sample every one hour for 8 hours.eir inhibitory effects on viral protease with microscopic imaging and spectrophotometric analysis of absorbance in relation with casein hydrolysis. Data is compared with the in silico model to assess the consistency of the antiviral property of a test drug. Data of the test and control wells are compared to assess norfloxacin inhibitory effect on viral papain-like protease. Such procedure could be repeated to assess other drugs for their protease inhibitor efficacy.

6. A drug repositioning step includes evaluating the level of similarity to an approved drug to be reassessed for antiviral use against that target protein.

7. Designing an in vitro model of antiviral assessment of the already preprocessed compounds and drugs. This model is formulated by incorporating the target COVID-19 papain-like protease into the lipid bilayer vesicles to evaluate the inhibitory efficacy of the experimental or approved drug on this enzyme function which causes casein opalescence.

8. Further surveying drug substructure and similarity to evaluate the optimal anti-COVID-19 drug.

9. Recommending further in vitro and in vivo study conduction and clinical study designs since the increase in antiviral treatment options is important for controlling viral infections

10. Data analysis with the compatible statistical tests for in silico and in vitro outcomes.

THE RESULTS

The outcomes of in vitro and in silico evaluation of compounds against COVID-19 were divided according the main pharmacological proteins targets related to this virus.

Figure (9): the 3D presentation of NSP9 (replicase associated protein) of COVID-19 with evaluating the target sites for drug design
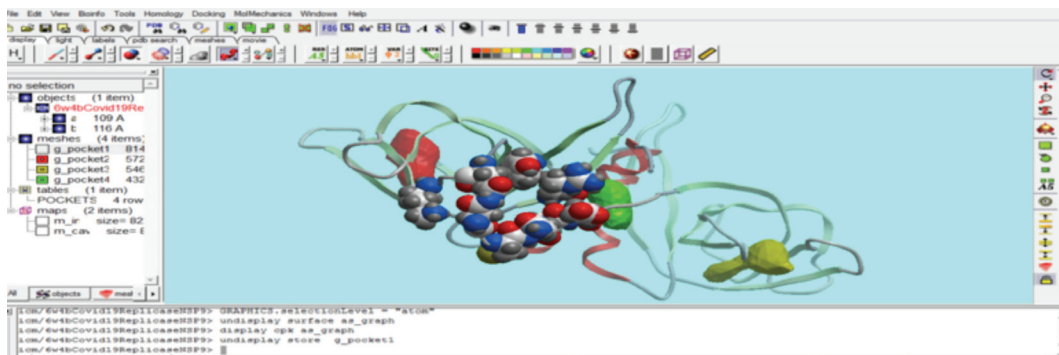
Figure (10): the 3D grid superimposition of the COVID-19 S protein (Rt) and Hcov S protein (Lt)
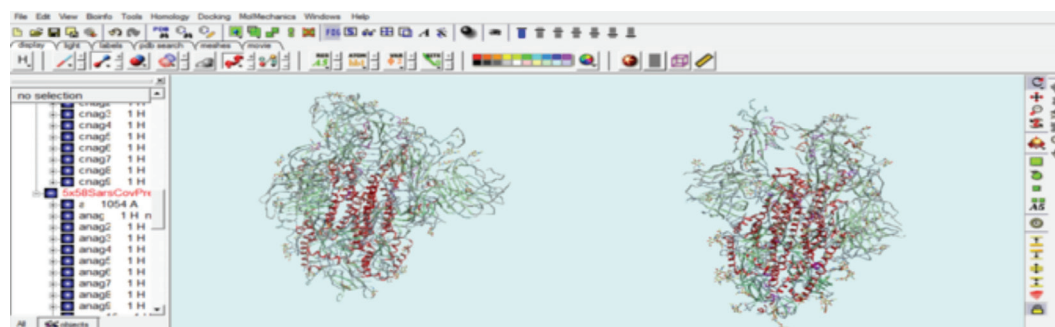
Figure (11): SARS Cov S protein connected to the host angiotensin converting enzyme ACE2 to mediate cell membrane penetration.
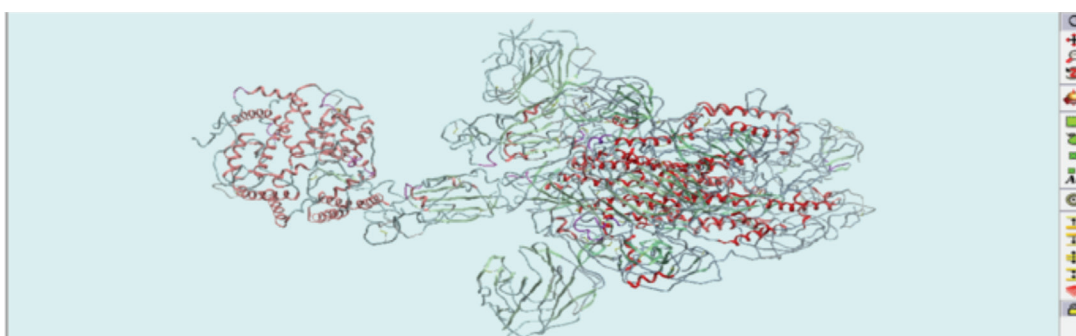
Figure (12): the ball and sticks representation of decaglucopyranose ligand of COVID-19 S protein.
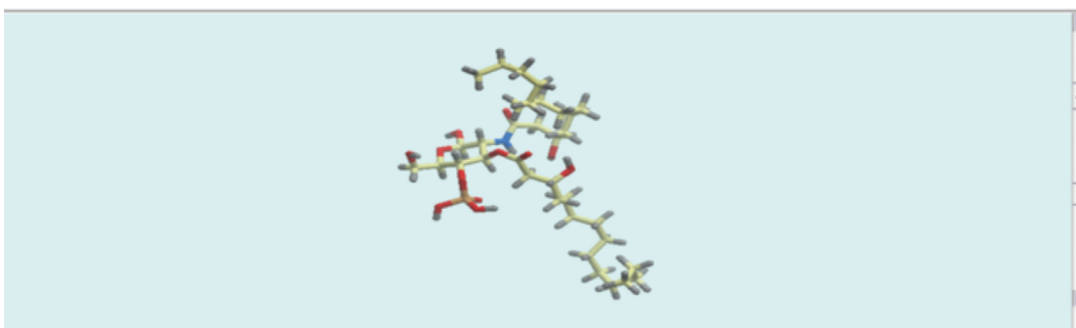
Figure (13): represent a prior assessment and visualizing the M protein related to SARS Cov linked to HLA (Rt) and free (Lt)
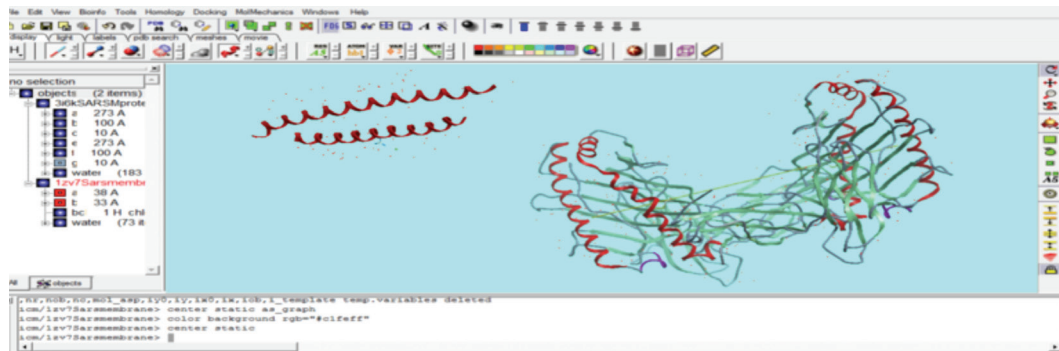
Figure (14): the binding sites of different tested drugs against COVID-19 protease PLP
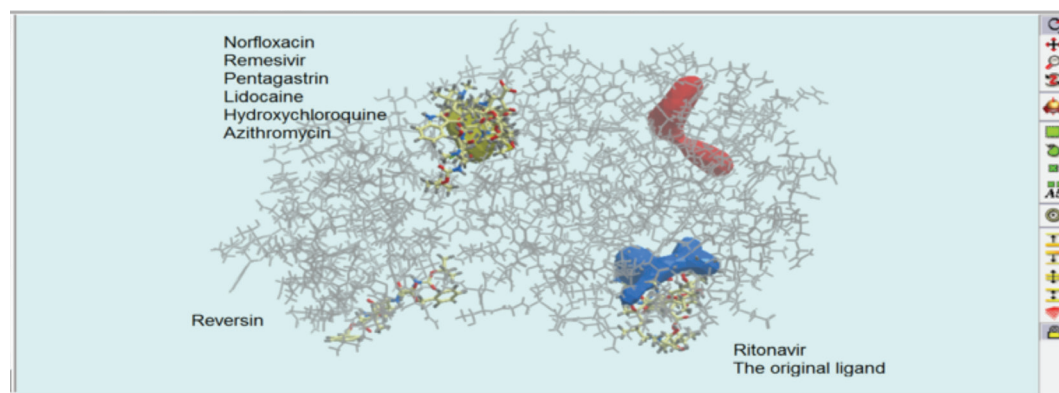
1-Findings of the inhibitory efficacy of test drugs repurposed against the COVID-19 main protease or the papain-like (PLP) protease.

Table (1) COVID-19 protease inhibition efficacy of the test drugs as a binding energy in kJ\mol as analyzed with Mol soft pro

Test drugs against COVID-19 protease	The inhibitory efficacy In kJ\mol	Statistics Z score	Drug approval
1- Reversin	-137.30	>1.9	approved
2- Tetrazapentadecanoate	-129.57	>4.0	experimental
3- Remdesivir	-119.50	>1.6	approved
4- Pentagstrin	-118.82	>1,9	approved
5- Nitazoxanide	110.29	>1.9	approved
6- Norfloxacin	-103.70	>1.9	approved
7- Ritonavir	-95.22	>1.6	approved
8- Hydroxychloroquine	-86.42	>1.6	approved
9- Azithromycin	-85.78	>1.6	approved
10- Lidocaine	-80.69	>1.6	approved
Red = very potent			

Figure (15): the comparative efficacy of drugs that were selected according to their skeletal superimposition to the predicted ligand of PL protease.

