

الإسراء

مجلة كلية

الجامعة للعلوم الطبية



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Scientific Research
Research & Development
Department



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كلية الاسراء الجامعة / السيد العميد المحترم

م/ مجلة كلية الاسراء الجامعة للعلوم الطبية

السلام عليكم ورحمة الله وبركاته ...

أشارة الى كتابكم المرقم ع/١٩٥٠ في ٢١ / ١١ / ٢٠٢٠ بشأن اعتماد مجلتهم التي تصدر عن جامعتكم الموقرة واعتمادها لأغراض النشر والترقيات العلمية وتسجيلها ضمن موقع المجلات العلمية الاكاديمية العراقية ، حصلت موافقة السيد وكيل الوزارة لشؤون البحث العلمي بتاريخ ٢٠٢١/٢/١٤ على اعتماد المجلة المذكورة في الترقيات العلمية والنشاطات العلمية المختلفة الأخرى وتسجيل المجلة في موقع المجلات الاكاديمية العلمية العراقية ، وحسب ما جاء بأعاماننا المرقم ب ت ٤/١٠٩٨٨ في ٢٤/١١/٢٠١٩ (تقرر اعطاء موافقة مؤقتة لمدة ٦ أشهر على ان يتم تزويدنا بالرقم المعياري الدولي المطبوع والالكتروني وانشاء موقع الكتروني للمجلة وبخلافه تلغى موافقه وأعلامنا الاجراءات لاحقاً).

للتفضل بالاطلاع وابلاغ مخول المجلة لمراجعة دائرتنا لتزويده باسم المستخدم وكلمة المرور ليتسنى له تسجيل المجلة ضمن موقع المجلات العلمية العراقية وقهرسة اعدادها ... مع التقدير .

السيد المدير العام المحترم

للتفضل بالتوقيع مع التقدير

أ.د. غسان حميد عبدالمجيد

المدير العام لدائرة البحث والتطوير

٢٠٢١/٣/١

د.هنا / المعاون

٣/١

نسخة منه الى:

- مكتب السيد وكيل الوزارة لشؤون البحث العلمي / اشارة الى موافقة سيادته المذكورة أعلاه والمثبتة على اصل مذكرتنا المرقم ب ت م ٤/١٠٩٣ في ١٧/٢/٢٠٢١ / للتفضل بالاطلاع ... مع التقدير .
- قسم المشاريع الريادية / شعبة المشاريع الالكترونية / للتفضل بالعلم واتخاذ مايلزم ... مع التقدير
- قسم الشؤون العلمية / شعبة المؤلفات والنشر والمجلات / مع الاوليات .
- الصادرة .

مهند ، أئس
٢٤ / شباط

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تعليمات النشر

في مجلة كلية الإسراء الجامعة للعلوم الطبية

- تصدر جامعة الإسراء (مجلة كلية الإسراء الجامعة للعلوم الطبية) في مجلد سنوي يضم عددين.
- تقوم المجلة بنشر البحوث العلمية للباحثين في تخصصات العلوم الطبية التالية:
 - الطب العام وطب الأسنان
 - العلوم الصيدلانية
 - تقنيات المختبرات الصحية والطبية
 - تقنيات الطب الحيائي
 - التمريض
 - العلوم البيولوجية.
- يشترط في البحث المقدم للنشر أن لا يكون قد نشر أو أرسل لجهة أخرى للنشر .
- تخضع البحوث المقدمة للنشر في المجلة للتقييم حسب الأصول العلمية المتبعة من قبل اثنين من المختصين في موضوع البحث ومن ذوي الكفاءة، وقد يستشار بثالث عند الضرورة مع حجب أسماء المقيّمين عند إرسال الملاحظات للباحثين.
- يلتزم الباحث بإجراء جميع التعديلات التي يراها المقيّمان ضرورية ويُرفض البحث إذا اتفق المقيّمان على رفضه، أو رفض من أحدهما وتعديلات جوهرية من الآخر، أو تعديلات جوهرية من كلا المقيّمين.
- يلتزم الباحث عند النشر في هذه المجلة بمليء استمارة التعهد الخاص ببيان ملكيته الفكرية للبحث وعدم نشره سابقاً في أي مجلة علمية أو مؤتمر علمي.
- تخضع البحوث المقدمة للنشر لتحديد نسبة الاستلال (الانتحال) Plagiarism باستعمال برنامج Turnitin.



- يعرض البحث قبل النشر للتدقيق من قبل مقيّم لغوي (اللغة العربية واللغة الانكليزية) ويجب على الباحث الالتزام بتعديلاتهما.
- تلتزم المجلة بسياسة نشر تعكس التزامها بأخلاقيات البحث العلمي وبنود لجنة أخلاقيات النشر Committee of Publication Ethics
- تلتزم المجلة بجميع الضوابط الصادرة من وزارة التعليم العالي والبحث العلمي / دائرة البحث والتطوير الخاصة بالمجلات العلمية.
- تحتفظ هيئة التحرير بحقها بإجراء التعديلات الشكلية واللغوية اللازمة.
- تحتفظ هيئة التحرير بحقها في عدم نشر أي بحث دون إبداء الأسباب وتعتبر قراراتها نهائية.
- لا ترد البحوث لأصحابها سواء قبلت للنشر أو لم تقبل.
- يزود صاحب البحث بنسخة ورقية واحدة من العدد الذي نشر فيه بحثه.

شروط النشر

1. يطبع البحث بواسطة الحاسوب بمسافات مفردة بين الاسطر وبحجم خط 12 ونوع (Simplified Arabic)، اما العنوان باللغتين العربية والانكليزية فيكون بحجم خط 14 شريطة ألا يزيد عدد صفحاته عن 15 صفحة بما في ذلك الجداول والأشكال والمراجع وعلى وجه واحد على ورق قياس A4 مع ترك هامش في حدود 2 سم من الاعلى والاسفل وهامش بحدود 3 سم من الجانبين الايمن واليسر.
2. لا يفضل نشر البحوث من قبل رئيس وأعضاء هيئة التحرير في المجلة سواء كان البحث منفرداً أو مشتركاً.
3. يقدم البحث بثلاثة نسخ ورقية عند تقديمه للنشر ونسخة محدثة وأخرى إلكترونية بعد قبول البحث للنشر، يسلم البحث بشكله النهائي مطبوعاً بالنظام الاعتيادي بمسافة منتظمة لكافة الصفحات عدا الصفحة الأولى التي تتضمن عنوان البحث وأسماء الباحثين وعناوينهم والبريد الإلكتروني للباحث الأول باللغتين العربية والإنكليزية وعلى قرص مدمج CD ببرنامج Microsoft Word 2010 .
4. تقبل البحوث باللغتين العربية والإنكليزية ويفضل كتابة البحث باللغة الإنكليزية.

دليل المؤلف Author Guidelines

أدناه الشروط والمتطلبات الواجب مراعاتها من قبل الباحث للنشر في هذه المجلة بشرط أن لا يكون البحث قد نشر أو سينشر في أية مجلة علمية أخرى ولم يمضِ على إنجازهِ أكثر من أربع سنوات.

1. يجب أن يكون عنوان البحث موجزاً قدر الإمكان ومعبراً عن البحث.
2. أسماء الباحثين: تكتب أسماء الباحثين وعناوين عملهم بصورة واضحة مع البريد الإلكتروني للباحث الأول.
3. يجب أن يتضمن المستخلص موجزاً واضحاً عن البحث مكون من 250-300 كلمة متبوعاً بكلمات مفتاحية 4-6. إذا كان البحث باللغة العربية فيكون المستخلص متبوعاً بالكلمات المفتاحية أولاً، ثم المستخلص متبوعاً بالكلمات المفتاحية باللغة الإنكليزية ثانياً والعكس صحيح.
4. المقدمة: تتضمن مراجعة المعلومات وثيقة الصلة بموضوع البحث الموجودة في المصادر العلمية وتنتهي المقدمة بأهداف الدراسة وأساسها المنطقي.
5. المواد وطرائق العمل: تذكر طرائق العمل بشكل مفصل إن كانت جديدة، أما إذا كانت منشورة فتذكر بشكل مختصر مع الإشارة للمصدر، يستعمل النظام العالمي للوحدات Standard International of Units (S.I.U.s) بكتابة الوحدات فضلاً عن استخدام مختصرات المصطلحات العلمية المعتمدة عالمياً، على أن تكتب بشكل كامل في أول مرة ترد في النص.
6. النتائج والمناقشة: تعرض بشكل موجز وهدف وبنظام متوالي وتعرض النتائج بأفضل صورة معبرة وتوضع الجداول والأشكال في أماكنها المخصصة بعد الإشارة إليها في النتائج.
7. يستعمل نظام الأرقام العربية وهكذا في البحوث المرسله للنشر وتمثل مناقشة النتائج تعبيراً موجزاً عن النتائج وتفسيراتها.
8. تكون كتابة المصدر في قائمة المصادر متضمنة الآتي: اسم أو أسماء الباحثين، سنة النشر وعنوان البحث كاملاً واسم المجلة ورقم المجلد والعدد وعدد الصفحات، مثال:
حمزة، عصام شاكر و جارالله، عزيز لطيف ورشيد، فرقد عبدالله وسلمان، سرحان علي (2018)، تقدير مستويات الزئبق في مصل دم مستخدمين لحشوات الأسنان.
مجلة كلية الإسراء الجامعة، المجلد الأول\ العدد الأول: 281-294.



9. المستخلص الإنكليزي يجب أن يكون وافياً ومعبراً عن البحث بصورة دقيقة، وليس بالضرورة أن يكون ترجمة حرفية للمستخلص العربي ومتبوعاً بكلمات مفتاحية 4-6.

دليل المقيّم Reviewer Guidelines

أدناه الشروط والمتطلبات الواجب مراعاتها من قبل المقيم للبحوث المرسلّة للنشر في هذه المجلة

1. ملء استمارة التقييم المرسلّة رفقة البحث المطلوب تقييمه بشكل دقيق وعدم ترك أي فقرة بدون إجابة.
2. على المقيّم التأكّد من تطابق وتوافق عنوان البحث باللغتين العربية والإنكليزية وفي حالة عدم تطابقهما اقتراح العنوان البديل.
3. أن يبين المقيّم هل أن الجداول والأشكال التخطيطية الموجودة في البحث وافية ومعبرة.
4. أن يبين المقيّم هل أن الباحث اتبع الأسلوب الإحصائي الصحيح.
5. أن يوضح المقيّم هل أن مناقشة النتائج كانت كافية ومنطقية.
6. على المقيّم تحديد مدى استخدام الباحث للمراجع العلمية الرصينة وحدثتها.
7. أن يؤشر المقيّم بشكل واضح على واحد من ثلاث اختيارات وهي:
 - البحث صالح للنشر بدون تعديلات.
 - البحث صالح للنشر بعد إجراء التعديلات.
 - البحث غير صالح للنشر.
8. يجب أن يوضح المقيّم بورقة منفصلة ما هي التعديلات الأساسية التي يقترحها لغرض قبول البحث.
9. للمقيّم حق طلب إعادة البحث إليه بعد إجراء التعديلات المطلوبة للتأكد من التزام الباحث بها.
10. على المقيّم تسجيل اسمه ودرجته العلمية وعنوانه وتاريخ إجراء التقييم مع التوقيع على استمارة التقييم المرسلّة له رفقة البحث المرسل له للتقييم.

المصادر

1. يشار إلى المصادر في متن البحث كما يلي:
اللقب أو الاسم الثالث للمؤلف والسنة إذا كان البحث بإسم باحث واحد، وإذا كان مؤلفين فيذكران والسنة وإذا كانوا ثلاثة فأكثر فيذكر اسم الأول وآخرون والسنة.
2. ترتب المصادر حسب الصيغة العالمية (APA) وكما بالأمثلة المذكورة :
 - أ. بحث في مجلة.
اسم الباحث أو الباحثون، (السنة)، عنوان البحث، اسم المجلة، المجلد، العدد وصفحتي البدء والانتهاه للبحث.
 - ب. كتب.
اسم المؤلف أو المؤلفون، (السنة) عنوان الكتاب، الطبعة، دار النشر وعدد الصفحات.
 - ج. الرسائل والأطاريح الجامعية.
اسم الباحث، (السنة)، عنوان الرسالة أو الأطروحة، العنوان (الكلية والجامعة) وعدد الصفحات.
 - د. بحث في وقائع مؤتمر أو ندوة علمية.
اسم الباحث أو الباحثون، (السنة)، عنوان البحث، اسم المؤتمر أو الندوة العلمية، مكان الانعقاد، صفحتي البدء والانتهاه للبحث.

ترسل البحوث إلى مجلة كلية الإسراء الجامعة للعلوم الطبية على العنوان الآتي:

جامعة الإسراء - قسم التوثيق والنشر

بغداد \ العراق

البريد الإلكتروني :

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(تعهد الملكية الفكرية)

إني\إننا الباحث\الباحثين صاحب\أصحاب البحث الموسوم
(.....)

أتعهد\نتعهد بأن البحث قد أنجز من قبلي\قبلنا ولم ينشر في أي مجلة أخرى في داخل
وخارج العراق وأرغب بنشره في مجلة (مجلة كلية الإسراء الجامعة للعلوم الطبية) في
جامعة الإسراء.

التوقيع:

التاريخ:



(تعهد نقل حقوق الطبع والتوزيع)

إني\إننا الباحث\الباحثين صاحب\أصحاب البحث الموسوم
(.....)

أتعهد\نتعهد بنقل حقوق الطبع والتوزيع والنشر إلى مجلة (مجلة كلية الإسراء
الجامعة للعلوم الطبية) في جامعة الإسراء.

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7. Use carefully studied mixtures of local plant waste powders in water treatment, eliminating specific pollutants, isolating its natural products (chemical compounds), and developing medical and pharmaceutical products.

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2. Spent olive and pine leaf powders can be used for two consecutive cycles to remove nitrate and phosphate from domestic wastewater with an acceptable reduction in removal efficiency. The plant powder remains free from damage, tearing, or destruction after two reused cycles.
3. Wastewater treated with leaf powders can be reused for irrigation without any toxic effects or inhibition of germination rates, based on phytotoxicity tests.

Recommendations

1. Develop the wastewater sector, find solutions to mitigate environmental and health damage, treatment of pollution, conserve water and agricultural land, and raise farmers' awareness of the dangers of irrigating crops with wastewater.
2. Improve the performance and efficiency of plant leaf powder in treating domestic wastewater, and develop low-cost methods for regenerating plant powders and increasing the economic feasibility of the treatment process.
3. Determine the toxicity of the plant leaf powder sludge produced after treatment and its potential for various uses.
4. Determine safe or long-term disposal methods.
5. Determine the efficiency of plant powders in removing heavy metal ions, chemical dyes, pharmaceutical compounds, and chemical pesticides from industrial and agricultural wastewater.
6. Determine the optimal conditions for achieving maximum efficiency in treating various types of wastewater for each plant species.



A temperature between 20-30°C is one of the most important external factors affecting germination. The germination rate of cress irrigated with water from different sources exceeded 90% (Bansal 2015). Research has shown that the best rate is achieved when irrigated with drinking water of moderate concentrations. Raw domestic wastewater has a negative effect due to its salinity and harmful substances (Nana *et al.*, 2019).

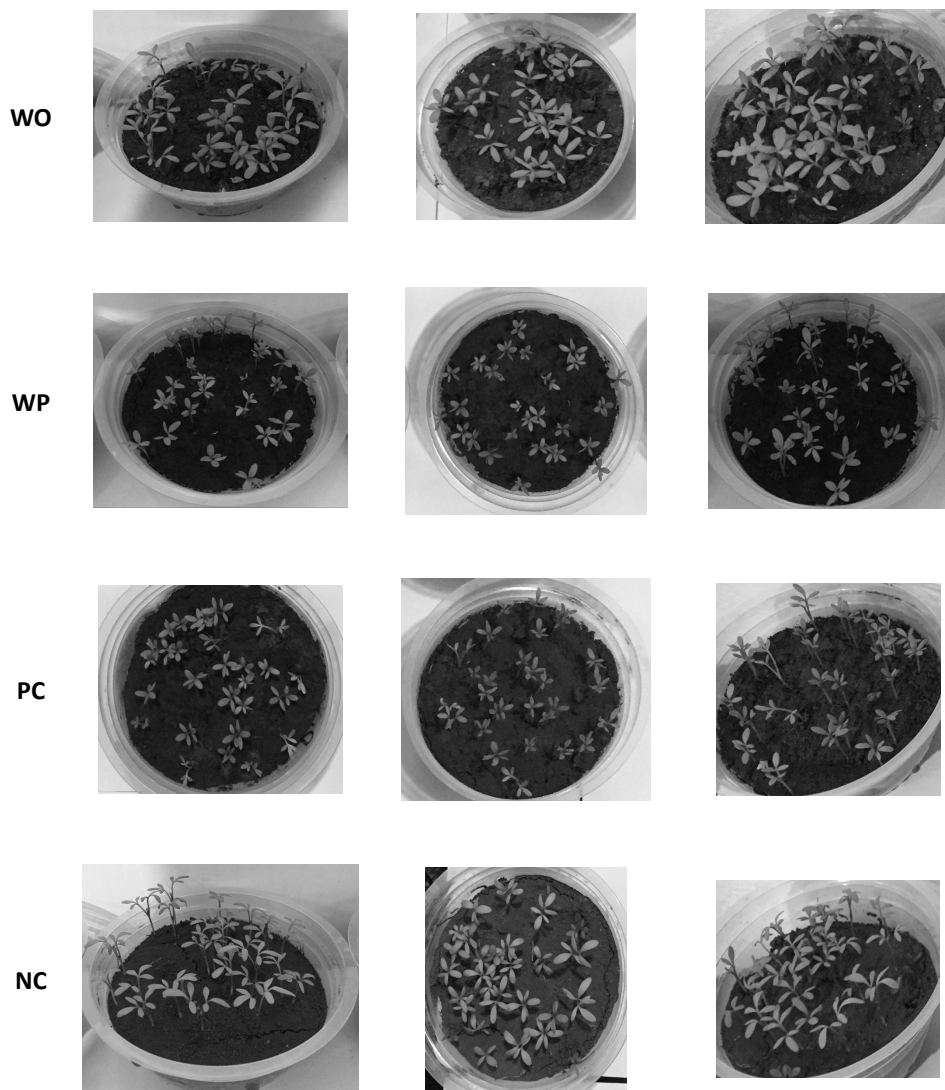
4.2 Phytotoxicity tests of spent plant powders

The results confirm that the use of spent plant powders is safe, as they are non-toxic due to their biodegradable nature (Zucconi and Bertoldi 1981). The germination rate using olive powder and pine powder reached 86.67% and 80%, respectively, after 3 days (Table 4), which is more than what others reported (Robalds *et al.*, 2016). Spent powders can be added as a soil quality improver; they increase permeability and water holding capacity, organic matter content, and are a source of nitrate and phosphate (Buenaño *et al.*, 2019).

Accordingly, the importance of using unmodified plant leaf powder in treating domestic wastewater can be emphasized. It is also environmentally friendly, low-cost, and reusable, which increases economic feasibility and achieves sustainable environmental development goals when it is needed.

Conclusions

1. The process of removing pollutants from water occurs quickly, depending on the type of pollutant, compared to other treatment methods such as plant treatment units or stability ponds, which require longer processing times. The odor of the treated wastewater is acceptable. Even if the BOD values of the treated wastewater do not decrease, it is considered non-toxic.



WO: wastewater treated by Olive powder// WP: wastewater treated by Pine powder //
PC: Positive Control (drinking water)// NC: Nigative Control (wastewater nontreated)

Figure 2. Phytotoxicity tests of treated domestic wastewater.



compounds at each treatment cycle, as well as external environmental factors (Zhang *et al.*, 2019), the accumulation of ions at the adsorption sites (Mohan and Sumitha 2008), and the disruption and damage to the structure of the plant powder (Kong *et al.*, 2015, Nag *et al.*, 2016).

4. Phytotoxicity

4.1 Phytotoxicity tests of treated domestic wastewater

The results of Table 4 show that the germination rate of cress seeds irrigated with domestic wastewater treated with olive and pine leaf powder was 96.67% on the tenth day. This is more than 6% higher than irrigation with raw wastewater, but is mostly like that of drinking water (Figure 2).

Table 4. Phytotoxicity testing of treated wastewater and used plant powders.

Phytotoxicity of treated domestic wastewater						
treated water	After 4 days		After 7 days		after 10 days	
	Seed №	GR%	Seed №	GR%	Seed №	GR%
<i>Olea</i> powder	22	73.33	26	86.67	29	96.67
<i>Pinus</i> powder	22	73.33	28	93.33	29	96.67
Row wastewater	20	66.67	26	86.67	27	90.00
Drinking water	26	86.67	28	93.33	29	96.67
Phytotoxicity of spent plant powders after 3 days						
GR%	<i>Olea</i>	<i>Pinus</i>	Soil			
	86.67	80.00	96.67			

Seed №: Number of germinated seeds/ GR%: germination rate %

The delayed seed germination in treated water may be due to the presence of plant polyphenols. Higher concentrations inhibit or delay seed germination, unlike lower concentrations.



compared to the initial value (cycle 0), and reached more than 73% after the third treatment cycle.

These results were not consistent with other studies; the phosphate removal efficiency from aqueous solutions decreased by 13.63% and 14.25%, respectively, after the third cycle using NaOH (0.2 M) as a regeneration solution for plant powders modified with layered double oxides (Mg-Al) (Zhang *et al.*, 2019). Amine-modified bagasse powders can be used for at least three consecutive cycles with a slight decrease in nitrate and phosphate removal efficiency (Ren *et al.*, 2016).

Table 3. Efficiency of plant leaf powder in removing nitrate and phosphate after three consecutive cycles.

testes	Crude WW	(Olive powder) treatment cycles			
		0	1	2	3
NO ₃ -N, mg/L	26.3	15.23	15.99	18.75	19.46
Uptake %		42.1	39.2	34.5	26.0
Phosphate, mg/L	29.2	18.63	19.41	20.82	25.14
Uptake %		36.4	33.4	28.7	13.9

testes	Crude WW	(Pine powder) treatment cycles			
		0	1	2	3
NO ₃ -N, mg/L	24.7	11.99	12.87	15.68	21.29
Uptake %		51.47	47.9	36.5	13.8
Phosphate, mg/L	26.5	15.93	16.77	18.10	23.93
Uptake %		39.9	36.7	31.7	9.7

The decreased removal efficiency after several cycles may be attributed to partial degradation of cellulose and hemicellulose under acidic or alkaline conditions, the decomposition of some surface functional groups or active adsorption sites (Ren *et al.*, 2016), the decrease in the amount of active



(Vunain et al., 2019). The effectiveness in reducing the concentration of dissolved solids is attributed to the active functional groups that contribute to the formation of organometallic complexes (Assaffii et al., 2012). The active functional groups in the plant powder, such as carboxyl, hydroxyl and amino, work to neutralize the charges on the outer surfaces of suspended materials and collect and precipitate the suspended materials (Lee *et al.*, 2014, Choy *et al.*, 2015).

The results of table 2 show that the nitrate and phosphate values decreased by 39.97% and 27.13% in olive powder, and by 28.16% and 19.09% in pine powder, respectively. This is consistent with the results of other studies (Ismail 2012, Qiao *et al.*, 2019). The increase in the value of BOD is due to the release of soluble organic compounds present in the plant powder (Kushwaha et al., 2013, Edogbanya et al., 2016); which is consistent with the results of other studies (Anastasakis 2009, Shan et al., 2017). In the acidic range, the powder surfaces acquire new active sites; due to the increased H⁺ ions and increased removal efficiency due to the electrostatic attraction between nitrate and phosphate ions and the active surface sites of the powder (Krishnan and Haridas 2008, Ismail 2012, Robalds et al., 2016).

3. Regeneration of plant leaves powder in wastewater treatment

The results show that the percentage of phosphate and nitrate removal using olive powder decreased by 7–21%, respectively, after two consecutive treatment cycles compared to the initial value (cycle 0). The percentage of removal decreased by more than 38% after the third treatment cycle (Table 3). Similarly, the percentage of removing by pine powder decreased by 8–21% and 7–29%, respectively, after two consecutive treatment cycles



depending on crop type. Turbidity value was 174 ± 4.7 NTU, and BOD value was 146.4 ± 11.3 mg/L, which represents the amount of oxygen consumed by bacteria aerobically at 20°C over five days. It often exceeded the maximum permissible limit. High values lead to depletion of dissolved oxygen (DO), making conditions anaerobic and increasing the toxicity of the medium.

2. Results of domestic wastewater treatment using plant leaves powder

Table 2 shows the results of water treatment using olive and pine leaf powder, respectively.

Table 2. Results of domestic wastewater treatment using plant leaf powder, 1 g/L.

Testes	Crude WW	treated WW using	
		<i>Olive</i>	<i>Pine</i>
Temperature, C°	23.7	25.0	25.0
pH	7.45	6.5	6.5
TDS, mg/L	568	501.83	448.83
EC, S/cm μ	820	730.78	687.73
Turbidity, NTU	176	82.09	41.82
NO ₃ -N, mg/L	21.53	12.92	15.47
Phosphate, mg/L	27.0	19.67	21.85
BOD, mg/L	149.3	204	191.6

The results of table 2 show a decrease in the TDS value when treated with olive leaf powder by 11.65%, and with pine leaf powder by 20.98%, and a decrease in EC value with olive and pine powders by 10.88% and 16.13%, respectively. The TDS values after treatment are within the maximum permissible limit in wastewater. The turbidity value of domestic wastewater treated with olive powder also decreased by 53.36% and 76.24% in the case of pine. Dissolved solids are directly proportional to electrical conductivity



Results and Discussion

1. Physical and chemical analysis of domestic wastewater

Table 1 shows the results of physical and chemical analyses of domestic wastewater.