COVID-19 papain-like protein inhibitors were evaluated by Mol soft pro for the binding energy in kl/mol
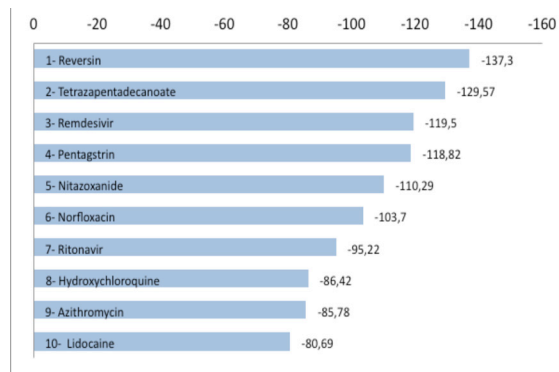
The parameter was evaluated as the binding energy to PL protease in kJ/mol. Reversin, remdesivir, pentagastrin and norfloxacin showed enthalpy > 100 kJ/mol.

The highest negative value the highest inhibitory efficacy

Reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-PLP binding energy with Mol soft pro and BioXLab analysis. The calculated inhibitory binding was -137.30 kJ/mol z > 1.9. as compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate anti PLP activity (>- 100 kJ/mol).

2- Findings of the in vitro study design of COVID-19 papain-like protease

Figure (16): in vitro antiviral model under 1000X microscopic image showing the casein micelles of the blank sample (upper left) and the artificial papain-like protease containing vesicles AV (upper right). The lower left sample represents casein sample mixed with AV and the lower right sample shows the casein sample mixed with AV and norfloxacin to assess its effect on papain-like protease.
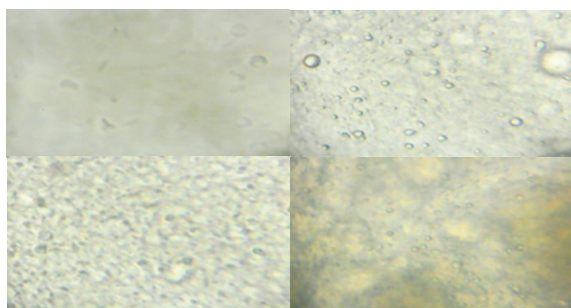
Figure (17) the relative protection with norfloxacin against casein decrease in opalescence. This decrease in opalescence is due to the effect of papain-like protease in hydrolyzing casein at cysteine residues. It was obvious that norfloxacin had a protective effect which indicates its papain-like protease inhibitor action. Casein opalescence was measured with Simulink image analysis to determine the relative loss in image intensity.
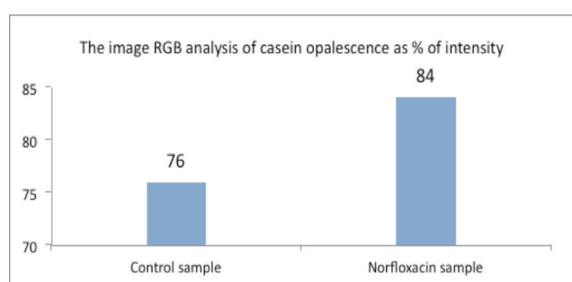
3- Findings of COVID-19 nucleocapsid N inhibiting ligands

Table (2) COVID-19 nucleocapsid N inhibiting ligands evaluated and represented by Mol soft pro and iGemdock

COVID-19 nucleocapsid N inhibitor drugs	Anti-N binding energy in kJ\mol	Statistics Z score	Drug approval
Phenanthridine glycenamide	-102.10	4.2	experimental
Morpholin quinoline	-90.70	4.0	experimental

Figure (18) the main COVID-19 nucleocapsid N binding ligands and their binding energy in kJ/mol
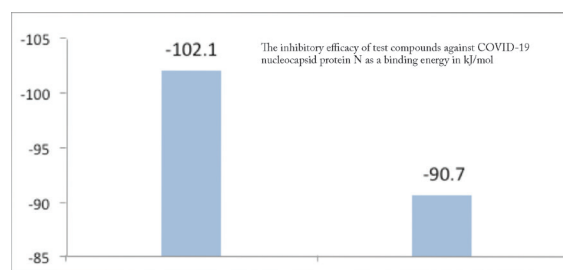
Table (3): the inhibitory efficacy of a group of drugs evaluated for their NSP12 (RDRP) binding energy with Mol soft pro

COVID-19 NSP12 inhibiting drugs	Anti-RNA polymerase Efficacy in kJ/mol	Statistics Z score	Change
1- Benzyl glutathione	-129.28	>1.9	experimental
2- Lopinavir	-118.80	2.2	approved
3. Hydroxymethyl-glutathione	-112.00	>1.9	experimental
4- Glutathione	-105.72	>1.9	approved
5- Favinavir	-93.10	2.2	approved
6- Ribavirin	-91.50	2.2	approved
red = highly potent			

Assessment of the drugs activity against NSP12 (RDRp) included selected ligand analogues benzyl glutathione, hydroxymethyl-glutathione, lopinavir, glutathione, Favinavir and ribavirin showed promising results against COVID-19 infection. Benzyl glutathione had the highest inhibitory efficacy against COVID-19 RDRp with a binding energy of

-129.28 kJ/mol z > 1.9 and lopinavir came second in efficacy with a bindind energy of -118.80 kJ/mol, z = 2.2. Glutathione had a moderate RDRp binding energy (> -105.72 kJ/mol).

Figure (19): the comparative efficacy of test drugs against COVID-19 RDRP, the parameter was evaluated as the binding energy to RDRP in kJ/mol. Benzylglutathion, lopinavir, hydroxymethylchloroquine and glutathione had a binding enthalpy > 100 kJ/mol and z score >1.9

Drugs that were tested with Molsoft against COVID-19 NSP12 the RNA polymerase or RDRP for their binding energy in kJ/mol

Drug	Binding energy
1- Benzylglutathione	-129,28
2- Lopinavir	-118,8
3. Hydroxymethylglutathione	-112
4- Glutathione	-105,72
5- Favinavir	-93,1
6- Ribavirin	-91,5
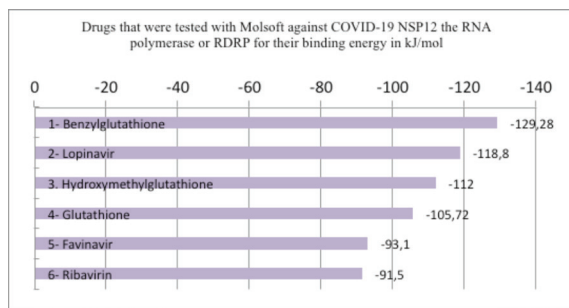
Discussion

Although viruses like COVID-19 having limited number of protein, however these proteins contain multiple pockets and binding sites to guide the rational for developing many antiviral compounds [25]. Among the COVID-19 genome set of proteins, the current study concerned with papain-like protease (PLpro) and RNA-dependent RNA polymerase (RdRp) as the main targets. The near future steps will include more presice optimization of the ligands against other COVID-19 proteins [26-28]. Ten drugs where assessed by in silico model of studying the PLP binding energy in kJ/mol. These drugs included reversin, pentagastrin, the original ligand tetrapentazadecanoate, remdesivir, nitazoxanide, norfloxacin, hydroxychloroquine, ritanovir, Azithromycin and lidocaine. The anti-resistance reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-PLP binding energy. The calculated inhibitory binding energy was -137.30 kJ/mol z > 1.9 as compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate PLP binding energy (>-100

kJ/mol). In vitro PLP inhibiting activity for norfloxacin was slightly consistent with the in silico outcomes. The designed in vitro model for COVID-19 was not highly reliable due to the limited facilities under the current epidemic, however adopting a more sophisticated in vitro models against risky viral infections is the key of development of new antiviral drugs decause this model is accessible for a wider number of researchers [29-31]. Other tested drugs against PLP showed just a weak binding energy (-80 to -95 kJ/mol). These drugs included hydroxychloroquine, Azithromycin, ritanovir and lidocaine. Antiviral activities of remdesivir, ritanovir, nitazoxanide, quinolones and some oligopeptides were confirmed by different studies against viral infections other than COVID-19 [32]. Assessment of the drugs activity against NSP12 (RDRp) included selected ligand analogues benzyl glutathione, hydroxymethylglutathione, lopinavir, glutathione, Favinavir and ribavirin showed promising results against COVID-19 infection. Benzyl glutathione had the highest inhibitory efficacy against COVID-19 RDRp with a binding energy of -129.28 kJ/mol z > 1.9 and lopinavir came second in efficacy with a bindind energy of -118.80 kJ/mol, z = 2.2. Glutathione had a moderate RDRp binding energy (> -105.72 kJ/mol). Glutathione, lopinavir, Favinavir and ribavirin had also antiviral effects on other viral infections [33-34]. Comparing the 3D conformational binding was also evaluated and showed that some of the evaluated drugs had different docking sites while others had the same docking sites with different binding residues and energies. Evaluation of the ligands against NSP9 COVID-19 replicase associated protein. The predicted ligands binding to NSP9 were evaluated using Mol soft pro and they showed no significant similarity in their structure or formula although the similarity cut-off was set at 0.4. Evaluation of the ligands against COVID-19 spike S protein. This protein was relatively large in size and it performs a structural unit and cell penetration mechanisms which mean it has a macromolecular surface of binding site so that direct inhibitory actions of small drugs may have a limited effica-

cy against S protein [35-37]. One ligand was predicted to be of applied value which was the decaglucopyranose.

The COVID-19 relevant host components furin and ACE2

Furin is an essential housekeeping enzyme for activating many metabolic and cellular proteins it needs for a highly selective mechanism of modifying its action in order to spare the physiologic effects [38]. On the other hand, angiotensin converting enzyme ACE2 is an essential cytoprotective enzyme although it's a one mediator of corona virus's penetration into the cell [39-40].