Table 1. Physical and chemical properties of domestic wastewater.

Testes		1	2	3
Temperature, C°	24.0 ± 0.3	-	-	-
pH	8.38 ± 0.07	6 - 9		
TDS, mg/L	570 ± 4.0	15000	1500	-
EC, S/cmμ	824 ± 3.5	-	-	-
Turbidity, NTU	174 ± 4.7	-	-	-
Nitrate, mg/L	26.50 ± 0.34	20	25	25
Phosphate, mg/L	28.3 ± 0.15	20	20	20
BOD, mg/L	146.4 ± 11.30	30	100	150

1: Maximum permissible level of wastewater used for irrigation of cooked vegetables, parks, and urban roadsides. 2: For fruit trees, lawns, cereals, and forage crops. 3: For industrial crops and forest trees. 1, 2, and 3, accordint to Syrian pstandards.

Table 1 shows that the pH value of domestic wastewater was alkaline, reaching 8.38 ± 0.07 . Water pH affects the status of nutrients, nitrifying bacteria, and microelements. Some substances in water may be toxic due to their interaction with high or low pH levels. Water temperature was 24.0 ± 0.3 °C. Temperature significantly affects the rates of biochemical reactions, treatment processes, and sedimentation efficiency (Mohammed *et al.*, 2012). Electrical conductivity (EC) value was 824 ± 3.5 S/cmμ, which is consistent with the total dissolved solids (TDS) value of 570 ± 4.0 mg/L (Noorjahan 2014). TDS values were within the maximum permissible limits



Preparation of plant extract

Aqueous extracts of plants powder were prepared separately. 50 g of leaf powder was placed in a Soxhlet apparatus and 500 ml of solvent was used at a ratio of 1:10 (v/w) for 7 hrs. The extracts were then filtered and evaporated using a rotary evaporator under reduced pressure at 40°C., they were stored in a refrigerator at 4°C until use (Bissa and Bohra 2008). All extracts were sterilized before by using 0.45 µm membrane filters.

Phytotoxicity tests

The germination percentage of cress seeds was calculated to determine the phytotoxicity of treated wastewater, and of the splet plant powders after the treatment process, as follows: (1) To determine the phytotoxicity of treated water: 40 g of sterile, moistened soil was placed in 50 g plastic containers with 30 ml of treated wastewater. 30 seeds were planted in each container. Drinking water was used as positive control, and crude wastewater was used as a negative control. The containers were placed at 25°C, alternating between light and darkness (10 hrs. of darkness versus 14 hrs. of light) for 10 days. (2) To determine the toxicity of splet plant powders after treatment: Plant powders were dried in the shade for 14 days until weight was relatively stable. Then, 20 g of the dried powders were placed in 50 g plastic containers, where 30 seeds were planted and watered with 30 ml of distilled water. The soil served as positive control. The plastic containers were then placed for 3 days at 25°C, alternating between dark and light (10 hrs of darkness versus 14 hrs of light). All previous experiments were conducted with three replicates each, and the germination percentage for all experiments was calculated (Darvishi 2014, Robalds *et al.*, 2016) using the following equation:

$$\text{Germination rate (\%)} = \text{N}_g \text{ of germinated seeds} / \text{total N}_g \text{ of seeds} \times 100$$



and treatment experiments were carried out according to the applied methods (Chiban *et al.*, 2012, Mokif *et al.*, 2018, Haghighizadeh *et al.*, 2020). 250 ml flasks containing 100 ml of domestic wastewater were used; according to the following steps (Figure 1): Plant powder dose 1 g/L, at 25 °C, pH 6.5, treatment time 60 minutes, and shaking speed 150 rpm. After treatment, the water was filtered with Whatman filter paper (No. 45) for the purpose of conducting pre- and post-treatment tests. As for the turbidity experiments, the treated water was left for 5 minutes to settle, then 10 ml was withdrawn from the upper layer of the water; for turbidity analysis. All experiments were conducted in three replicates. A control was used by adding 100 ml of domestic wastewater without plant powder. The pH values of the domestic wastewater were adjusted using HCL (0.1 M) and NaOH (0.1 M) after adding the plant leaf powder. The removal efficiency was calculated using the following equation:

$$\text{removal efficiency (R \%)} = (C_i - C_f) / C_i \times 100$$

where, C_i and C_f are the initial and final concentrations of pollutants in the domestic wastewater, respectively.

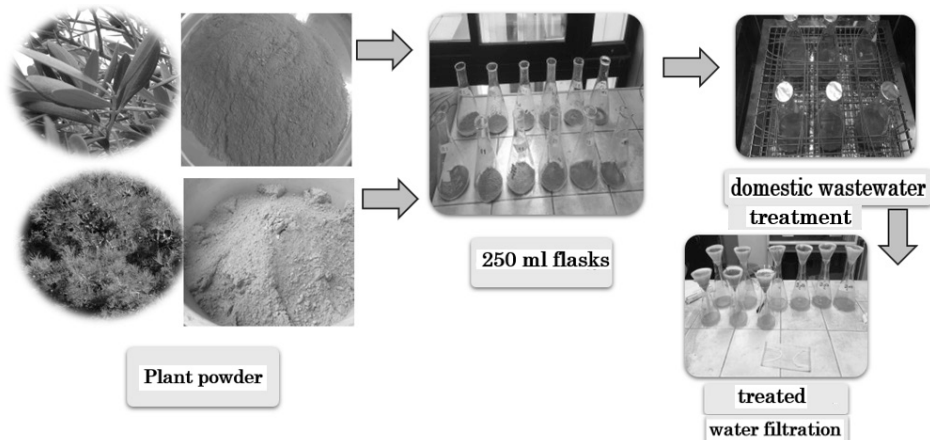


Figure 1. Stages of domestic wastewater treatment using plant powders.



amino, sulfhydryl, phosphoryl, and phenols, presence of a rough and heterogeneous surface structure, and polar chemical compounds such as phenols, tannins, flavonoids, and saponins that are characterized by bioactivity (Omojate *et al.*, 2014). Plant waste consists of materials of organic origin such as cellulose, hemicellulose, lignin, pectin, and extractives, which are useful in removing various pollutants (Malik *et al.*, 2017, Escudero *et al.*, 2019).

Several researchs have been conducted to evaluate the efficiency of different plant powders in removing pollutants from water (Chandran *et al.*, 2015, Ahmadi and Mostafapour, 2017, Rehman *et al.*, 2019), as well as to use plant waste powder in multiple cycles in removing pollutants (Adeniyi and Ighalo 2019, Tran *et al.*, 2019). This method is a promising alternative to wastewater treatment due to its low cost and high efficiency. It is widely available and has a low economic value. Furthermore, it can be regenerated and reused in treatment. It is environmentally friendly and achieves environmental, health, and sustainability development goals. The research aims to evaluate the toxicity of treated wastewater to the germination of cress, as well as the toxicity of the spent plant powder to germination.

Methodology

$$\text{removal efficiency (R \%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where, C_i and C_f : initial and final concentrations of pollutants in domestic wastewater, respectively.

The leaves of olive and pine used in experiments were collected, in different sites in Damascus, according to the applied methods (Sharma and Bhattacharyya 2005). Domestic wastewater samples were collected and analyzed, in Damascus city, according to standard methods (APHA 2017),



Introduction

Health and ecosystems face significant pollution problems on a wide scale. Wastewater pollution is of particular concern due to the presence of pathogens such as viruses, bacteria, fungi, protozoa, and helminths (Cabral 2010, Rose *et al.*, 2015, Al-Gheethi *et al.*, 2018). Untreated wastewater is often discharged directly into waterways, freshwater, and agricultural lands in most developing countries, causing serious health and environmental problems. More than two billion people live in countries with water scarcity, and the situation is expected to worsen in some regions due to climate change and population growth. An estimated 1.7 billion people drank contaminated water in 2022, in addition to chemical contamination. Contaminated water causes approximately 500,000 diarrheal deaths each year (WHO 2023). The water quality of the Tigris and Euphrates rivers in Iraq is deteriorating due to the low discharges from upstream countries, return water from drains and wastewater, and nature of the lands through which the rivers pass (Obaid 2024).

There are many technologies applied, such as oxidation, ozonation, coagulation, chemical precipitation, electrochemical treatment, ion exchange, and activated sludge, to remove organic and inorganic pollutants, chemical toxins, and pathogens from wastewater. However, they have disadvantages, the most important of which are: high cost, especially when treating large quantities of water, need for energy, and high operating skill. Process of removing pollutants from wastewater using plant waste powder involves a set of mechanisms; including physical and chemical adsorption, complexation, ion exchange, and precipitation on the surface of the plant powder, in addition to containing numerous functional groups such as hydroxyl, carboxyl, carbonyl,



المستخلص

تواجه الصحة والنظم البيئية مشكلات مؤثرة على نطاق واسع؛ فمياه الصرف الصحي تُصرف دون معالجة إلى المجاري المائية والأراضي في معظم البلدان النامية، وهي تحتوي على المواد الضارة ومسببات الأمراض. تشير منظمة الصحة العالمية WHO إلى أن نحو 1.7 مليار شخص يشربون مياه ملوثة في البلدان النامية وأن تلوثها يؤدي إلى وفاة نحو 500 ألف شخص بالإسهال كل عام. لكن طرائق المعالجة المطبّقة غير كافية لإزالة المواد الذائبة والملوثات النوعية، مكلفة جداً وبحاجة للطاقة، وتتطلب تقنيات متطورة ومهارة عالية في التشغيل. تنطوي عملية إزالة الملوثات من مياه الصرف الصحي بمساحيق المخلفات النباتية على مجموعة من الآليات؛ بما في ذلك الامتزاز والتعقيد complexation والتبادل الشاردي والترسيب على سطح المسحوق، فهذا يتميز بوجود المجموعات الوظيفية والمركبات الكيميائية النشطة والبنية السطحية؛ ما يؤكّد أهمية استعمالها في المعالجة. يهدف هذا البحث research aims إلى تقييم سُمية المياه المعالجة بالمسحوق النباتي وكذلك سُمية المسحوق النباتي المستهلك اعتماداً على الإنبات. فقد جُمعت أوراق نباتات الصنوبر *Pinus brutia* والزيتون *Olea europaea*، وأُجريت تجارب معالجة مياه الصرف الصحي وتحاليلها، وتجارب السمية النباتية على الرشاد (*Lepidium sativum*) بمراقبة معدّل الإنبات أو تأخيره أو أي تأثير ضار على النباتات، وأُجريت مقارنة بنتائج مياه الصرف الخام وماء الشرب وبالقيم القصوى المسموح بها في مياه الصرف المستعملة لأغراض مختلفة. أظهرت النتائج أن استعمال مسحوق أوراق الزيتون والصنوبر غير المعدّلة يفيد في تحسين كفاءة عملية معالجة المياه، ويمكن استعمال المساحيق المستهلكة دورتين متتاليتين في المعالجة مع انخفاض مقبول في كفاءة الإزالة وإزالة الملوثات، وتبيّن أن طريقة المعالجة أو المسحوق المستهلك آمنة، مقارنة بطرائق محطات المعالجة أو برك الاستقرار التي تحتاج إلى وقت أطول، وكانت رائحة المياه المعالجة مقبولة. وهذه طريقة صديقة للبيئة تحقق أهداف التنمية البيئية والصحية والاستدامة، متوافرة بكميات كبيرة، الكلفة المنخفضة والكفاءة الجيدة. الكلمات المفتاحية: السمية النباتية، مياه الصرف المنزلي، أوراق النباتات، المساحيق، المعالجة الخضراء.



Abstract

Health and ecosystems face widespread, impactful problems. Untreated wastewater is discharged into waterways and lands in most developing countries, containing harmful substances and pathogens. WHO estimates that nearly 1.7 billion people drink contaminated water in developing countries, and that contamination leads to nearly 500,000 diarrheal deaths each year. However, existing treatment methods are insufficient to remove dissolved substances and specific pollutants; they are very expensive, energy-intensive, require advanced technologies, and high operational skills. The process of removing pollutants from wastewater using plant waste powders involves a combination of mechanisms, including adsorption, complexation, and ion exchange. These processes are characterized by the presence of functional groups, active chemical compounds, and surface structures, which underscores their importance in treatment. This research aims to evaluate the toxicity of water treated with plant powders and the toxicity of spent plant powders based on germination.

Using powder of Pine (*Pinus brutia*) and olive (*Olea europaea*) leaves, wastewater treatment, and analysis experiments were conducted. Phytotoxicity experiments were also conducted on cress (*Lepidium sativum*), monitoring the rate of germination, its delay, and any harmful effects on plants. Results were compared with raw wastewater and drinking water, and with the maximum permissible values for wastewater used for various purposes. The results showed that the use of unmodified olive and pine leaf powder improves the efficiency of the water treatment process. The spent powders can be used for two consecutive treatment cycles with an acceptable reduction in removal efficiency, and contaminant removal. The treatment method or the spent powder proved safe, compared to treatment plant or stability pond methods, which require longer time. The odor of the treated water was acceptable. This is an environmentally friendly method that achieves the goals of environmental, health, and sustainability development. It is available in large quantities, at a low cost, and with good efficiency.

Keywords: phytotoxicity, domestic wastewater, plant leaves, powders, greenly treatment.



Evaluation of Phytotoxicity of Greenly Treated Domestic Wastewater Using Spent Plant Leaf Powders / Original article

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تقييم السمية النباتية لمياه الصرف الصحي المنزلية المعالجة
بطريقة خضراء باستخدام مساحيق أوراق النباتات المستهلكة

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and symptom are good markers to treated UTI infection, also the current finding highlight is the need to identify the specific pathogens in different population to commonly use specific antibiotic to prevent extra problem to women and baby.

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(28%), *E. coli* and *Klebsiella pneumonia*, other researcher has found that *E. coli* have higher frequency about (55.1%). while *Pseudomonas aeruginosa* and *Acetobacter baumannii* give negative test (Chateen *et al.*, 2007) (Ranganathan ,2014), also results showed that fungi of *Candida albicans* had (6%) frequency. (Fisher *et al.*, (2011) which related to many environmental factors.

In this study, maximum number of UTI cases is 15% that found in the group (18-20) years of age (young patients) followed by (24-26) age group 13% , the high incidence of UTI in young reproductive age group is due to early pregnancy particularly in remote setting (Emmanuel , 2023) ,also similar results showed by Naziz 2020 ,or this high frequency ,may due to an increased sexual activity in this age or, it may related to other environmental factors, like region or Socio-economic factors (Lucas,1993)(Emmanuel, 2023). The antibiotic sensitivity studies revealed that Cefepime, Nitrofurantion and Ceftriaxone antibiotics are most effective in bacteria and most useful antibiotic for treatment of UTI followed by Amoxyclav which shows intermediate effective in more samples, while Cefalexin antibiotic give resistant in most sample respectively, this results little differ from studies carried by (Hani, *et al.*, 2013),also this study are differ from studies and findings in Caucasian women where ampicillin and Sceptin remain the most useful antimicrobial agents(Ronald *et al.*, 1987), while, Chateen and his coworkers in 2007, found the most effective antibiotic was Nalidixic acid on *E. coli* and *Proteus mirabilis*, while Amoxicillin and Ampicillin were effective on *Staphylococci*.

The current study results show that urinary tract infection in pregnancy women is a vary frequent medical problem along the 9 months of maternity

**Table 10: Antibiotics examination**

antibiotics	Discontent	Sensitive	intermediate	Resistant
Amoxyclav (Aomxycillin/ Clavulanic acid) AMC	30mcg	34	28	1
Cefalexin CN	30mcg	23	20	20
Cefepime CPM	30mcg	43	12	8
Ceftariaxone CTR	30mcg	44	16	3
Nitrofurantoin NIT	100mcg	46	15	2

Discussion

The aim of the present study was to investigate the infection of urinary tract among pregnant women attending *Al Elywiah Hospital for Maternity-Baghdad / Iraq*.

The study revealed that the frequency of symptomatic UTI in present sample about 57% (40) (Table 2) while the asymptomatic patients about 33% this finding is smaller to results finding by Chateen and his coworkers (2007) in Kirkuk city, Iraq.

Also Results of chemical examination by dipstick, showed that most of women have negative sugar test except two, as they are young in age, for diabetic ,they are normal or it my related to pregnant diabetic, also specific gravity showed high positive results for all women which mean that most of pregnant have different type of amorphous and crystals that effect medically in pregnant ,as most of them felling difficulty in urination and some fell pain in a part of urinary system, while results for nitrite test shows ten samples positive result, this result was similar to results carried by Hani,2013.which its sign for infection. From the data obtained it is found that *Enterococcus faesialis* had highest frequency (40%) followed by *Staphylococcus aureus*



Table 8: Represents Crystals, Mucus and Fungal analysis by microscopic examination (40X) of urine of pregnant women with UTI

	Number of cases	Percentage %
Crystal		
Present	43	61
Absent	27	39
Mucus		
Present	30	43
Absent	40	57
Fungi		
Present	4	6
Absent	66	94
Total	70	100

Table 9: Number of bacterial and fungal culture cases using (UTI agar)

	Number of cases	Frequency %
Bacteria		
<i>Escherichia coli</i>	6	9
<i>Proteus mirabilis</i>	0	0
<i>Kelbsiella pneumoniae</i>	5	7
<i>Pseudomonas aeruginosa</i>	0	0
<i>Enterococcus faecalis</i>	28	40
<i>Staphylococcus aureus</i>	24	34
Fungi		
<i>Candida albicans</i>	4	6
Total	70	100



Frequency of UTI-associated microbial agents

Out of the 70 UTI pregnant women study, it was found that *Enterococcus faecalis* was the most frequently isolated pathogen, it's about (40%) followed by *Staphylococcus aureus* (34%). On the other hand, *E. faecalis* and *Staph. aureus* were the most frequently isolated pathogens (25% each) from the 23 UTI cases (Table 9) (Figure 2) (Al-Dujaily, 2000) (Akerelle, et al., 2001) (Yveline, et al., 2023) Smail et al., (2019),",

Results showed that *Candida albicans* fungi was found in two samples only, The sensitivity test of bacteria, results showed that antibiotics *Cefepime*, *Nitrofurantion* and *Ceftariaxone* are the most effective in bacteria which shows sensitive, also *Amoxyclav* shows intermediate in more samples, While *Cefalexin* is resistant in most samples .(Awaness et al.,(2000)(Table 10).

Tables: 6 & 7 Represents Pus cells and RBCs analysis by microscopic examination (40X) of urine of pregnant women with UTI

Pus cells/HPF	Number of cases	Percentage %
0-2	40	57
3-5	8	11
6-8	6	9
9-11	11	16
Numerous >11	5	7

HPF\RBCs	Number of case	Percentage%
0-5	48	69
6-10	12	17
Numerous>10	10	14
Total	70	100



Results of chemical examination, showed that most of pregnant are negative for sugar test except three, as most of pregnant are young in age, they have no diabetic and 2-3 have pregnant diabetic, also specific gravity showed highly positive in results for most pregnant which mean that most of them have infection with different type of amorphous and crystals, as they have some symptom in difficult urination and effect their health as shown under microscope (Chateen, *et al.*, 2007), while results for bilirubin showing normal for all sample except two sample, test for nitrite shows positive for (11) sample, which mean these women had infection with negative bacteria, while leukocyte esters showed positive in some sample (Table 5).

Results of microscopic examination showed RBC and Pus cells from (5-30 HPF) in the most sample. (Tables 6, 7 and 8).

Table 5: Some Chemical examination (Urobilinogen, Bilirubin, Glucose...etc.) by reagent strip test of urine of pregnant women with UTI

Test	Number of cases			Percentage %		
	Present	Absent	Total	Present	Absent	total
Urobilinogen	1	69	70	1	99	100
Bilirubin	2	68	70	3	97	100
Glucose	3	67	70	4	96	100
Ketones	3	67	70	4	96	100
Blood	27	43	70	39	61	100
Nitrite	11	59	70	16	84	100
Leukocyte esterase	35	35	70	50	50	100
Protein	26	44	70	37	63	100

**Table 2: Age group of pregnant women with UTI**

Age group of pregnant women (in years)	Number of cases	Percentage %
18-20	15	21
21-23	9	13
24-26	13	19
27-29	7	10
30-32	11	16
33-35	8	11
36-38	4	6
39-41	8	4
Total	70	100

Table 3: Prevalence of UTI among pregnant women

Investigated population	Asymptomatic UTI		Symptomatic UTI		TOTAL	
	No.	%	No.	%	No.	%
Pregnant women. 70	23	33%	40	57%	63	100%

Table 4: Physical examination (color, appearance, odor) of urine of pregnant women with UTI

Color and appearance and odor	Number of cases	Percentage %
Pale yellow to clear odorless	8	11
Yellow to clear odorless	23	33
Yellow to cloudy or turbid foul smell	33	47
Dark or deep yellow to turbid foul smell	2	3
Reddish yellow to cloudy foul smell	4	6
Total urine of	70	100

Result of physical examination showed that samples color of urine range from pale yellow, yellow to dark with turbid except two samples show reddish color (Table 4). The turbid samples may refer to infection by different source like bacteria or amorphous.



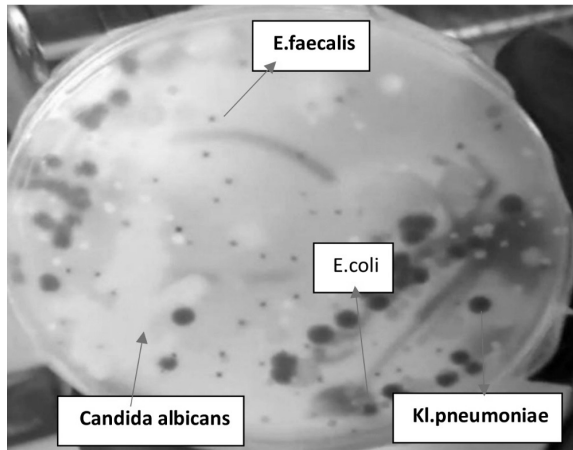
Table 1: (Microbiology sensitivity test).

Antibacterial		
1- Amoxyclav(Aomxycillin/Clavulanic acid) (30mcg(20/10mcg)	AMC	Disc-content
2-Cefelxine		Disc-content (30mcg (
3- Cefepime CPM		Disc-content (30mcg(
4 -Ceftariaxone CTR		Disc-content (30mcg(
5- Nitrofurantoin NIT		Disc-content (100mcg (
Antifungal		
1- Nystatin NS		Disc-content (50mcg(
2- Clotrimazole CC		Disc-content (10mcg(

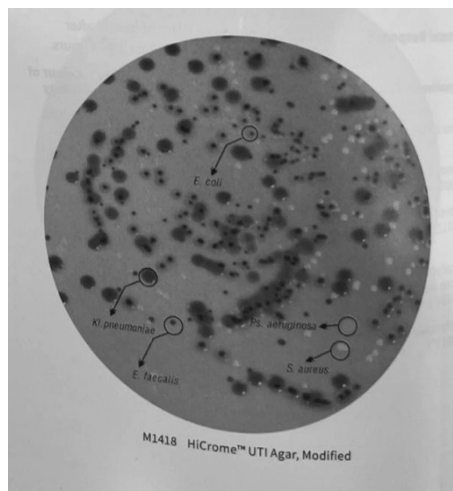
Results and Discussion

Prevalence of UTI among pregnant women

In this study a sample of (70) pregnant women that registers in Al Elywiah Hospital for maternity / Baghdad, medical data results taken from attended pregnant women with age range (18-39 year old). These pregnant were grouped as (18-20) , (20-23 ,..... (39-41) years ,as shown in (Table 2). Based on the clinical symptoms and doctor questioner, Out of 70 pregnant women test, about 40 women (57%) have positive symptom for urinary tract infection, patients are classified as symptomatic, and 23(33%) UTI-positive patients that associated with medical questioner classified as a symptomatic UTI. While 7 patients have negative test (Fatima and Ishrat, 2006) (Table 3).



(Figure 1) HiCrome™ UTI Control agar



(Figure 2) UTI Culture media



(All women were instructed how to give a clean catch midstream sample. Briefly, they were asked to clean the area around urethral opening with clean water and alcohol swab, then dry and collect sample by discarding the first part of urination and collect 10-20 ml of midstream in clean sterile disposable container, each sample carry information for pregnant patient's name and age. each sample was divided into two parts were carry for lab analysis.

Urine analysis

The urine samples were divided in two part, for the first part, different analysis were carried out for each sample starting by physical test which include (color, clarity and odor), while chemical test examined by dipstick ,10 reagent test strip (CYBOW 10, Gimhee, Kora) that have panels to detect glucose, Bilirubin, Ketones, specific gravityetc. (smith, 2003). Furthermore, wet preparation was made for sedimentation (using centrifugation at 5000 rpm for 5 minute), in Ultra centrifuge (Heltich Zentrifugen/EBA20). Than urine sample were microscopy examined at 40x for detection of WBC, RBC crystals and Amorphous types. While, the second part of the urine sample was cultured on plates with media, these media are modifications of HiCrome™ UTI Agar, which can be used in place of MacConkey Agar for isolation, and confirmation of various microorganisms (Figures 1 and 2). It facilitates and expedites the identification of some gram negative and positive bacteria on the basis of different contrasted colony color produced by reactions of genus or species specific enzymes with two chromogenic substrates. The list of antibiotic use for urine infection examination was shown in (Table 1).



prevalence of Bacteriuria during pregnancy is similar to that in non-pregnant women, pregnancy enhances the possibility of infection among women.(Dafnis, *et al.*,1992) The occurrence of the infection is at its peak during pregnancy which in turn relies on several other factors.