Other host components like INF gamma and ILs and vaccines

Immunotherapy is critically important in treating and controlling viral diseases. Vaccines may comprise the versatile health care measures against future viral epidemics; however several weak points are correspondent with immunotherapy in the future of viral infections. Of these drawbacks of immunotherapy is its expiry of protection since most of pathogens have the virulent strategy to change their antigens, moreover, host immune response has its own duration of action which may extend from weeks to few years, however immunoprotection is uncommonly to be lifelong [41]. As it was confirmed by bioinformatics of different databases, another critical point in viral immunotherapy is that most of the viral infections will eventually exaggerate immune system. This response is at most the cytotoxic and pathogenic event that in many instances gives rise to the seriousness of even simple viral infection so that immunotherapy will remain risky and in many times it is cautious [42-46]. For all these reasons the antiviral drug therapy is highly promising to compact the epidemic infections.

Conclusion

From the overall results quinolones, antiviral drugs, glutathione and peptides like reversin and pentagastrin had a promising inhibitory

efficacy against COVID-19 protein targets so that developing COVID-19 proteins blockers from the approved drugs is an accessible approach and could provide rapid and safe therapeutic option against the risky viral epidemics.

Statement of novelty

Quinolones, antiviral drugs, glutathione and the small peptide compounds are promising inhibitors for different COVID-19 proteins and provide the rational to be repurposed in further studies against corona virus infections.

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Appendices

Appendix I
The red blood cell membrane camouflaged nanoparticle

Preparation methods of RBCM-NPs Various methods including physical and chemical property-based techniques have been reported for encapsulating drugs or other bioactive agents in erythrocytes, such as hypotonic hemolytic, hypotonic dilution, hypotonic dialysis hypotonic preswelling and osmotic pulse or chemical perturbation of the membrane along with electrical breakdown. In addition, endocytosis, lipid fusion, and intrinsic uptake of substances by erythrocytes are used to encapsulate different compounds. To achieve successful covering, the encapsulated compounds may require a considerable degree of water solubility as well as erythrocyte inactivity; i.e., lack of physical and chemical interactions with erythrocyte membranes to avoid leakage from the loaded RBCs, which could result in toxicological problems. Therefore, drugs are commonly prepared into anano preparation with less toxicity and higher stability as the core, followed by the use of RBCMs to disguise the nano-preparations to avoid identification by immune systems. Preparation of RBCM-derived vesicles (RVs) In general, the optimized and common preparation of RBCM-NPs can be divided into two parts: membrane-derived vesicles from RBCs and vesicle-particle fusion. RVs are obtained by combining two steps, hypotonic treatment and sequential extrusion. Fresh whole blood obtained from the organism (e.g., mouse) is centrifuged at 4 1C to maintain protein activity, and then the serum and buff coat are removed to collect erythrocytes. The resulting RBCs are repeatedly washed with phosphate buffered saline (PBS) and re-collected by centrifugation to remove residual plasma and other unwanted cells. RBC ghosts are then acquired by hypotonic treatment, where in the washed RBCs are gently mixed with an excess of 0.25_ PBS and held to release the intracellular RBC components. Following high- speed centrifugation to remove hemoglobin, the RBC ghosts comprising the resulting pink precipitate are sonicated in a bath Sonicator, and then passed through different pore size polycarbonate porous membranes using an Avantimini-extruder to obtain the target size of RVs. To keep the membrane bio-active, protease inhibitors are usually added to the samples and the samples are stored at 4 1C.(47)

Appendix II
Studies on the opalescence in sodium-caseinate solution developed by the milk coagulating enzymes

Casein was prepared from unpasteurized cow's skim milk by the method of Hipp.2)

a-Casein was prepared by the method of War-ner S) and J3-casein by the method of Hipp4) with the use of urea, specifically. Both of them were electrophoretically pure at **pH** 7.6.

The enzymes used were Hansen's Rennet Tablet and Mikuni's pepsin preparation. Hy-drolysis was carried out at **pH** 6.5 on 2.5~;; so-dium caseinate solution at 35°C.(48)

Appendix III

List of abbreviations
NSP: non-structural protein
PLP: papain-like protease
Orf: open file read of gene
N: nucleocapsid protein
M: membrane glycoprotein
S: spike viral glycoprotein
E: envelope protein
RV: red blood cell derived vesicles
AV: artificial viral vesicle
SARS: Severe acute respiratory syndrome co-rona virus
COVID-19: corona virus disease 2019
HLA: human lymphocytes antigen
PDB: protein data bank
ICM: internal coordinate mechanics of Mol soft pro
ACE2: angiotensin converting enzyme 2
RBCs: red blood cells
RDRp: RNA dependent RNA polymerase

Appendix IV'

Whole genome FASTA of COVID-19 from NCBI database

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Urinary tract infections in unexplained neonatal hyperbilirubinemia: Prevalence and predictive risk factors

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ABSTRACT

Introduction: Urinary tract infections (UTIs) are widespread clinical disorder among early neonates. Neonates with UTIs were susceptible to higher rates of morbidity and mortality, particularly when presented with hyperbilirubinemia. Early diagnosis may help in **complete** recovery**rather than being threatened in terms of complications.** The study aimed at determining the prevalence and predictive risk factors of UTIs in neonates with an unexplained hyperbilirubinemia.

Method: A cross-sectional study was carried out in the NICU of Aswan University Hospital, Egypt from August 2018 to February 2019. The study was conducted on 140 newborns who were diagnosed with indirect hyperbilirubinemia in the first 4 weeks of life after exclusion of unrelated criteria. Demographic and clinical data were collected by an interview questionnaire. Biochemical markers including bilirubin level, CBC, urine analysis and urine cultures and sensitivity were determined.

Results: The prevalence rate of UTIs in the studied newborns was 25%. Escherichia -coli was the dominant organism isolated. Amikacin was the most common antibiotic sensitive to the isolates. There was a significant difference between the UTI positive and negative neonates in the univariate analysis regarding some studied variables. While, an increase in the number of WBCs in the blood (OR = 6.90, P = 0.001), small for gestational age (OR = 4.07, P = 0.021), prolonged phototherapy (OR = 3.50, P = 0.034), and presence of maternal complications (OR = 2.92, P = 0.001) were statistically associated with a positive urine culture in multivariate analysis.

Conclusions and recommendations: The prevalence rate of UTIs was 25%. The study indicated the importance of routine screening of UTI (urine culture) as part of the clinical assessment of unexplained hyperbilirubinemia in neonates with an increase in the number of WBCs in their blood, small for gestational age, prolonged duration of phototherapy, and neonates born from mothers who had a history of obstetric complications.

Key words: Neonates, Urinary tract infection, UTI, jaundice, Hyperbilirubinemia.

INTRODUCTION

Bacterial infections are widespread clinical disorders among early neonates. One-third of these bacterial infections have occurred in the urinary tract. [1] The signs and symptoms of urinary tract infections (UTIs) are extremely diverse, varying from acute illnesses to nonspecific manifestations. [2] One of the clinical nonspecific signs identified with UTIs is neonatal jaundice, sometimes it is the main manifestation of UTI in the neonatal period. [3] The prevalence rate of the UTI associated with hyperbilirubinemia in the early neonates varies between 3% and 21%. [4]

The morbid physiology of hyperbilirubinemia in UTI cases is infection-related hemolysis induced by bacterial endotoxins, cytokines, and others. Heme released from hemolysis provokes the heme oxygenase-1 enzyme, which degrades heme to biliverdin, and others. [5]

Neonates with urinary tract infections (UTIs) were susceptible to higher rates of morbidity and mortality, particularly when presented with jaundice. [6]

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Early diagnosis of UTIs in neonates with hyperbilirubinemia may help in complete recovery after appropriate management of UTI rather than a being threat in terms of complications. [5,7]

Although the association between neonatal jaundice and UTIs are emphasized, there is no proof recommendation for screening UTIs in all neonates with jaundice. It is only restricted to neonates with prolonged hyperbilirubinemia with high bilirubin level. [8] On the other hand, there are incompatible data between health care staffs on the effect of associated factors on UTI induced hyperbilirubinemia. [9] Providing health care professionals with clear insights in the light of current clinical and epidemiological studies could help them to identify jaundiced neonates requiring early urine culture and treatment strategies. [10] Screening for UTI in jaundiced neonates is significant as it is a cost-effective strategy for early detection of cases. Moreover, it helps the NICU hospital authority to create prevention and intervention strategies. [9]

Accordingly, the current study aimed at determining the prevalence and the predictive risk factors of UTI in neonates with an unexplained hyperbilirubinemia in the first 4 weeks of life in Neonatal Intensive Care Unit (NICU) of Aswan University Hospital in an attempt to help in reducing neonatal morbidity and mortality and to make recommendations to improve pediatric practice in Upper Egypt.

Methods

A cross-sectional study was carried out in the neonatal intensive care unit (NICU) of Aswan University Hospital, Aswan Governorate, Egypt from August 2018

to February 2019. The study was conducted on full term (≥ 37 weeks) and late preterm neonates (≥ 35 weeks) who were diagnosed with indirect hyperbilirubinemia in the first 4 weeks of life. Neonates with hemolytic jaundice, sepsis, congenital anomalies, or neonates with evidences of hematomas and other pathologic causes of indirect hyperbilirubinemia were excluded.

Using G Power program (version 3.1.9.2), a sample size of 128 neonates was the required sample to detect a prevalence of 12.2% for UTI among jaundiced neonates [11], at a confidence level of 90%, and a precision degree of 5%. The sample was increased to 140 neonates. Consecutive sampling was adopted till attainment of the required sample size.

Data collection

All the included neonates in the study were subjected to the following:

1. Parents filled an interview questionnaire, which included demographic data, jaundice-related data with a special focus on obstetric history, perinatal events and postnatal data including feeding pattern.
2. Full clinical examination and ultrasonography using a 7.5 MHz probe.
3. Laboratory investigations included C.B.C with reticulocytes, ESR, blood group and RH, Coomb's test, serum bilirubin level (total and direct bilirubin), liver enzymes and CRP. In addition to urine analysis and urine culture and sensitivity. We combined microscopic urine analysis with urine culture to increase the reliability of our findings.

For blood sampling, 5 ml of peripheral blood was taken and divided into two parts; the first 3 ml was taken in EDTA vacutainer for CBC with reticulocytes %, blood group, RH, and Coomb's test. The remaining 2 ml was taken in plain vacationer for serum bilirubin level, liver enzymes, and CRP.