The diagnosis of UTI may be made on the basis of clinical sign and symptoms in combination with urinary results. The urine culture remain as important test in diagnosis of UTI, to determine type of infection (Stam, *et al.*, 1993) (Wing, *et al.*, 2000). The aim of current study is to scope chemical, physical and microscopic tests for pregnant women with UTI in addition to bacterial test drug resistant profile that associated with such infections among pregnant women attending the Specialized Hospital for Maternity. (Al- Elywiah Public Hospital – Baghdad / Iraq).

Materials and Methods

Study sample

A total of 70 pregnant women with age ranging from 18 – 39 years were subjected to study after obtaining their consent, the pregnant women were admitted in **Al Elywiah Maternity Hospital** (public hospital) during period from December 2024 to April 2025. Urine samples for analysis were collected from pregnant patients in sterile container that follow up in hospital, all study cases, analysis and culture were used for the detection of UTI infection.

Sampling collecting

A sample of urine of clean catch mid –strem specimen used to minimize contamination with normal flora of genitourinary tract (Betty, *et al.*, 1998)



treated for UTI during pregnancy (Johnson, *et al.*, 2021). Untreated asymptomatic or symptomatic UTIs are associated with negative outcomes for both mother and baby (Azami *et al.*, 2019), which include pyelonephritis, which can progress to sepsis necrotizing infection and acute renal failure in pregnant women (Belyayeva and Jeong, 2022)

As the name indicates, the infected urinary tract parts coming in different region and symptoms, upper and lower urinary tract. The infection is named after the part gets infected and is referred to as cystitis (bladder infection) and phylonephritis (kidney infection). The symptoms associated with the bladder and kidney infections are contrasting that includes painful and frequent urination, in case of cystitis as a result of bladder infection. Whereas conditions like high fever and flank pain are commonly experienced, in case of kidney contagion which is referred to as Phylonephritis (Tanvi, *et al.*, 2024). Bacteria are the prime perpetrator responsible for conferring the infection among humans of UTI, but the role of certain fungi and viruses cannot be over looked. However, the incidence of UTI as a result of viral or fungal infection is considered to be rare phenomena (Sheikh, *et al.*, 2000).

The infection of urinary tract seems to be not harmless in initial stages, then, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances (Parveen, *et al.*, 2011) (Demilie, *et al.*, 2012) Urinary tract infection can be a consequence of poor diagnosis and is regarded as the common hospital acquired infection. (Kolawole *et al.*, 2009) (Koffuor, *et al.*, 2012) The infection encompasses a diverse group of clinical syndromes and diseases that differ in epidemiology, etiology, location and severity of the condition.(Lucas, *et al.*, 1993) The onset of the infection in pregnant, is in the 6th week of pregnancy through 24th week (Rahimkhani *et al.*, 2008) Although the



Introduction

Urinary tract infection(UTI), are the most common infection among pregnant women and have been associated with maternal and fetal complication, where one or more part of urinary system become infected, usually after bacteria overcome the natural defense mechanism of urinary tract (Yveline, *et al.*, 2023)(Al-Dujaily, 2000) (Akerelle, *et al.*, 2001).

UTI in female had higher frequency than men due to recurrent infection, and restrictions antimicrobial agents use during pregnancy, also in women the ease of urinary tract contamination by faecal flora and various other reasons (Blastein`s, 2019) (Fowler, 1986) Pregnant women are at high risk of UTI. It have been estimated that, all types of UTIs combined effect approximately 2%- 15% of women and this percentage increase in pregnant (Yveline, *et al.*, 2023) . In women, in general, the incidence of Urinary tract infection is more frequent, especially in pregnant time as compared to non-pregnant woman, due to so reasons, that pregnancy associated with physiological and anatomical changes, extended abdomen and difficulty of personal hygiene (Awaness, *et al.*, 2000). Also, increased bladder volume and decrease tone with decrease urethral tone, that contribute to increased urinary stasis and Uretherovesical reflux additionally, the physiological increase in plasma volume during pregnancy decrease urine concentration up to 70% which may develop glycosuria ,that encourage bacterial growth in urine(Patterson and Andriol 1987). On the other hand, the increase in urinary progesterin's and estrogen hormones may lead to decrease the ability of lower urinary tract to resist invading bacteria (Lucas, and Cunningham, 1993). Using data from National Birth Defects Study Population from (41,869 women) found that 11%- 26% of women reported being diagnosed and / or



على الطفل، بينما كانت نتائج فحص البيليروبين طبيعية لجميع العينات باستثناء عينتين. أظهر اختبار النترات نتيجة إيجابية لحدي عشر عينات، في حين أظهرت كريات الدم البيضاء نتائج إيجابية في خمس وثلاثون عينة.

أظهرت نتائج الفحص المجهرى نتائج كريات الدم الحمراء وكريات الدم البيضاء من (5-30 خلية) في معظم العينات، بينما أظهرت معظم العينات احتواءها على خلايا صديدية او قححية. وكانت فحوصات العينات للفطريات طبيعية في جميع العينات باستثناء اثنتين. أظهرت نتائج فحص البكتيريا أن العزل الأكثر للبكتيريا هو الإنيتيروكوكوس فيسياليس، والمكورات العنقودية الذهبية، والكلبسيلا الرئوية، والإشريشا كولاي القولونية. كما تشير النتائج إلى وجود فطريات كانديدا ألبيكانس في عينتين. كما أظهر اختبار حساسية البكتيريا أن المضاد الحيوي سيفيبيم، والنيتروفورانتوين، والسيفتاريكسون هي الأكثر فعالية ضد البكتيريا، بينما أظهر المضاد الحيوي أموكسيسكلاف نتائج متوسطة في المزيد من العينات، بينما كان السيفاليكسين مقاومًا في معظم العينات.

الكلمات المفتاحية: التهاب المجاري البولية، النساء الحوامل، المضادات

الحيوية و البكتريا المرضية



results shows present of *Candida Albicans* fungi in two samples, the sensitivity test of bacteria showed that antibiotic Cefepime , Nitrofurantion and Ceftriaxone are most effective in bacteria, while Amoxyclav antibiotic showed intermediate in more sample, while Cefalexin is resistant in most samples.

Keywords: Urinary Tract Infection (UTI), Pregnant women, UTI antimicrobial resistance.

المستخلص

التهاب المسالك البولية هو من الالتهابات الخطيرة التي يجب على النساء الحوامل الانتباه لها حيث تؤثر على صحة المرأة الحامل والجنين خلال فترة الحمل وبالباغة التسعة أشهر. هذه الأعراض تؤثر بطرق مختلفة على صحة الأم والطفل. أجريت الدراسة على النساء الحوامل من خلال فحص عينات من الادرار تم أخذها من الأمهات المسجلات في مستشفى العلوية للولادة، ثم أجريت تحليلات مختبرية متنوعة. بما في ذلك الفحوصات الفيزيائية والكيميائية والميكروسكوبية، ومعلومات واسئلة صحية للمرضى بالإضافة إلى اختبارات حساسية البكتيريا والمضادات الحيوية. تم أخذ حوالي 70 عينة من الادرار من النساء الحوامل اللاتي يتابعن حالتهم الصحية في مستشفى العلوية للولادة، وكانت أعمار النساء المشاركات في الدراسة تتراوح بين (18-39) عاماً. أظهرت نتائج الفحص الفيزيائي أن لون العينات يتراوح بين الأصفر الشاحب، والأصفر، إلى الداكن مع وجود عكارة، باستثناء عينتين أظهرتا لوناً مائلاً للأحمر، أظهرت نتائج الفحص الكيميائي أن معظم النساء الحوامل لديهن نتائج سلبية في اختبار السكر باستثناء ثلاث،، ويعزى ذلك الى إنهن صغيرات في السن، والبعض لديهن مرض سكر الحمل ، كما أظهر فحص (الأملاح والرمل) نتائج إيجابية عالية لمعظم الحوامل مما يعني أن معظم الحوامل لديهن عدوى من أنواع مختلفة من المواد غير المتبلورة والبلورات (رمل وأملاح)، حيث تعاني الحوامل من بعض الأعراض مثل صعوبة التبول والألم الذي يؤثر على صحتهم وقد يؤثر



Abstract

Urinary tract infection is a vary series symptom that pregnant women must be looking for treat as it effects in pregnant woman health baby for a long of 9 months of pregnant, this symptoms effect in different way on baby and mother health. The study on pregnant women carried out by examination of fresh samples taken from registered mothers in Maternity hospital and then carried varies analysis, physical, chemical and microscopic tests with bacterial and antibiotic sensitivity tests About 70 Urine sample taken from pregnant women that follow up their health in *Al Elywiah maternity Hospital*, the rested women with average age (18-39) years old. Results of physical examination showed that samples color of urine range from pale yellow , yellow to dark with turbid except two sample shows reddish color, although results of chemical examination, showed that must of pregnant have negative in sugar test except three, as they are young in age, or they have pregnant diabetic , also specific gravity showed highly positive results for most pregnant which mean that must of pregnant have infection with different type of amorphous and crystals in which pregnant have some symptom of difficult urination and pain that effect in their health and may effect baby , while result for bilirubin showing normal for all samples except two . Test for nitrite shows positive for eleven samples, while leukocyte esters showed positive in 35 samples.

Results of microscopic examination showed the results of RBC, WBC from (5-30 Cells) in the most sample, while for mucus most of samples show few, , fungi examination is normal all sample except two, the results of bacterial culture showed that more isolation of bacteria are *Enterococcus faesialis*, *Taphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, also



Study of Urinary Tract Infection Among Pregnant Women Attending Al Elywiah Maternity Hospital in Baghdad / Iraq, Original study

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دراسة التهاب المسالك البولية بين النساء الحوامل المراجعات لمستشفى العلوية للولادة \ بغداد \ العراق

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treatment of infected individuals, with clean supply of water and food, (Todd, 2023). The impact of these strategies would be further enhanced via organized health education programs, which may encourage healthy attitude and lead to a declining of gastro-intestinal parasite infections.

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contrast to the study in Baghdad, which observed ,that the male was more infected than female. With exception of *E. coli*, which showed high recovery whether in Bagdad or Babylon, others parasitic infection discovered, like, *T. tichura*, *Ancylostoma duodenal*, *Ascaris lu,mbriciodes* ,*Chiolomestic meselinae*, *H. nana*.

The high infection in Babylon than Baghdad, may be due to, several reasons,health education, patient logged in rural or urban, No facilities to get clean water, number of sample taken, expert of examiner ,equipment used beside others(Östan, *et al.*, 2017).

The routes of infection can also reflect the age-specific occurrence especially for *G. lamblia* and *E.histolytica/dispar* (Abdel Kareem, *et al.*, 2019), . They are generally transmitted by the fecal-oral route and are most common in children, while the lower occurrence in adults suggesting acquired immunity after repeated infections (Al-Qadhi, 2011). Most of the parasites included in this survey were reported as having high occurrence in male individuals. This result was similar to the previous findings (Todd, 2023). The gender difference may be due to male-specific behavioral factors (Hamza, 2009) such as taking risks with their work or during outdoor activity. While high occurrence of *E.vermicularis* was seen in female individuals this is agreed with findings of others (Al-Sadoon,. and Ali, 1993 and AL-Warid et al,2022). Some cities showed high occurrence of parasites, < 10000 cases for each. Some of these cities are big cities (Baghdad and Basra) and they are more urbanized. Finally our findings from the current survey revealed high occurrences of *E. histolytica/dispar*, pin worm and *G.lamblia*, and in the surveyed population especially among big cities and warrants long-term control strategies to improve sanitary and some living conditions, including



are cosmopolitan in distribution, more common in warm climates (Bahir Dar, Amhara,2019). So infections with these parasites were expected to be high in this survey. This can be attributed to the easy routes of transmission and the nature of direct fecal-oral contamination particularly through drinking water, food or reinfection for pin worm. Increasing of travel and immigration, due to bad circumstances, from region to other has been recently reported very frequently in Iraq which is most likely related with the high number of cases diagnosed. These findings were in consistent with others who showed that these three parasites are the top parasites in Iraq (Mahdi, 2022). Naqid (2023) found less total of infection(18.65%) whether in Babylon or Al-alwiyah Children Hospital ,but discovered high infection percentage of *E.histolytica* (68.61%),on the other hand ,found higher infection with *G. lambelia*(25.31%) comparing with AL-Alwia hospital, and less what was discovered in Babylon Hospital (45%), also the same author, declared ,the most infection occurred in the age group more 15 year ,comparing with others, this current study, registered ,high infection in age less than 10 years ,in both hospital. According to percentage of infection in Sulimania governorate registered the highest (70.6% with *E. histolytica* and 28.7% with *G.lambelia*, then followed by Salah-Aldine , Qadesia ,62.1% ,37.9% and 37.9%,28.5% respectively *G.lambelia* ,flowed registered high infection , Also , the same authors, recorded high infection with *E. vermiculais* (35.8%)Which was higher than noticed in Baghdad (21.8%) and less than in Babylon Hospital(55%),also found in female than male ,which may be due to social behavior and anatomy of geneto-Urinary system. In comparison with age, found more infection in age over 10 years, than other groups. (Mehdi, 2022).

Which is in contrast of current study, also their study found infection in male was higher than in female with in paroral of the study in Babylon, with



Table (11) Protozoa, and metazoan ,which observed in Al-Alwiyah children hospital with number and percentage of recovery

Parasite protozoa	Examined of samples	Infected of samples/ %of infection
<i>E. coli</i>	116	66/56.89
<i>E.histolytica</i>	109	51/46.78
<i>G.lambliia</i>	89	23/25.84
Total	314	44.5/140

Parasite metazoan	Examined of samples	Infected of samples/ % of infection
<i>E.vermicularis</i>	87	19/21.83
<i>H.nana</i>	70	11/15.71
<i>A.lumbeicoides</i>	49	9/18.36
Total	206	18.9/39

Discussion

There were many differences in outcome of this study between people attended Babylon hospital and AL-Alwiyah Children hospital, which may be due to ,socioeconomy, overcrowding, health education and the age of people who attended the above institute. This study indicated high occurrence of gastro-intestinal parasites in Babylon hospital and AL-Alwiyah Children Hospital and was high in the former (48.42%) than the later (34.49%). The most common protozoa infections in the survey of Babylon Hospital were *E. coli* 66/56%, *E. histolytica/ dispar* 51/46% and *G.lambelia* 23/25%, while the metazoan ,*E.vermicularis*49/55%,*A.duodenale* 28/41 and *A. lumbricodes* 21/25%. While the result of the study in Al-alwiyah Children hospital is nearly the same with parasites outcome, *E. coli*,66/56, *E. histolytica/ dispar* 51/46% and *G.lambelia* 23/25% while the metazoan , it recovered ,*E. vermicularis* 19/21% and *A. lumbercoids* 9/18%.These parasites



Table (10) Examined samples, number, percentage of protozoa and metazoan which registered in (Babylon hospital)

Parasite protozoa	Examined of samples	Infected of samples %
<i>E.histolytica</i>	104	61 58.6
<i>G.lambliia</i>	93	42 45.1
<i>E.coli</i>	111	77 69.3
<i>Chilomastix mesnili</i>	70	44 62.8

Parasite metazoan	Examined of samples	Infected of samples %
<i>E.vermicularis</i>	89	49 55
<i>A.lumbericoides</i>	82	21 25.6
<i>H.nana</i>	77	24 31.3
<i>Anclyostoma duodenale</i>	68	28 41.1
<i>Trichuris trichiura</i>	102	45 44.1
Total	418	157 37.5

Regarding total infection of parasites during the months of the year in AL-Alwiyah Children hospital and with gender. Highest infection recorded during October 28(57%) and the lowest observed in June 6(10.9%). High infection 19 (61%) registered in male during October, while high infection in female 4(80%) was pointed in November as mentioned in Table (10).

On the other hand, the same as in Babylon hospital, percentage of discovered protozoa was higher 140(44.58%) than the metazoan 39(18.93). In case of protozoa ,also ,*E.coli* was in the high rank,66(56.89),then followed by *E. histolytica/ dispar* 51(46.78%),while in the metazon,*E.vermicularis* noticed in high percentage 19(21.83%) then followed by *A.lumriciodes* 9(18.36%), as mentioned in Table (11)



In general, the infection in the year of study, was 168 (32.3%), the percentage of infection of the male 123 (36.39) was nearly the same 69 (37.91%) in female, high % of infection recorded in October 27 (65.85%) followed in August, 33 (63.82%), the lowest was registered in April 6 (12.5%) as mentioned in Table (9).

Table (9) Parasitic infection according to the months of the year in AL-Alawiyah children's hospital.

Months	Sample of examined	Sample of infected	%	Male of examined	Male of infected	%	Female of examined	Female of infected	%
Jan.	46	15	32.6	26	11	29.72	20	4	20
Feb.	49	7	14.28	37	5	14.07	12	2	13.33
Mar.	45	11	24.44	25	6	18.18	20	5	20
Apr.	48	6	12.5	32	4	10.25	16	2	12.5
May	46	15	33.33	31	11	34.37	15	4	26.66
Jun.	38	15	39.47	25	10	40	13	5	38.46
Jul.	47	30	32.6	28	16	42.10	19	11	57.89
Aug.	59	33	63.82	38	17	40.47	21	10	47.61
Sep.	69	34	49.27	45	18	38.29	24	11	45.83
Oct.	41	27	65.85	26	19	61.29	15	11	73.33
Nov.	32	10	31.25	25	6	23.07	7	4	57.14
Total	520	168	32.3	338	123	36.39	182	69	37.91

Generally, according to protozoa and metazoan spread in Babylon hospital, the protozoa was more prevalent 224 (58.5%) than metazoan 157 (37.55%). Accordance of, the species of protozoa most spread, *E. coli*, *C. meselinae*, and *E. histolytica/ dispar*, in 77 (69.3%), 44 (62.8%) and 61 (58.6%) respectively. While in metazoan, *E. vermicularis* registered high infection, 49 (55%), then *T. trichura* 45 (44.1%)



Table (7) Examined and infected with parasitic infection for both males and females in AL-Alwiyah hospital.

Parasite of infection	Sample of examined	Infected sample	%	Male of examined	male of infected	%	Female of examined	Female of infected	%
<i>E.coli</i>	116	66	56.89	81	42	51.85	35	24	68.57
<i>E.histolytica/ dispar</i>	109	51	46.78	77	39	50.64	32	12	37.5
<i>G.lamblia</i>	89	23	25.84	61	17	27.86	28	6	21.42
<i>E.vermicularis</i>	87	19	21.83	63	14	22.22	24	5	20.83
<i>H.nana</i>	70	11	15.71	52	9	17.30	18	2	11.11
<i>A.lumbeicoides</i>	49	9	18.36	32	5	15.62	17	4	23.52
	520	30.57	366/126	34.42	154	43/27			33.8

The most spread parasites, during the year of study in Babylon Hospital, were *E. coli*(77),*E. histolytica / dispar* (61),beside equal spread of *G.lambelia*(42) and *G.meslinnaein* (47),while the spread of helminthes ,was *E. vermicularis*(39) and *H. nana*(24).On the other hand, the most discovered ,parasites ,during months of studying ,was 60 cases ,during May, and 73 case in November as showed in Table(8)

Table (8) Parasitic infection according to the months of the year in Babylon hospital.

Months	E.coli	E.histolytica	G.lamblia	E.vermicularis	A.lumbericoides	H.nana	Ancylostma	Trichuris	chillomastix
Jan.	15	11	5	2	0	0	0	5	9
Feb.	0	3	7	0	4	0	1	0	0
Mar.	19	6	0	6	0	3	5	0	12
Apr.	0	7	5	3	3	2	1	10	5
May	12	5	16	11	4	0	0	9	3
Jun.	4	10	3	0	2	7	5	0	2
Jul.	8	9	2	2	2	5	1	6	0
Aug.	9	7	3	8	5	3	9	3	11
Sep.	6	3	0	0	0	4	6	8	0
Oct.	4	0	1	7	1	0	0	4	5
	77	61	42	39	17	24	28	45	47



The distribution of the recovered parasite in patients examined and its relationship with male and female in Babylon hospital listed in Table (6), which indicated ,that most prevalent protozoa were *E.coli*, *E. histolytica / dispar* and *C.meseline*,77(69.3%,61(58.6) and 47(64.2%0 respectively. While the most discovered helminthes were *T. tirchura* and *E. vermicularis*, which found in 45(44.1%) and 39(43.8%) respectively. In general, the male was more affected than female. On the other side the results Baghdad hospital was clarified in Table (7) Regarding percentage of distribution of parasites in patients in and its relationship with male and female hospital whether in male or female. Which indicated that the most prevalent parasites recovered were *E.coli* 66(56.8%),followed by *E. histolytica / dispar* 51(46.7%),while the lowest 11(15.7%) In case of helminthes, the *E.vermicularis* recovered in n19((21.83%) then *H. nana* in 11(15.71%).The distribution of the parasites in infected patient was nearly the same in male and female, but the rate of infection of parasites Al-Alwiah Children Hospital was lower than in Babylon Table (6 and 7)

Table (6) Examined and infected with parasitic infection for both males and females in Babylon hospital

Parasite infection	Samples examined	Samples infected %	Male examined	Male infected	%	Female examined	Female infected	%
<i>E.histolytica/dispar</i>	104	61(58.6)	76	41	42.63	28	20	20.8
<i>E.vermicularis</i>	89	39(43.8)	73	27	24.03	16	12	10.68
<i>A.lumbericoides</i>	82	21(25.6)	67	12	9.84	15	9	7.38
<i>G.lambliia</i>	93	42(45.1)	68	27	25.11	25	15	13.95
<i>E.coli</i>	111	77 (69.3)	72	51	56.61	39	26	28.86
<i>H.nane</i>	77	24(31.3)	5.1	17	13.09	26	7	5.39
<i>Ancylostoma duodenale</i>	68	28(41.1)	49	21	14.28	19	7	4.76
<i>Trichuris trichiura</i>	102	45(44.1)	78	34	34.68	24	11	11.22
<i>Chilomastix mesnili</i>	70	47(64.2)	44	36	25.2	26	11	7.7



266(46.2%(53.47) in Babylon hospital, also the age group. In Baghdad hospital in less than 10 year old ,was higher169(51.19%) than others group. On the other hand the male in later (hospital showed high infection 91(37.7%) than female 32(36.0%) as in the Tables (4 and 5)

In general ,the total infection in Babylon was 554(69.59%),the highest infected group was, the less than 10 years old 254(71.75%).Male registered high infection 73% than female 66%.While in Al-alwiyah, the total of infection was less 179(48%) and the female infected was higher 150(50%) than male 129(35%) as in Tables (4 and 5)

Table (4) Relationship between age groups and gender in Babylon hospital

Age of group years	Male			Female		
	Total number of examined	Exam. Number of infected	%infection	Number of examined	Number of infected	% total of infection
1-10	231	143	62.17	88	51(57.9%)	83.98
Above 10 years	232	80	34.44	90	49(54.4%)	55.6
Above 20 years	261	43	16.47	40	18	45
Total	578	266	46.2	218	118	54.12

Table (5) Relationship between age group and gender in Al-alwiyah children hospital.

Age of group Years	Male			Female		
	Number of examined	Number of infected	% of infection	Number of examined	Number&% of infected	%total of infection
1-10	317	131	41.31	93	32-34.4	163-51.19
Above 10 years	191	48	25.13	61	18-29.5	66-21.57
Total	366	129	35.24	154	50	169-19.23



Table (1) Number of examined people in Babylon and Al-alwiyah children's Hospitals laboratories and their percentage of infection

Name hospital	Total number of examined	Number of infected	%INFECTION
Babylon hospital	796	384	48.42
Al-Alwiyah children's Hospital	520	179	34.49

According to gender ,female was more infected in Babylon118(54.12%) than male 265(45.84%) while in in Al-alwiyah children's Hospital, the opposite occurred ,Male infection was higher 256(70.32%) than those of female 65(45.18%) respectively ,Tables (2 and 3)

Table (2) Number and percentage of infection of female and male in Babylon hospital

Sex	Samples examined	Infected samples	% Infection
Male	578	265	45.84
Female	218	118	54.12
Total	796	384	48.24

Table (3) Number and percentage of infection of female and male in AL-Alwiyah Children's Hospital

Sex	Number of examined	Number of infected	% of infection
Male	364	256	70.32
Female	166	65	45.18
Total	520	179	63.6

According the relationship of age and infection, the age group (1-10 year) was high infected than other groups ,whether in Babylon 194(83.98%) or in AL-alwiyah Children's Hospital 163(36.8%) comparing to other groups .The percentage of infection in female was higher 118(54.12%) than male



Sample collection: Samples were collected in dry, clean, sterilized, wide-opening plastic containers, taking care not to be contaminated with urine. For detection of pin worm, Scotch Tap Technique was used especially when itching or irritation of the anal regions according to (Gun and Pitt, 2021).

Macroscopic examination: Fecal samples were examined macroscopically before being detected by microscope. The description includes the quantity, forms, color, odor and density of the stool in terms of texture. It may be soft, watery, solid, semi-solid or diarrhea, as it is considered evidence of the type of parasite present in it, taking into account the examination of soft samples in 30 minutes. Trophozoite phases were found in the soft sample; while cyst is appears in solid samples. It is also necessary to contain the sample to contain blood or mucus, as in some cases parasitic worms such as round worm, like pin worm.,tape worm can be seen by necked eye.