Urine samples from neonates were collected by urinary catheterization following the standard procedures including ensuring enough amounts of urine volume (usually >10ml), using protective equipment and, sterile laboratory tools to avoid contamination. Complete urine analysis was done on the urine specimens by microscopic examinations where centrifuged urine was investigated microscopically for pus cells, WBCs and RBCs morphology. Pyuria is defined by ≥ 10 leukocytes per high power field. [12] Bacteriuria is defined by the presence of any bacteria per 10 oil immersion field of Gram-stained smear. [13]

Urine culture (diagnostic test for UTI**)** was done to all neonates. The presence of bacteria

in a fresh, Gram-stained specimen of uncentrifuged urine correlates with 10^5 colony-forming unit/mL in culture. The threshold for bacteriuria was the presence of at least 5×10^4 colony forming unit/mL of a single urinary pathogen. [14)

The sensitivity of isolated bacteria to antibiotics was done through diagnostic sensitivity test agar. The bacterial isolates were subjected to in vitro susceptibility tests against some common antibiotics. First the organisms showing bacteriuria were inoculated into peptone water then they were plating on Mueller-Hinton agar. Antimicrobial sensitivity discs were pressed on the surface of the sensitivity agar using sterile techniques. The plates were incubated at 37°C for 24 hours and the areas of growth inhibition were measured. [15) The discs used were ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, ceftazidime, ceftriaxone, cefotaxime, cefepime, cefaxone, norfloxacin, ciprofloxacin, levofloxacin, gentamycin, amikacin, and piperacillin. Quality control in all stages was assured.

Statistical analysis

Data were analyzed by SPSS version 26. The quantitative variables were shown as mean, standard deviation (SD) and range, while the qualitative variables were expressed as frequencies. Two groups were compared with the independent sample t-test for the quantitative variables. Chi-square test and Exact test were applied to compare qualitative variables. A P-value< 0.05 was accepted as statistically significant.

Ethical consideration

The study followed the principles of the Declaration of Helsinki and approved by the Ethical Review Committee of Aswan Faculty of Medicine. The purposes, steps, and benefits of the study were discussed with the parents. Written informed consent was obtained from all parents before the enrollment of their neonates in the study, In case parents were not able to read, an impartial witness had to be there at that time to explain accurately the content of the informed consent and sign it on the behalf of them. Confidentiality of all data had been ensured. Ethical matters including plagiarism, data fabrication, double publication have been discerned by the authors.

Results

During the study period, 140 neonates with unexplained hyperbilirubinemia were investigated. The mean age for the studied cases was 5.61 ± 3.4 days, male to female ratio was 67.1%: 32.9%. The mean gestational age was 37.37 ± 1.5 weeks. And mean birth weight was 3006 ± 505 grams. Caesarian section deliveries represented about sixty percent of the investigated sample (58.6%). Nearly half of them (48.6%) were fed with breast milk, 38.5% were fed with formula, and 12.9% were mixed fed.

Urine culture was done for all the studied neonates (Figure 1) and identified positive culture in 36 neonates (25.7%) and negative culture in 104 neonates (74.3%). Table (1) showed that Escherichia coli was the dominant organism isolated; it was isolated in 66.7% of positive specimens, Klebsiella was isolated in 22.2%, while Enterobacter was isolated in 11.1%.

Amikacin was the most common antibiotic sensitive to the isolates as it was identified in two-thirds of neonates (33.3%). Whereas, Ampicillin was the utmost resistant antibiotic as it was identified in (44.4%) of neonates with (+) urine culture.

In univariate analysis, the demographic and jaundice-related data of the positive UTI neonates compared to negative UTI neonates are outlined in Table 2 and Table 3 respectively. Most of the cases with UTI (72.2%) were males, neonates with UTI had smaller gestational age in weeks (36.89 ± 1.1) compared to those without UTI (38.21 ± 1.6) with a P-value = 0.040. The mean age of presentation was 4.63 ± 2.0 days in the UTI group in comparison to 5.51 ± 1.5 in non-UTI group, P-value= 0.022. The hospitalization period was significantly longer in the UTI positive cases than UTI negative cases (6.21±1.2 Vs. 4.6±1.5 days, P=0.042).

Regarding the mean duration of phototherapy, the study data showed that UTI positive neonates received phototherapy for 75.91 ± 19.8, hours, while UTI negative neonates re-

ceived 63.33 ± 21.0 hours of phototherapy, with statistically associated with a positive urine culture, P-value = 0.033.

Cesarean section delivery in the UTI group was 55.5% in comparison to 59.9% in non-UTI group, P-value = 0.091. The presence of maternal complications was detected among the mothers of positive neonates. Premature rupture of membrane was the most frequently observed complication with an occurrence of 22.2%.

Regard to lab results (Table 3), the mean of total serum bilirubin level was significantly different between the UTI positive and negative neonates (18.68 ± 0.9 mg/dl vs. 16.01 ± 1.4 mg/dl respectively, P value= 0.044).

An increase in the number of white cells in the blood (WBCs) was high in the UTI (+) neonates in comparison to the UTI (-) neonates (13.65 ± 0.7×103 vs. 7.56 ± 0.3×103, P-value < 0.001). Other values of CBC parameters were within normal levels in both studied groups. Liver parameters like ALT and AST levels also were normal. C reactive protein (CRP) was positive in 11.1% of UTI group whereas it was positive in 13.5% of non UTI group, with P-value= 0.580.

The mean of pus cells in urine was 26.72 ± 1.8 HPF in the UTI group compared to 4.69 ± 0.5 HPF in the non-UTI group with P-value< 0.001. Pyuria was positive in all UTI subjects, while sterile pyuria was shown in 19.2% in the non-UTI subjects.

All UTI positive neonates were evaluated by abdominal ultrasonography and it revealed normal finding for all of them.

In multivariable logistic regression analysis (Table 4), jaundiced neonates with an increase in the number of WBCs in their blood had 6.9 times higher odds of developing UTI compared to those with low values [AOR= 6.903; 95% CI (2.79- 17.28), P= 0.001]. The odds of developing neonatal UTI is significantly higher also with the small for gestational age neonates [AOR = 4.07; 95% CI (1.06-11.05), P= 0.021], neonates who received prolonged duration of phototherapy [AOR = 3.50; 95% CI (1.99-8.62), P= 0.034], neonates born from mothers who had history of obstetric complications [AOR =2.92; 95% CI (1.02- 5.99), P= 0.001].

Figure (1): Magnitude of UTI among studied cases

- Neonates with positive urine culture; n=36
- Neonates with negative urine culture; n=106
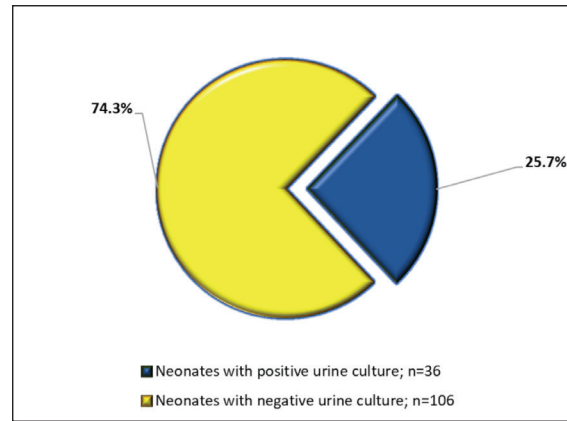
Table (1): Isolated organisms in urine cultures, antibiotic sensitivity and resistant

Variable	Category	N = 36
Organism	E. Coli	24 (66.7%)
	Klebsiella	8 (22.2%)
	Enterobacter	4 (11.1%)
Antibiotic sensitivity	Amikacin	6 (33.3%)
	Cefaxone	2 (5.6%)
	Cefepime	4 (10.9%)
	Cefotaxime	2 (5.6%)
	Ceftazidime	2 (5.6%)
	Ciprofloxacin	2 (5.6%)
	Gentamycin	8 (22.2%)
	Levofloxacin	2 (5.6%)
	Norfloxacin	2 (5.6%)
Antibiotic resistant	Ampicillin	16 (44.4%)
	Amoxicillin-clavulanic acid	8 (22.2%)
	Piperacillin	6 (16.6%)
	Ampicillin & Sulbactam	2 (5.5%)
	Ceftriaxone	2 (5.5%)
	Cefaclor	2 (5.5%)

Discussion

Urinary tract infection (UTI) is a microbial invasion of the urinary tract tissues expanding from the renal cortex to the urethral meatus. Some studies have indicated that hyperbilirubinemia may be the first sign of UTI in early neonates. [3, 16,17] Hyperbilirubinemia also can lead to UTI by altering bactericidal activity in the blood serum of jaundiced newborns making them more susceptible to infections. [18]

In this study, the frequency of UTI among the studied jaundiced neonates was 25%, which agrees with the studies done in Leba-

Table (2): Demographic and clinical characteristics of the studied neonates

Variable	Neonates with UTI (No=36)	Neonates without UTI (No=104)	P-value
Gestational age in weeks (Mean ± SD)	36.89 ± 1.1	38.21 ± 1.6	0.040*

Gender
    Female
    Male
	
	10 (27.8%)
	26 (72.2%)
		
		36 (34.6%)
		68 (65.4%)
			0.0411**

Birth weight (gram) (Mean ± SD)	2592 ± 504	3011 ± 405	0.094*
Onset of jaundice (day)	2.61 ± 0.2	4.10 ± 0.3	**0.031***
Age at presentation (day)	4.63 ± 2.0	5.51 ± 1.5	**0.022***
Hospitalization period (day)	6.21±1.2	4.61±1.5	**0.042***
Duration of phototherapy (hour)	75.91 ± 19.8	63.33 ± 21.0	**0.033***

Mode of delivery
    Caesarian section (C.S)
    Normal vaginal delivery (NVD)
	
	20 (55.5%)
	16 (44.5%)
		
		62 (59.9%)
		42 (40.1%)
			0.091**

Feeding type
    Breast feeding
    Formula feeding
    Mixed
	
	16 (44.4%)
	14 (38.8%)
	6 (16.8%)
		
		52 (50%)
		42 (40.4%)
		10 (9.6%)
			0.053**

Maternal obstetric complications
    Hyopthyroidism
    Oligohydramniosis
    Preeclampsia
    Gestational diabetes mellitus
    Premature rupture of membrane≥18 hour
	