Microscopic examination: A sample of feces was taken by means of a wooden stick and divided into two parts and placed on both ends of the glass chip, then a drop of normal solution at a concentration of 0.9% is added to one end to detect trophozoite, cyst, red blood cells and white blood cells. The second end is added a drop of iodine solution to dye Glycogen in dark brown color and the nucleus in red and the cytoplasm in light yellowish brown, and examined according to (Riaz ,*et al.*, 2020). In some cases, concentration methods were used in case of presence of small amount of feces present in the fecal sample.

Results

Current study revealed high infection 384/796 (48.42%) occurred in patients attended to Babylon hospital lab than those who arrived to Al-alwiyah Hospital-Baghdad, 179/520(34.4%) as showed in Table (1)



of the occurrence of potentially pathogenic intestinal parasites in Iraq. Infections with gastro-intestinal parasites are associated with poor sanitation, unsafe human waste disposal, inadequate of safe drinking water, low socioeconomic status, and demographic factors and spatial. The aims of this survey was to compare the presence of parasites which prevail in Babylon Hospital patients attended to its lab and compared it with AL-Alwiyah Children Hospital patients, during the year starting from the beginning g of January 2003 till the end of December 2023, taking in consideration, age, gender, month of the year. This study is retrospective depending on data received from both Hospitals, for comparing the infection in people who attended, Babylon Hospital and Al-Alwiyah Children's Hospital in Baghdad.

Materials and Methods

This current study depends on data received from Babylon hospital and AL-Alwiyah Children Hospital. Parasite infection was arranged according to gender of patients, age, seasonal variation from the beginning of January 2023 to the end of December, 2023. Data for each parasite were arranged according to the age distribution with groups as follow: Group 1: less than 10 year age, Group 2: more the 10 year to less than 20year ,group 3 more than 20.(in case of AL-Alwiyah ,children, group one was 10 year and the second group above 10 year Alawiyah Children's Hospital. 796 stool samples were collected from Babylon Hospital and (520) stool samples from Al-Alwiyah Children's Hospital

A survey study was conducted to investigate intestinal parasites for patient's attendance to both hospitals. Babylon Teaching Hospital and AL-Alwiyah Children hospital laboratories.



Introduction

Parasitic diseases are of great importance all over the world, as a result of its high prevalence and potential pathological effects, which sometimes reach the point of death mainly in tropical areas, such as parts of Africa, where the population suffers from malnutrition, overcrowding, and deterioration of the public health infrastructures and poor personal health situation, especially in rural areas (Garcia and Brucker, 1993). Parasites have caused the revenge and misfortunes of the human race for centuries (Bilharzia, recognized before 2000 ago) and continue to cause a lot of death and suffering in the world, especially in developing countries' (Ichpujani and Bhatia, 1994). According to the World Health Organization (2020) Estimated, the infections were, 800–1000 million *A lumbricoides*, 700–900 million Hookworm infections, 500 million *T trichiura*, 200 million *Giardia lamblia*, and 500 million *Entamoeba histolytica/dispar* cases globally. Regardless of recent efforts to control intestinal parasite infections, the diseases are still the main causes of mortality and morbidity in the world. In Iraq, gastro-intestinal parasitic infections caused a series of public health issues such as malnutrition, anemia, and growth retardation [AL-Sadoon, 1996, AL-Saqur, *et al.*, 2015 AL-Saqur, 2016, Mehdi, 2022]. Also, overall prevalence rate was about 22% with varying prevalence rates for individual parasites. It is acknowledged that gastro-intestinal parasites are of considerable public health importance in Iraq and throughout less developed Asia [AL-Taie, 2009]. But the geographical distribution and regional burden remain to be determined and updated regularly. However, most reports were based on single samples from a variety of patient groups and controls, and the community prevalence or occurrence has not been documented in details. The present study reports a detailed analysis



المستخلص

شملت الدراسة مقارنة معدل الاصابات بالطفيليات المعوية للمرضى المراجعين لمختبرات , مستشفى بابل التعليمي ومستشفى اطفال العلوية في بغداد للفترة الممتدة منذو بداية شهر كانون الثاني 2023 ولغاية نهاية شهر كانون الاول 2023, كان عدد المفحوصين في مستشفى بابل 796 وعدد المصابين 384 (48.49%). اعلى نسبة اصابة بالطفيليات كان ب بالانتامبيا كولاي (85% ز47) و يليه طفيلي الزحار الاميبي – الانتامبيا هستولاتكا (63.44%) وبعدها الديدان السوطية ترايكورس تراكيورا (45.9%), اما بقية الطفيليات فكانت كالاتي, الجيارديا اللمبلية (39.09%), الانتروبيس فيرميكيلارس (34.73%), جالومستك مسيليني (31.5%), الشريطية القزمة (18.48%), والديدان المعوية الكبيرة الاسكارس – الصفر الخراطيني (11.22%). بينما كانت نتائج فحص 520 من المراجعين لمستشفى العلوية للاطفال, وجدت اصابة 179 (34.4%). ايضا اظهر طفيلي الانتامبيا كولاي الاعلى نسبة اصابة من غيره, اذ سجل (69.3%) و يليه طفيلي الزحار الاميبي (58.6%) و يليه الديدان اسوطية ((45.9%), الجيارديا اللمبلية (25.84%), الديدان الديوسية (21.83%), دودة الصفر الخراطيني (18.36%) والشريطية القزمة (15.71%) واطهرت النتائج بان الاناث كانت الاكثر اصابة (52%) من الذكور في بابل كذلك كانت الاعلى (38%) في بغداد, وبينت الدراسة بان الاعمار 1-10 سنة الاعلى اصابة (56.81%) مقارنة ببقية الاعمار في بابل وبغداد ((51%). بينت النتائج بان الاصابات المعوية في بابل اكثر مما هو في بغداد

الكلمات المفتاحية: طفيلي الزحار الاميبي، الجيارديا الليمبية، الاوالي المعوية و الديدان الدبوسية.



Abstract

This study included detecting the rate of human infection with intestinal parasites on patients arriving the laboratories of Babylon Teaching Hospital and Al-Alawiya Children's Hospital In Baghdad for the period from beginning of January 2023 to the end of December 2023. The total number of examined people in the former hospital was 796, infected patients were (384 with 48.49%), The highest percentage of parasites recorded were *Endameba coli* (85.47%) followed by *E. histolytica* (63.44%) then *Trichuris trichura* (45.9%) the others parasites were *Giardia lambelia* (39.09%) *Enterobius vermicularis*(34.71%), *Chilomastix mesnili* (31.5%), *Ancylostoma duodenale* (19.04%), *Hymenolpis nana* (18.48%), *Ascaris lunmbricoides* (17.22%).While the result of the study in Al-Alawiya Children's Hospital In Baghdad, the total number of examined people was 520 ,with infected patients was (179 with (34.4%). The highest percentage of infection also was with *E.coli* (69.3%) followed by *E.histolytica* (58.6%) , *Trichuris trichiura* (45.9%), *G.lambalia* (25.84%), *E vermicularis* (21.83%), *A lumbericoides* (18.36%) *H nana* (15.71%), and %).Female patients were more(52%) infected in Babylon than male also the percentage of female infection in Baghdad was higher in(38%). Age group(1-10 year) was more (56.81%) than the others groups in Babylon ,also in Baghdad the former group recorded the highest (51%) . The current study proved that the infection of parasites in Babylon was higher than in Baghdad.

Keywords: *Entamoeba histolytica*, *Giardia lamblia*, intestinal protozoa and *Enterobius vermicularis*



Comparative Study Between Attendance Patients to Babylon Teaching Hospital in Babylon and Al-Alawiyah Children's Hospital in Baghdad Infected with Intestinal Parasites / Original article

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دراسة مقارنة بين المراجعين لمستشفى بابل التعليمي في محافظة بابل ومستشفى العلوية للاطفال في محافظة بغداد والمصابين بالطفيليات المعوية

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plant. It can also be used in finished products by small or large milk processing industries, distributors, and even government regulatory entities to promote the quality and protection of the authenticity of milk, as a product for daily consumption, which is considered a basic in the family diet.

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by evaluating only the physico-chemical parameters of the samples. parameters alone. Thus, milks A, B and C were shown to be chemically adequate when comparing their values with those of the the standard and numerically they do not differ from those of brand D milk, this suggests that the detection of GMP by SDS-PAGE is a specific and sensitive parameter as a marker of whey milk adulteration.

Conclusion

The extraction and run conditions in PAGE-SDS used in this work indicate that GMP is evident as a band of 20.9 KDa which probably represents a trimeric form of this peptide. The results obtained indicate that the investigation of GMP in milk, represents a specific and sensitive specific and sensitive parameter to detect adulteration levels of milk with whey, from 1%: what cannot be prevented. The presence of GMP in four brands of pasteurized milk analyzed in this study reveals adulteration by whey; however, this was only a descriptive study which needs to be carried out in a more complete study in future research, to carry out a more complete study to evaluate the quality of pasteurized milk sold in the region.

Recommendation

The presence of GMP in the signal of interest collected and analysis by HPLC and mass spectrometry of adulterated samples comparison of the chromatographic run with a commercial GMP standard (retention time), and by the recognition of GMP-specific monoclonal antibodies on an immunostick strip. The presented method is easy to implement and develop in the laboratory; it can be applied to routine tests of milk arriving at the dairy



percentages of 10 and 15%, showed lower total protein values (2.95 and 2.85%, respectively) than those established by the established in the current legal regulations. On the other hand, milk class E also exhibits relatively low percentage of caseins (1.6%) compared to the other commercial brands (around 2.5%); similar to what was found by (Faría and Bocan,[5]) who describe values of 1.99 and 1.85% casein for the samples previously adulterated with 10 and 15% serum, respectively; this is probably a consequence of the dilution of the caseins of the caseins with the addition of whey. The percentage of whey protein, obtained by difference between total proteins and caseins, were similar (about 0.8%) for brands A, B, C, and D, while brand E milk, showed a relatively lower value (0.55%) compared to the resulting value in fresh raw milk (1.25%). This corresponds to the low value obtained for total protein for this brand of milk.

Table 2. Physicochemical average values of pasteurized milk

Parameters	Type of pasteurized milk					Raw milk
	A	B	C	D	E	
Total protein	3,36	3,36	3,50	3,25	2,15	3,75
Caseine %	2,50	2,50	2,70	2,50	1,6	2,50
Serum protein %	0,86	0,86	0,80	0,754	0,55	1,25
Fat %	3,60	3,70	3,40	3,10	2,30	4,00
Total solids %	12,0	12,0	12,6	11,9	9,7	13,0
Acidity	17,0	18,0	15,0	18,0	15,0	19,0
Freezing point	0,545	0,525	0,535	0,538,	0,513	0,531

These findings, in correlation with the results of (Faría and Bocan, [5]), milk samples adulterated with whey between 1 and 10% cannot be detected



summarizes the results obtained with pasteurized milk type A, B, C and E, while class D milk did not show the presence of GMP. Based on the results as described in (Figure 2) the percentage of adulteration in milk samples where adulteration in the milk samples where GMP was found to be present was estimated presence of GMP was evidenced. In that sense, milk B corresponded to with 1%; brands A and C with 10% and brand E showed a little more than 50% adulteration (Table 1).

Table 1. Glycomarcopeptide (GMP) levels in five types of pasteurized milk

Type	Evidence of GMP in Adulterated Samples as a Percentage	Estimated percent of whey added
A	100	5
B	66,6	10
C	66,6	10
D	0	0
E	100	50

Physiochemical parameters of the analyzed milk

Table 2 shows the average values of the physiochemical parameters of the pasteurized milks A, B, C, D and E analyzed in this study and fresh raw milk, compared with the values established by current legal regulations, for pasteurized milk. It can be seen in this table that the milk of brand E, which showed the highest degree of adulteration, has values for total protein, fat, total solids and other physicochemical parameters, lower than the rest of the brands studied and lower than the value established in the value established in the standard, which was the product of an evident dilution of the milk as its lowering of critical point was observed. These results correlate with those observed by (Briñez et al., [1]; Léonil and Mollé, [20]). that milks whey adulterated with cheese whey in



be seen in this gel that there is a gradual increase in the 20.9 kDa band of GMP, proportional to the concentration of whey in each mixture. In this sense, if lines 4, 5, 6, and 7 of this figure are compared, it can be observed that the intensity of this band increases as the proportion of whey in each mixture increases. The results of this gel show that the 20.9 kDa band corresponds to GMP as it is absent in line 3 where fresh raw milk treated with ATC while in lines 8 and 9 it is clearly present in samples of sweet serum treated with ATC.