	2 (5.5%)
	2 (5.5%)
	2 (5.5%)
	4 (11.1%)
	8 (22.2%)
		
		0 (0.0%)
		0 (0.0%)
		0 (0.0%)
		4 (3.8%)
		0 (0.0%)
			0.001***

*T-test was used to compare the difference in means between the two groups
**Chi-square analysis was used to compare the difference in proportions
***Exact test was used to compare the difference in proportions
--Significance level is considered when p ≤ 0.05

Table (3): Lab investigations among the studied cases

		Neonates with UTI (No=36)	Neonates without UTI (No=104)	P-value
Total serum bilirubin (mg/dL)		18.68 ± 0.9	16.01 ± 1.4	**0. 044***
Direct serum bilirubin (mg/dL)		0.49 ± 0.03	0.52 ± 0.02	0.510
white blood cells WBCs *10³		13.65 ± 0.7	7.56 ± 0.3	**< 0.001***
HB g/dl		16.31±1.8	15.21±2.1	0. 110
PLT × 10³.		261.54 ± 64.7	266.50 ± 54.0	0. 150
ALT level (U/L)		36.61 ± 8.8	36. 11 ± 9.1	0.940
AST level (U/L)		27.51 ± 7.3	28.21 ± 7.1	0. 850
CRP	**Negative**	32 (88.9%)	90 (86.5%)	0.580
	Positive	4 (11.1%)	14 (13.5%)	
Pus cells in urine		36.72 ± 10.8	4.69 ± 2.5	**< 0.001***
Pyuria	**No**	0 (0%)	84 (80.8%)	**< 0.001***
	Yes	36 (100%)	20 (19.2%)	

*Chi-square analysis was used to compare the difference in proportions
**T-test was used to compare the difference in means between the two groups
--Significance level is considered when p <0.05

non and Egypt by Omar et al. (2011) study, [19] and Rashed et al. (2014) study.[9] They reported a UTI prevalence of 21% and 25% respectively. Mutlu et al. (2014),[4] and Ozcan et al. [20] reported rates of 18% and 16.7% in Turkey. Lower rates of 5.5%, 3.8% respectively were detected by Chen et al. (2011), [21] and Zarkesh et al. (2015). [22]

Table (4): Multivariable logistic regression analysis for UTI predictors of the studied cases attending NICU in Aswan

	P-value	AOR	95 % CI
Gestational age in weeks	**0.021**	4.07	1.06-11.05
Gender (male)	0.070	1.10	0.43-3.29
Onset of jaundice (day)	0.951	0.53	0.11-2.78
Age at presentation (day)	0.066	1.10	0.60-1.41
Hospitalization period (day)	0.091	0.80	0.20-2.56
Duration of phototherapy (hour)	**0.034**	3.50	1.99-8.62
Maternal obstetric complications (yes)	**0.001**	2.92	1.02-5.99
WBCs *103	**0.001**	6.90	2.79-17.28
Pus cells in urine	0.050	2.91	1.09-4.62
constant	**<0.001**		

AOR: Adjusted odds ratio   Adjusted R2 = 0.34   Model sensitivity = 76.1%
Note. Variables with a P-value of <0.05 in bivariate analysis were entered in the multivariate model.

In neonates with UTI, the dominant organism indicated by urine culture was E. coli. This result was similar to the results obtained from prior studies conducted in Baghdad by Hussien et al. (2010), [23] and in Turkey by Mutlu et al. (2014), [4] and Ozdogan et al. (2018). [11] Other researchers reported that klebsiella was the common organism in jaundiced newborns with UTI [16,19] While Enterobacter was the main isolated organism in Bilgen et al. (2006) study. [24]

In agreement with Nejad et al. (2010), [25] our study showed that Amikacin was the most common antibiotic sensitive to the isolates. In contrast, meropenem was the most sensitive antibiotic in Trihono et al. (2012) study. [2]

Most cases of positive UTI (72.2%) were males. This comes into agreement with several studies.[2, 4, 11,16, 22] In contrary to our finding (Table 4), male gender was a significant predictive risk factor in the development of UTI among jaundiced neonates admitted at the NICU in Zagazig and Menofiya governorates, Egypt. [9]

In line with Mutlu et al., (2014) [4], the study was noticed that neonates with UTI had smaller gestational age in comparison to those without UTI. Some studies observed that neonates with UTI had greater gestational age [11,26]. In general infants with small for gestational age have an immature hepatic system that reduced bilirubin excretion, and RBCs lifecycle. As well as the associated delay in feeding entry in those less mature infants accelerates the increase in the serum bilirubin level [27].

Similar to those of the previously reported studies, [11,16,19, 22, 23, 28] this study reported no significant difference between weight in UTI and non-UTI neonates.

Although the study declared that no significant difference between the two groups of the studied neonates concerning the mode of delivery. Vaginal delivery was considered as a predictive risk factor for UTI by some investigators. [2,11] On the other hand, high frequency of cesarean section deliveries were observed among UTI jaundiced neonates in Iran. [26]

The current study in accordance with the other studies [17, 19, 24] that found newborns with the early onset of jaundice were more likely to have UTI. This result was inconsistent with the other studies' findings. [21, 23]

The time of presentation in positive UTI jaundiced neonates was significantly earlier than the time of presentation in negative UTI neonates (Table 2). Our result is in accordance with the results from the earlier studies. [20, 21] and in contrast to two studies [11, 26] which asserted that positive UTI infants had late presenting time.

The study established that jaundiced neonates with a mother history of obstetric complications have more positive UTI results. In line with this study, several researchers agreed that presence of maternal complications was a strong predictive risk factor for the occurrence of UTI. [2,9,17]

In agreement with prior studies, [19,20] our study detected a significant high serum bilirubin level in the studied infants with UTI

compared with those without UTI. Other studies [3,17] revealed significant low serum bilirubin levels in their studied subjects with positive UTI results. While, some studies [4,16, 21, 28] showed that no significant differences between the UTI group and non-UTI group.

Hyperbilirubinemia associated with UTI may be due to hemolysis and increased red cell fragility produced by certain strains E. coli and other gram-negative microbes. Any slight hemolysis may elevate the serum bilirubin levels due to immature liver conjugating processes in neonates [18]. Hyperbilirubinemia also can overload through the effect of direct bacterial and endotoxin mediated products that could enter the biliary system through several mechanisms. triggers cholestatic jaundice causing microcirculatory problems in the liver. [5]

White blood cells count was significantly increased in UTI group compared to non UTI group as a response to the inflammatory processes. This finding agrees with earlier studies. [17,28, 29] They recommended that CBC investigation could be included as a routine screening tool even in asymptomatic hyperbilirubinemia.

No significant difference between the two studied groups regarding CRP results. Rashed et al. (2014) [9] reported positive CRP results among jaundiced newborns with UTI. Mutlu et al. (2014) [2] reported negative CRP results. Malla et al. (2016) [28] reported significant CRP in non-UTI subjects with prolonged jaundice. The reason for the differences of such results may be related to that CRP is used as screening indicators of inflammatory processes with low sensitivity. [29]

All UTI cases had positive pus cells in urine analysis while the presence of pus cell (pyuria) in non-UTI neonates was present in 19.2% in non-UTI group. Sterile pyuria is the presence of inflammatory cells with a negative urine culture. [30] Shahian et al. (2012) [16] indicated that dehydration in the jaundiced neonates even minor may be responsible for the presence of WBC in their urine. Pyuria cannot serve as a sensitive marker in the evaluation of UTI in jaundiced infants, it could give over-estimation numbers of UTI cases. [31] However, bacteriuria was deemed as a more reliable parameter in the identification of UTI cases. [1]

The mean duration of phototherapy in neonates with UTI is elevated than that found among neonates without UTI (Table 2). Early detection of the UTI in these neonates may lead to a reduction on the time needed for phototherapy. The presence of UTI should be considered in case of a poor response to phototherapy. [32]

Contrary to previous studies that limited the screening of UTIs for neonates with prolonged jaundice, [2,4, 16, 21] Our study in line with Saudi Arabia study, [32] that advocated on the early screening for all neonates with unexplained hyperbilirubinemia mostly in case of all other causative factors of hyperbilirubinemia are ruled out.

Conclusions and recommendations

In view of our results, the prevalence rate of urinary tract infections in the studied newborn with unexplained hyperbilirubinemia was 25%. E-coli was the frequently isolated microorganism. Amikacin was the effective first-line therapy for the treatment of UTI. For early detection and treatment of the newborns and avoid higher morbidity, this study indicated the importance of routine screening of UTI (urine culture) as part of the diagnosis of unexplained hyperbilirubinemia in neonates with leukocytosis, small for gestational age, prolonged duration of receiving phototherapy, and neonates born from mothers who had a history of obstetric complications.

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Four-Dimensional Echocardiographic Volumetric and Functional Assessment of the Left Atrium in Healthy Male Subjects in Relation to Age

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ABSTRACT

Background: Four-dimensional echocardiography technique results in marked improvements in terms of quality and accuracy in assessing the size and function of heart chambers, especially the left atrium as its geometric variation has a notable impact on the results when using traditional echocardiographic techniques alone. Thus, this study aimed at investigating the probability of significant functional and morphological changes in the left atrium with advancing age using Four-dimension echocardiography. **Method**: Four-dimensional technique derived left atrial phasic volumes and functions were assessed in (30) healthy subjects with mean age of 73.6 (±3.6) years and (30) young subjects with mean age of 23.17 (±3.5) years. **Results:** Old age group showed a significant increase in maximum left atrial volume index, minimum left atrial volume index and pre-atrial contraction left atrial volume index compared to young age group (31.52±1.09 vs. 26.44±1.03, $P < 0.001$), (17.93±0.43 vs. 15.89±0.44, $P < 0.00$) and (25.73±1.003 vs. 22.34±0.77, $P < 0.01$), respectively. In the old age group, the passive left atrial emptying function (LAEF) significantly decreased (20.43±0.25% vs. 24.96±0.93%, $p<0.00$), while the active LAEF significantly increased (37.36±1.33% vs. 32.65±1.13%, $P< 0.009$) in comparison to the values of the young subjects. **Conclusions:** These results suggest that left atrial structural as well as functional changes occur with advancing age in absence of evident pathological causes and 4-dimentional echocardiography can be used to evaluate these changes.

Keywords: Four-dimensional echocardiography, Atrial volume, Atrial function

Abbreviation: LA: left atrium, LV: left ventricle; 4D: four dimensional, 3D: three dimensional, LAVI: left atrium volume index, LAEF: left atrium emptying function, BSA: body surface area, Pre-AC: pre-atrial contraction, FR: frame rate.

Introduction

Diastolic dysfunction and ventricular stiffness are associated with aging process (Redfield, Jacobsen, Borlaug, Rodeheffer, & Kass, 2005). It is important to evaluate left atrial function as well as its size as they are considered good predictors of cardiovascular outcomes (Hirose et al., 2012). More recently, 4D-echocardiography had been used to assess the size and function of LA. This is because 4D-echocardiography technique outperformed 2D-echocardiography in terms of giving more precise results. In this concern, 2D-echocardiography technique assumes that the LA geometry is unchanging (de Isla et al., 2009). Recent reports documented that three dimensional (3D) echocardiography is more dependable than 3D-computed tomography (CT) (Avelar et al., 2010; Nagaya et al., 2013). Hence, this study aimed at using 4D-echocardiography technique to illustrate the possible LA changes that occur with advancing age.

Methods

Two groups with matched body surface area (BSA) were involved in this study. Group A consisted of 30 subjects (with mean age of 73.6 (±3.6) years, mean BSA of 1.58 (±0.22) m²). Group B consisted of 30 subjects (with mean age of 23.17 (±3.5) years, mean BSA of 1.67(±0.24) m²). All subjects were subjected to thorough medical history and physical examination to exclude other conditions (hypertension, lung disease and diabetes) that might affect the studied parameters. The presence of arrhythmia as in atrial fibrillation, a paced rhythm, and valvular heart disease were al-

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so considered as exclusion criteria. 4D-echo-cardiography machine (Vivid, E9, USA) with 4V probe were used. This could result in a time–volume curve of the left atrium. A FR of 30–40 frames/second was used (Fig. 1). Electrocardiogram (ECG) was connected to each participant. Recall a full volume image and then LA volume quantification were carried out by proper alignment of view in apical two, three and four chamber views. Several marks were set on the endocardial border of LA in the apical two, three and four chamber view. Automatic tracing of LA endocardium with manual adjustment for proper tracing was then performed. After that, automatic tracking and analysis of wall motion within entire cardiac cycle was performed. LA phasic function could be measured from time–LA volume curves during sinus rhythm. **LA total emptying function (EF) (reservoir function) was defined as "(maximum LA volume – minimum LA volume)/ maximum LA volume × 100%. LA passive EF (conduit function) was defined as (maximum LA volume – pre-atrial contraction LA volume)/ maximum LA volume × 100%. LA active EF was defined as (pre-atrial contraction LA volume – minimum LA volume)/ pre-atrial contraction LA volume × 100%"** (Hoit,

2014; Kowallick et al., 2014; Vianna-Pinton et al., 2009). LA volume was indexed to body surface area. The Ethics Committee of our institution has approved this study. Additionally, a written consent was taken from all participants before participation.

Statistical analysis

Calculations of mean and standard error of mean were done for the parameters of interest. The groups under study were compared by employing independent T-test. The value of probability (P) of <0.05 was regarded as significant at (α=0.05). A linear regression test was conducted to define the correlation between different parameters of echocardiography and age. The statistical analysis was performed by utilizing IBM SPSS 18 software.

Results

Results of this study showed that elderly individuals (group A) had significantly higher maximum LA volume index (P<0.00), higher minimum LA volume index (P<0.00) and higher pre-atrial contraction LA volume index (p<0.01) than the younger age group

Table 1: The values of maximum LAVI, minimum LAVI and Pre-AC LAVI according to study groups

		Group A N: 30 Age:73.6±3.6 BSA (m2): 1.58±0.22	Group B N: 30 Age:23.17±3.5 BSA (m2): 1.67±0.24	*P* value
Maximum	LAVI, ml/m2	31.52±1.09	26.44±1.03	0.001
Minimum	LAVI, ml/m2	17.93±0.43	15.89±0.44	0.00
Pre-AC	LAVI, ml/m2	25.73±1.003	22.34±0.77	0.01

Table 2: The passive LAEF and active LAEF according to study groups

	Group A N: 30 Age:73.6±3.6 BSA (m2): 1.58±0.22	Group B N: 30 Age:23.17±3.5 BSA (m2): 1.67±0.24	*P* value
Passive LAEF, %	20.43±0.25	24.96±0.93	0.00
Active LAEF, %	37.36±1.33	32.65±1.13	0.009

Data are presented as mean ± SEM. BSA; body surface area, LAEF; left atrium emptying function

(group B) (table 1). Passive LA emptying function was significantly lower ($P < 0.00$) in group A compared to group B; while active LA emptying function was statistically higher in group A (table 2). Linear correlation regression test showed positive linear correlation of maximum, minimum LA volume and active LA emptying function with age in the two groups (figures 2, 3 & 4); while negative correlation was documented between age and passive LA emptying function (figure 5).

Data are presented as mean ± SEM. BSA; body surface area, LAVI; left atrium volume index, Pre-AC; pre atrial contration.

Figure 1: Four-dimensional echocardiography and time–left atrial volume curve during one cardiac cycle

Figure 2: linear regression plot of age in (years) and maximum left atrial volume index (Max LAVI) in (ml/ml2)
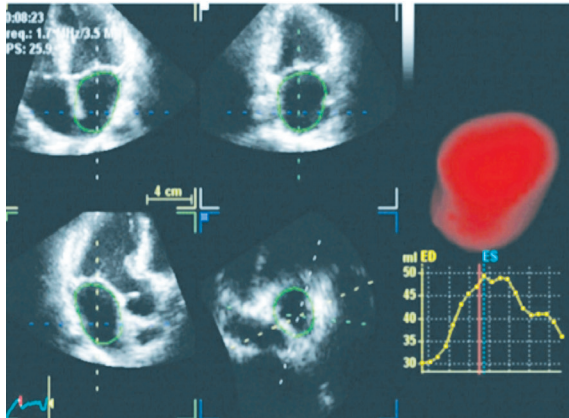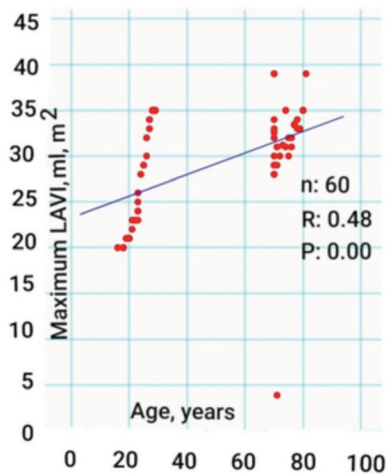

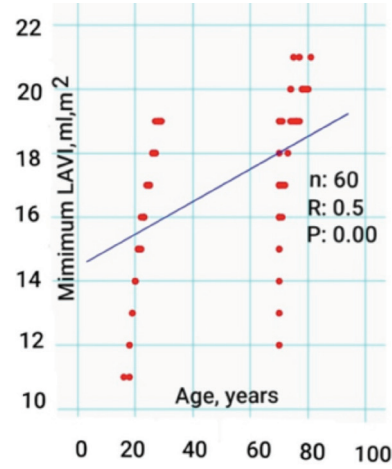**Figure 3:** linear regression plot of age in (years) and minimum left atrial volume index (Min LAVI) in (ml/ml2)

Figure 4: linear regression plot of age in (years) and active emptying function (EF %)
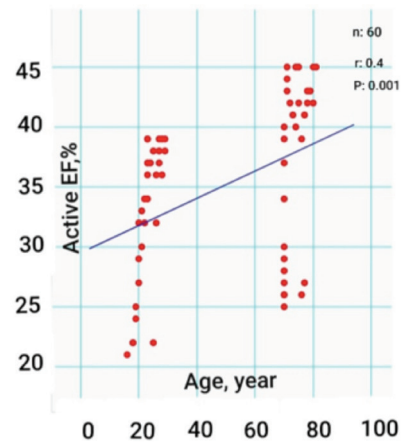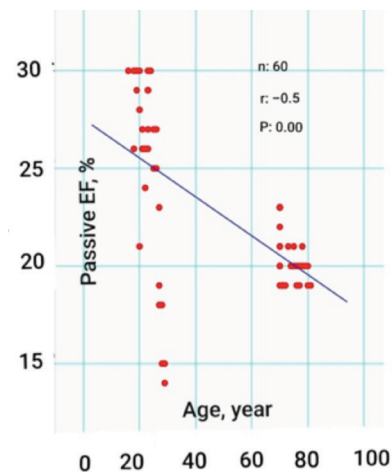

Figure 5: linear regression plot of age in (years) and passive emptying function (EF %)

Four-Dimensional Echocardiographic Volumetric and Functional Assessment of the Left Atrium in...

Discussion

The development of 4D-echocardiographic technique is considered a major development in the field of echocardiography. It allows more accurate assessment of volumes and functions of cardiac chambers than previous methods (Kawaguchi, 2008; Seo et al., 2009; Takeguchi, Nishimura, Abe, Ohuchi, & Kawagishi, 2010). Emphasizing this fact, there has been an increasing number of studies utilizing the technique to evaluate the phasic volume and function of LA as the main investigative tool (Kawai et al., 2004; Warita, 2012). In this concern, Rohner et al. (2011) reported a good correlation between 3D-echocardiography and cardiac CT imaging in the volumes of LA and LAEF calculations (r = 0.92 and r = 0.82, p < 0.001, respectively). Yet, according to the researchers' knowledge, no study has been conducted to evaluate the validity and accuracy of 4D-echocardiography when measuring the volume and function of LA.

There is rising evidence that the evaluation of the enlargement and functional impairment of LA by using echocardiography predicted significant cardiovascular consequences with high accuracy (Moller et al., 2003). Hence, 3D-echocardiography gives proper evaluation of the volume and function of LA than 2D-echocardiography by utilizing 3D-CT as a gold standard (Nagaya et al., 2013).

In the current study, the assessment of the volume and function of LA was performed by using 4D-echocardiography. The use of 4D-echocardiography allows the evaluation of the features that are affected by aging process.

It is proposed that the increase in the maximum volume of LA represents the morphological marker of LV diastolic dysfunction since its diastolic pressure is directly exerted on LA phasic volumes (Tsang, Barnes, Gersh, Bailey, & Seward, 2002).

When LV relaxation becomes abnormal, the conduit function reduces with the increase in the booster pumping of LA (Spencer et al., 2001). Because the filling pressure of LV gradually rises with the progression of diastolic dysfunction, LA functions largely as a conduit (Hirose et al., 2012). The result found in the current study shows agreement with those found in the study of Aurigemma et al. (2009) that the volume of LA increases with age.

Boyd, Schiller, Leung, Ross, & Thomas (2011) reported changes in LA volumes with age in a larger study. They demonstrated that LA volume index increases by 0.05 ml/m² yearly. On the other hand, D'Andrea et al. (2013) revealed that the size of LA differs with age, but it is considerably larger only in persons aged 50 years and older.

Those reports, however, employed traditional 2D-echocardiographic measures rather than the more advanced 4D-echocardiographic ones employed by the current study. This study found that elderly people have LAEF that is significantly greater than in young people. Concerning the passive LAEF, it was found that elderly people have significantly lower values than the young people. Moreover, Boyd, Richards, Marwick, & Thomas (2011) and Sun et al. (2013) revealed increase in the atrial contractile function with advancing age. However, only few studies stated no significant differences among different age groups.

Conclusions

Left atrial structural as well as functional changes occur with advancing age in absence of evident pathological causes and 4-dimentional echocardiography can be used to evaluate these changes.

Limitations
It was difficult to obtain a large number of healthy individuals, especially after age of 70 as most people in this age group have chronic medical diseases. Also, image quality posed a problem with older age group because 4D-echocardiography needs good image resolution for proper border delineation.

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Alopecia Areata And Serum Vitamin D in Iraqi Patients: a case-control study

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ABSTRACT

Background: Alopecia areata (AA) is a typical hair issue, which may have obliterating mental and social outcomes and is portrayed by the nearness of nonscarring alopecia.

Objective: This examination has targets to assess the serum nutrient D levels , with AA; contrast the outcome and clearly sound control; and confirm relationship between AA types and serum nutrient D levels.

Patients Also Methods: the examine might have been led clinched alongside Tikrit educating healing facility throughout those time starting with June 2019 of the limit for January 2020. Irrefutably the quantity of subjects associated with the assessment was ninety individuals isolated in two social events; the patients bundle were forty five the people who whimper of AA while the resulting gathering including a forty five age and sex-made solid volunteers were picked as a benchmark gathering. The degree and movement of the alopecia were noted and the patients were meticulously broke down for signs of various ailments. Research center assessments were led to patients and also to those control population, these included serum vitamin D levels were measured as 25-hydroxyvitamin D {25(OH)D} using a chemiluminescence microparticle immunoassay. Blood models were gotten starting with patients and control subjects after totally taught consent was gotten.

Results : An essential complexity may have been found for serum 25-OH Vit D levels between patients other than controls. Vitamin D sufficiency were more common in controls than in patients. Serum Vitamin D was deficient in both cases and controls group; but, the deficiency was significantly more throughout AA group (35. 6%) compared to the handle group (11. 1%). Among the list patients gathering, levels associated with nutrient D were totally higher in guys in contrast with females.

Conclusions: AA might be related with nutrient D deficiency as mean degrees of nutrient D of patients were seen as fundamentally lower than typical sound controls.

Key words: Alopcia areata (AA); Alopcia areata totalis (AT); Alopcia areata universalis (AU); 25-hydroxyvitamin D {25(OH)D}; Venereal Disease Research Laboratory test (VDRL); The Treponema pallidum haemagglutination test (TPHA); The antinuclear antibody test (ANA); anti-double stranded DNA (anti-ds-DNA); Diabetes mellitus (DM); Rheumatoid arthritis (RA); Systemic lupus erythematosus (SLE).

INTRODUCTION

Alopecia areata (AA) is the most well-known reason for irritation intervened male pattern baldness influencing roughly 0.1–0.2% of everyone, with a lifetime danger of 2% [1]. Alopecia areata (AA) is a typical hair issue, which may have crushing mental and social outcomes and is described by the nearness of nonscarring alopecia [2].

Alopecia areata may clinically give a single or possibly couple round or even oval patches of men example sparseness on the particular scalp or any various other hair-bearing zone from the entire body.

Alopecia areata may in like method present as a band-like alopecia on the occipital scalp or even diffuse alopecia, and can progress to be able to lost all terminal curly hair on the scalp totalis (AT) or lack all scalp and curly hair (AA universalis (AU) [3].

Trademark hairs, known as "exclamation perspective hairs," might be seen inside or around those territories from claiming alopecia. The hairs would decreased at the scalp end for thickening toward those distal end. Hair draw tests led during the fringe

of the lese greatness might make emphatically corresponded (six hairs alternately more) for illness movement [4].

The pathophysiology of AA is indistinct and a few lines of proof help the theory that AA is an organ-explicit immune system ailment with hered-

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itary inclination and an ecological trigger [5]. Numerous hypotheses have been created with respect to the charge of AA since its depiction by Sauvages within 1760. At long last, during the 1960s, the autoimmunity speculation picked up fame. The thought of autoimmunity in AA is maintained by the substantial pace of relationship together with other safe mediated afflictions like vitiligo and thyroiditis, and the reaction observed to safe altering prescription drugs [6].

Nutrient D3 has been known as the 'daylight' nutrient in light of the fact that the arrangement of nutrient D is intervened by introduction to daylight. Expanded sun based bright (UV) presentation is legitimately identified with expanded nutrient D creation. Accordingly, over 90% of people get their nutrient D necessity by easygoing introduction to daylight [7].

In keratinocytes and other cellular sorts, 1,25(OH)2D3 oversees advancement and separation. Consequently nutrient D analogs have been presented for the remedy of the hyperproliferative skin malady psoriasis. As of overdue, sebocytes had been outstanding as 1,25(oh)2d3-responsive goal cells, exhibiting that supplement d analogs is most likely convincing inside the treatment of acne. Other new components of supplement D analogs remember significant impacts for the resistant framework just as in different tissues insurance against malignancy and different ailments, including immune system and irresistible sicknesses. It very well may be expected that dermatological suggestions on sun assurance and wellbeing efforts for skin malignancy anticipation should be rethought to ensure an adequate nutrient D status [8].

Nutrient D assumes a significant job at the same time as the guideline about affecting safe framework moreover the multiplication and differentiation of keratinocytes and sebocytes. It has been entangle in the origination and development of a disease and therapy of numerous dermatological matter, for symbol, psoriasis, atopic dermatitis, vitiligo, acne vulgaris as well as alopecia areata [9].

Since nutrient D is basic considering encouraging the torso's reaction to couple cor-

poreal along with conceivably unsafe boosts, depressed nutrient D situation expands effective danger as concern numerous interminable sicknesses. Our own selves won't have striking genuine weight in this regard ailments before privately reestablish populace equivalent nutrient D position through farcical specimen height a well-known won for the time being advancement as concerns personal functions and activities of life [10].

Patients and methods

precise assent was seized in distinction to every patient affected role after full clarification about the chance of the present appraisal and a moral endorsing was earn from the Scientific Ethical Committee of Tikrit University College of Medicine.

The investigation was directed in Tikrit Teaching Hospital all the while duration from June 2019 as far as possible of January 2020. All patients were inspected clinically, then interviewed and detailed questionnaires were completed for each of them. Those analysis about AA might have been made for fact from claiming historical backdrop furthermore physical examination.

The hard and fast total of proposal associated with the questioning was ninety individuals isolated in two social events; affecting patients assortment were forty five effective human being who gripe of AA meantime the consecutive collection counting forty five an age and sexuality-facilitated strong enlist were picked as a criterion gathering none of these gave a history of increased hair loss or had alopecia clinically.

Consideration criteria: Know patients for a AA not getting any medicine to AA for at any rate a half year before incorporation in the investigation.

Avoidance criteria:
- All patient catching supplement D complement, iron courses of action, supplement B, folic destructive or calcium (Ca) complement over the latest a half year; systemic corticosteroid therapy and previous systemic therapy; immunosuppressive agents, phototherapy during last one

year, pregnant and lactating women, have autoimmune disorders, sarcoidosis, renal disease, malignancy and malabsorption were excluded from study; Patients with any associated disease and chronic systemic disease.

- Systemic and/or scalp disease that might be related to hair loss.
- Patients with different reasons for male pattern baldness, for example, androgenetic alopecia, telogen effluvium, tinea capitis, cicatrical alopecia were barred from the study. Patients with recurrent AA and those already on treatment for AA were excluded.

The level of scalp male example hairlessness was directed by isolating the scalp within 4 portion, trailed aside ostensibly choosing the degree about scalp male example sparseness current any portion and including the statistic well-adjusted, alongside a most outrageous account of one hundred percent. The prsent was settled by the reality of alopecia earnestness in alopecia instrument (severity of alopecia tool score) amount [11]. Scalp male pattern baldness (S) was named pursue: S0=no male pattern baldness; S1=25% male pattern baldness; S2=25–49% male pattern baldness; S3=50–74% male pattern baldness; S4=75–99% male pattern baldness, a= 75–95% male pattern baldness, b=96–99% male pattern baldness; and S5=100% male pattern baldness. Patients were grouped by the seriousness of AA in the direction of: mellow AA – patients which obtain S1; mild AA – patients which obtain S2; and extreme AA – patients which obtain S3, S4 and S5. Patients were additionally charac-

terized by the example of AA through to inconsistent AA or broad AA [alopecia totalis/alopecia universalis (AT/AU)].

Additionally, patients were arranged by length of the malady into patients inside a half year or over a half year.

All subjects study gave a solitary blood test at the main daytime belonging to the assessment, along with serum nutrient D height were estimated as 25-hydroxyvitamin D {25(OH) D} utilizing a chemiluminescence microparticle immunoassay.

Nutrient D height were grouped considering a spellbinding examination as per American Endocrine Society criteria as adequate (≥30 ng/mL), inadequate (21–29 ng/mL), or insufficient (≤20 ng/mL) [12].

Other investigations included complete haemogram, liver function, kidney function, thyroid profile, VDRL, TPHA, ANA and anti-ds-DNA was done whenever indicated.

The factual investigation of the information was finished utilizing understudies' t test for the distinction of means, chi-square test and Fisher's accurate test for proportions. These tests were referenced for p esteems and p estimation of under 0.05 was taken to be noteworthy.

Results

the segment information and clinical information of patients and control are delineated in Table 1.

Forty five patients and forty five sound people were selected in this body of evidence control

Table 1: Segment and clinical information of the considered gatherings.

Factors		Sufferers (n = 45)	Controls (n = 45)	P esteem < 0.05.
Age (years)	Range	10-50	12–49	Not significant (P = 0.1450)
	Mean ± SD	35.53 ± 7	33.2 ± 8	
Gender, n(%)	Males	29 (64.4%)	28 (62.2%)	Not significant at p < .05.
	Females	16 (35.6%)	17(37.8%)	
Duration of disease, n (%)	<6 months	20 (44.4%)		
	>6 months	25 (55.6%)		
Severity of AA, n (%)	S1(Mild)	14(31%)		
	S2 (Moderate)	31 (69%)		
	S3+S4+S5 (severe)	0		

contemplate who met those consideration criteria of this consider. The age range of cases and controls in our study was 10-50 years with mean age of 35.53 ± 7 years in cases and 33.2 ± 8 years in controls which was not statistically significant. There were 29 (64. 4%) guys What's more 16 (35. 6%) females in the situations What's more 28 (62. 2%) guys and 17 (37. 8%) females in the control bunch. Contrast was not measurably critical among cases and controls.

The duration of hair disease below 6 months were 20 (44.4%) and 25 (55.6%) over 6 months duration.

Amongst 45 patients of alopecia areata, 14 (31%) had mild alopecia areata, 31 (69%) had moderate alopecia areata and we did not get any cases of alopecia totalis (AT), alopecia universalis (AU) or ophiasis.

Table 2 reveals to a foremost contrast might have been discovered Previously, serum 25-hydroxyvitamin D levels Around patients Furthermore controls (P<0. 05). Nutrient D adequacy and inadequacy were more typical in controls than in patients however the thing that matters was not factually huge among cases and controls.

Serum Vitamin D was inadequate in the two cases and controls gathering; be that as it may, the lack was altogether more in AA gathering (35.6%) contrasted with the benchmark group (11.1%).

Amidst the patients gathering, height of nutrient D abide essentially higher mod guys contrasted with females, while in control bunch no huge distinction was found among guys and females. Serum 25-OH Vit d levels were a considerable measure of more level clinched alongside female parts over in male parts in the two get-togethers. There was no measurably critical relationship between serum 25(OH)D levels and term of the sickness in patients with AA.

Table 3 shows the patients with moderate type of AA tend to have lower serum vitamin D however, they were statistically not significant.

According to our findings, serum Vitamin D level was found to be 23.2± 6.5 ng/mL in mild AA, levels dropped to 20.7 ± 9.2 ng/mL in moderate AA. Constant drop was noted also in serum Vitamin D levels when correlated with the gender of alopecia areata and severity type of AA although the difference was not statistically significant.

Table 2: connection the middle of those examined bunches Likewise respect Vitamin D Supplement height.

Nutrient 25(OH)D		Sufferers	Controls	P esteem < 0.05.
Gender mean ± SD	Males	29.02±19.64	24.2±19.04	Significant (P = 0.0456) only between patients.
	Females	17.03±16.81	20.24±16.81	
Duration of disease, (ng/mL)	<6 months	13.25±4.08	not significant (P = 0.4315.)	
	>6 months	12.34±3.60		
Nutrient D level (ng/mL),n(%)	Sufficient (≥30 ng/mL)	15 (33.3%)	24 (53.3%)	not significant (P = 0.0569)
	Insufficient (21–29 ng/mL)	14 (31.1%)	16 (35.6%)	*not* significant (P = 0.6525)
	Deficient (≤20ng/mL)	16 (35.6%)	5 (11.1%)	significant (P = 0.0063)
Vitamin D (ng/ml): mean ± standard deviation		22.24±15.81	29.5±14.08	significant. (P = 0.0238)

Table 3: Relationship between mean 25-hydroxyvitamin D esteems with seriousness of AA in guys and females

Severity of AA	S1 (Mild AA)		S2 (Moderate AA)		Total	P value < 0.05.
	No. n(%)	Mean nutrient D (ng/ml)	No. n(%)	Mean nutrient D (ng/ml)		
Male	8(27.6%)	26.3± 17.5	21 (72.4%)	21.9± 9.8	29 (64.4%)	Not significant (P= 0.3957).
Female	6(37.5%)	24.6± 8.4	10 (62.5%)	21.8± 7.6	16 (35.6%)	Not significant (P=. 0.5034).
Total	14(31%)	23.2± 6.5	31(69%)	20.7 ± 9.2	45	Not significant (P= 0.3647).

Discussion

AA may be viewed as will make a T-cell-mediated autoimmunity happening for hereditarily predisposed people. Perplexing cooperations the middle of predisposing hereditary and Ecological elements prone help the incitement for immune-mediated reactions done AA [13].

The dynamic type of nutrient D, 25-hydroxyvitamin D, has to play with modulation impacts on the inherent and adaptive immune arrangement. Nutrient D restrains the arrangement of dendritic cells including diminishes T-cell enactment. Moreover, nutrient D has been accounted for to be included in restraining Th1 differentiation, yet in addition in creating resistance to auto-antigens, expanding CD4+ CD25+ regulatory T-cell action [14]. An association between some immune system maladies, including Type 1 diabetes, Rheumatoid arthritis, Systemic lupus erythematosus, vitiligo, psoriasis, multiple sclerosis (MS), inflammatory bowel disease (IBD), and nutrient D inadequacy acquire been accounted for. The present discovering recommends a particular vitamin d insufficiency could be an Ecological generate for those incitement of autoimmunity [15].

The effect of vitamin D deficiency on causation of AA seems to be associated mainly through its role in immune system regulation. Vitamin D causes shift of TH1 immune response to TH2 response. It also suppresses proinflammatory TH17 response, therefore decreasing the production of proinflammatory cytokines (IL-17 and IL-21). It may also suppress B cell proliferation. Therefore, vitamin D deficiency leads to loss of self-tolerance. AA is predominantly TH1 reaction interceded sickness, in this manner nutrient D insufficiency may assume a causative job [16]. Studies have indicated that nutrient D insufficiency was progressively regular in psoriatic patients and in vitiligo [17]. Another examination has demonstrated that sickness seriousness of rheumatoid arthritis connects with nutrient D insufficiency. Nutrient D substitution has improved SLE in another investigation [18].

Right now, assessed aggregate of 45 AA patients and surveyed their serum nutrient D (25-Hydroxyvitamin D (25(OH)- D)) level.

Then, we compared those level for serum vitamin D from claiming AA patients with that of age and sex matched coordinated solid controls. Mean period of case and control bunches were similar, number of male and female were also comparable between case and control group.

In this study, data of ninety subjects (forty five cases and forty five matched controls) was included and analyzed. In our study was the majority of the cases were males 64.4%. Comparable male power was additionally found by Yilmaz N et al [19]., Cerman AA *et al* [20]. , Attawa EM *et al* [21] and Nassiri S *et al* [22]. Mean age of subjects in cases and controls was found to be 35.53 ± 7 years and 33.2 ± 8 years respectively. Findings of this study was not quite similar to another study from India[23]. In that study, the subjects were two hundred subjects (one hundred cases and one hundred matched controls) that may explain the difference of our result.

In the introduce examination, AA patients shown Generally decreased about serum supplement d level contrasted with controls. This is in concurrence with different investigations [19, 20,22,24,]. In the present examination lower levels of nutrient D were more as often as possible found in female patients as opposed to guys in the two gatherings. Inside the case gathering, there were huge contrasts in regards to serum 25(OH)D levels and the sexual orientation. Similar to this, different examinations [20,22] revealed that serum height of nutrient D were reduced in female AA patients and controls, aforementioned might be expected to by the restricted presentation of females to daylight because of strict and social concerns.

We observed a drop in serum Vitamin D aligned between mild and moderate AA and also between the gender patients. Furthermore, no significant correlation was found between severity of AA, disease duration and nutrient D levels. aforementioned bolstered by Yilmaz et al. [19] and d'Ovidio et al. [24] which discovered no association betwixt those convergences of 25(OH)- D, 1,25(OH)2D3 Also different analytic criterion counting degree of the male design baldness, ailment compass Furthermore number for patches. Another examination likewise found no critical contrasts among the patients with various examples of male pattern baldness

together with their serum groupings of 25(OH) D [22]. In the inverse a progressing report card by Cerman et al. [20] found An foremost rearward association between's low 25(OH) d levels Also reality for AA Concerning illustration for every salt scores. There might a chance to be a couple clarifications for this unpredictability the middle of these examinations. There might be methodological varieties. Serum25 (OH) D displays extraordinary regular variety. Various components can impact serum nutrient D levels, and few have been assessed or investigated in logical examinations.

An investigation of the circulation of serum nutrient D inadequacy among cases and controls indicated a factually critical higher level of nutrient D lack among cases than in controls. The level of nutrient D insufficiency in southern India was assessed to be about 40%–70%.. [25] It is advantageous to make reference to that different investigations which have assessed nutrient D insufficiency when all is said in done populace have utilized distinctive cut-offs to characterize nutrient D inadequacy. A few investigations have characterized values under 35 ng/mL as insufficiency.[26]

There are certain potential limitations of this study like small sample size. More studies on this subject with larger cohorts are certainly warranted. Secondly, patients may be followed up after replacement of vitamin D for their severity scores. In conclusion AA might be related with nutrient D lack as mean degrees of nutrient D of patients were seen as fundamentally lower than ordinary solid controls.

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Alopecia Areata And Serum Vitamin D in Iraqi Patients: a case-control study

MANUAL DE SALUD MENTAL

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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.

Este libro se ha ideado como una síntesis didáctica de los conocimientos nutricionales que prevalecen actualmente. Se ha procurado aislar en cada capítulo lo más trascendente del tema de manera tal que no se desvirtúe la información en aras de la brevedad.

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