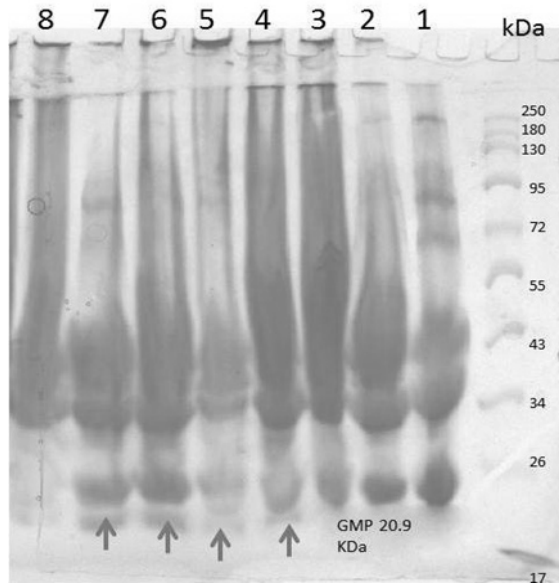


Figure 2. Protein profile in whey (milk mixtures), by SDS-PAGE

GMP as an index of milk adulteration

Fifteen (15) samples of pasteurized milk were classified to (A, B, C, D and E) according to the regions from which the samples were collected, were subjected to TCA treatment and analyzed by SDS-PAGE in order to evaluate the efficiency of the method in the detection of sweet whey adulteration of milk. (Table 1)



(Nakano and Ozimek, [17]). The mechanism of aggregation is unknown, and it is also unclear why the GMP aggregate does not dissociate on gels in the presence of SDS. It has been reported that SDS binds to the peptide moiety of a GMP molecule and avoids hydrophobic interactions with other GMP molecules. However, the glycine chains of the different GMP molecules can interact with each other through hydrogen bonding, which resulting in molecular masses greater than 18,000 Da, for this peptide in PAGE-SDS. Furthermore, the affinity of SDS for GMP is too weak to dissociate the GMP trimer, which may contribute to the predominance of the aggregated forms in the presence of SDS (Oria, [18]).

It has also been pointed out that at the level of the milk, α -lactoglobulin and casein-, can form bonds due to the effect of heat treatment and this may eventually block the binding of the peptide portion of GMP and SDS (Neelima et al., [2]). The 20.9 kDa in the TCA precipitate of the sweet whey, probably represents an aggregated form (trimer) of this peptide, which corresponds to that reported in previous research on GMP where its monomeric form has rarely been observed. . In this sense, several researchers have analyzed the structure of GMP, using various extraction, purification and characterisation methods, finding that in SDS-PAGE, sweet whey GMP appears as 2 bands with molecular masses of 20.740 Da (26.1%) and 18.380 Da (73.9%) (Bottacini et al., [19]). The structural heterogeneity of GMP, due to the variation in its glycidic composition, determines that the reported molecular mass values for GMP depend in part on its depend on the type of extraction and the method used to detect it (Chen et al., [10]). In order to observe a gradual increase in the 20.9 kDa band, corresponding to GMP, as a function of the degree of systematic adulteration of fresh raw milk with sweet whey, electrophoretic runs were carried out on mixtures of whey: milk. Figure 2, shows the protein pattern of whey: milk mixtures treated with TCA to extract GMP, It can



allows the precipitation of GMP, therefore, in the PAGE-SDS of raw milk and acid whey which not containing the glycomacropeptide treated with this treated with this acid, as expected, no band corresponding to GMP is observed in the PAGE-SDS of raw milk and acid whey. On the other hand, if we compare the protein profile of acid whey (line 5) and sweet whey (line 7) we can see that they are practically identical except for the 20.9 kDa band. This observation suggests that this band corresponds to the GMP.

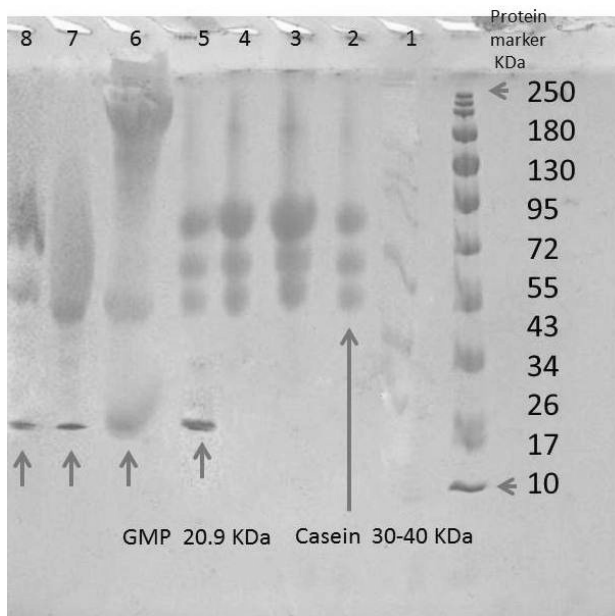


Figure 1. Protein profile of milk, acid whey and sweet whey by (SDS-PAGE)

It has been reported that the monomer of GMP has a molecular mass of 6.8 kDa without considering the glycidic portion; It has also been reported that it forms aggregates at neutral pH and that the molecular mass of the aggregate depends on the pH at which it is found (Kawasaki et al., [16]). GMP present in bovine whey has been observed in its aggregated form when analyzed by gel chromatography (Alemka et al., [13]) or electrophoresis



buffer containing SDS and β -mercaptoethanol as reducing agent (McKee and McKee, [15]). Electrophoresis was performed in a Mini- GEL preparation, at a constant voltage of 200 V, with a current ranging between 85 and 35 mA, using 4% and 15% acrylamide stacking and separation gels, respectively. The run was performed using tris-glycine buffer pH 8.3 for 60 to 120 minutes. A broad molecular mass range marker Bio-labs (New-England) was included in all runs. Visualization of the protein bands was performed by staining the gels with Coomassie blue R-250, 0.2% in methanol: acetic acid: water (25: 10: 85) at room temperature for a minimum time of 6 hours followed by decolourisation in a solution of methanol: acetic acid: water (85: 10:50).

Results and Discussion

Detection of GMP in SDS-PAGE

In order to recognize the band corresponding to GMP, the electrophoretic profile of fresh raw milk, acid whey (negative control) and sweet whey (positive control) before and after treatment with trichloroacetic acid were analyzed on the same gel (Figure1). The protein profile of authentic milk, acid whey and sweet whey, respectively, prior to treatment with TCA is shown in lines (3,5 and 7). In the milk sample, a highly coloured and extensive area between 30 and 40 kDa, which corresponds to the casein zone, whereas in the whey samples, the protein bands in the serum samples are 19 kDa and 13.5 kDa, corresponding to -lactoglobulin and -lactoalbumin monomer, respectively. Lines 4, 6 and 8 show the same samples after TCA treatment. The obvious difference between the protein profile in these three lines is given by a band with an apparent molecular mass of 20.9 kDa present in the line 8 which is also present in the sweet serum (line 7). The TCA treatment



The whey used to prepare the mixtures with milk was previously heated at 80°C for 10 minutes to remove any residual chymosin activity in the whey (Chow and Harper, [11]; Souza et al., [12]).

Glycomacropeptide extraction

GMP extraction was performed following the methodology of (Alemka et al., [13]) with some modifications: A 50 mL of sample (fresh raw milk, sweet whey, acid whey, whey-milk and whey-water mixtures), were added slowly and under constant agitation for 2 minutes, 25 mL of a 24% TCA solution was added slowly and under constant stirring for 2 minutes. This mixture was allowed to stand at room temperature for 60 minutes. After this time, the precipitate (caseins) was separated from the supernatant (whey proteins) by filtration using Whatman 42 paper, of 50% TCA was added to 30 mL of the filtrate, and allowed to stand under refrigeration (4-6°C (for 60 minutes. The resulting suspension was centrifuged in at 7000 rpm at 4°C for 10 minutes. The supernatant obtained was discarded and the precipitate was washed twice with 10 mL of a 1:1 ethanol-ether mixture and then centrifuged under the conditions of the previous centrifugation. The supernatant was discarded and the tubes were drained on absorbent paper to remove excess wash mixture. The final pellet was resuspended in 300 µL of Tris-HCl 0.05 M Buffer; EDTA 1mM, pH 7.2 and stored under freezing at -20°C until use (Laemmli, [14]).

Polyacrylamid gel electrophoresis (SDS-PAGE)

Samples resulting from precipitation with Trichloroacetic acid (TCA) were analyzed by SDS-PAGE, using the Laemmli discontinuous buffer system (Laemmli, [14]). In this system the proteins are denatured by heating in



Several studies worldwide (Rosas, [9]; Chen et al., [10]), have shown the presence of GMP in milk samples. This is an indicator of adulteration of raw milk for commercial purposes, representing a fraud for the consumer or the industry, but it means an increase in the volumes of profit for those who practice it, since this for the consumer or industrialist, but means an increase in profit volumes for the practitioner, as this whey has a cost very low compared to the price of milk. This practice is made possible because whey is a natural component of milk and therefore its colligative properties are similar, which makes its presence almost imperceptible when it has been added to milk. So far, several methods have been developed to detect adulteration of milk by whey addition, differing in their sensitivity and versatility of the equipment used in the analysis (Chen et al., [2012]). The isolation of GMP by trichloroacetic acid precipitation (TCA) followed by polyacrylamide (SDS-PAGE) gel electrophoresis has been widely used to detect the presence of GMP in processed milks for human consumption (Chow and Harper, [11]). In order to detect the presence of GMP as a marker of sweet whey milk adulteration, in the present work, it was proposed to standardize extraction and detection of this peptide by precipitation with TCA and SDS-PAGE respectively.

Materials and Methods

Sample Collection

Freshly milked raw milk (fresh raw milk) free of whey as a negative control, liquid whey was included as a positive control. Acidic whey was used as a negative control, the which was obtained by acid coagulation of fresh raw milk with 30% acetic acid Sweet whey-milk and sweet whey-water mixtures were prepared in the following proportions: 1: 5: 10 and 80 (%V/V).



the suspension; and in the suspension; undergoes hydrolysis by chymosin in a specific peptide bond, which resulting in destabilization of micelles and formation of micelles precipitates; furthermore, it is the only one of the casein that contains cysteine and carbohydrate residues in its structure (Valbuena et al., [4]; Faria and Bocan, [5]:).

Due to its status as a perishable foodstuff and the high risk of economic loss that this entails, the quality of milk is affected in certain aspects, such as microbiological, due to lack of hygiene, and physicochemical, due to adulterations, with the consequent nutritional, economic and legal consequences (B (Briñez et al.,[1]; O'Riordan et al., [6]). Among the most frequent adulterations that are made to milk are milk include: the addition of chemical substances not permitted by legislation, such as the addition of water and chlorides; the addition of preservatives or neutralizers; inadequate heat treatments that alter the native conformation of proteins; the addition of sweet whey, among others (Kavanaugh *et al.*, [7]). These adulterations occur at any stage of production, such as during transport or processing, and are intended to increase milk volumes in order to increase yields.

Sweet whey is the milk by-product that is separated from the curd during the cheese-making process. It is obtained by enzymatic coagulation of the milk, by the action of the enzyme chymosin, which hydrolyses the kappa λ -casein in milk at the Phe-Met bond (105-106), producing two fragments: a hydrophilic, acidic portion, consisting of 64 amino acids, called glycomacropeptide (GMP), which remains in solution in the serum, and the other portion is hydrophobic, basic in character, consisting of 105 amino acids , called Para casein, which is retained in the clot (Pinto, [8]; Hua et al., [3]).



Introduction

Milk is a foodstuff of great importance for its nutritional properties and high biological value. for its nutritional properties and high biological value. Approximately 1,400,000,000 litres of milk are produced each year. milk per year, which are used in the production of different dairy different dairy products, including pasteurized milk, powdered milk, cheese and other dairy products that are part of the daily diet. dairy products that form part of the Venezuelan diet (Briñez et al., [1]; Neelima et al.,[2]). The most important constituent elements in the milk of the different mammalian species are proteins, which are present in 3.2% of this food. mammals are proteins, which are present in 3.2% of this foodstuff. Of these proteins, 80% are caseins and the remaining 20% is represented by serum proteins - lactoglobulin, -lactalbumin, bovine serum albumin, euglactosomal albumin, euglactosomal serum albumin, bovine serum albumin, euglobulin, pseudoglobulin, mucins, globulin membrane proteins, and proteins, fat globule membrane proteins, other albumins, as well as numerous enzymes (Hua et al., [3]).

Caseins are a group of proteins that contain phosphorus and are specific to milk, where they are almost always found as micelles and precipitate at pH almost always in the form of micelles and precipitate at a pH of 4.6. Six types of caseins are known: s1 (alpha s1), s2 (alpha s2), (beta), (gamma), (kappa) and (lambda) (Hua et al., [3]). Casein has unique physico-chemical properties, when compared with other milk proteins. Some of these properties are insensitivity to precipitation by calcium ions, so it remains soluble in calcium solutions (Hua et al., [3]).With concentrations that precipitate all other caseins; stabilising effect on the other caseins when calcium is present in the suspension; undergoes hydrolysis of calcium ions in



المستخلص

تم استخدام دوديسيل كبريتات الصوديوم (SDS-PAGE) على نطاق واسع لفصل البروتينات الأصلية وغير الطبيعية. كان هذا في بلدان مختلفة. مفيد للكشف عن غش الحليب بالشرش ، وهي عملية احتيال وفقاً للمعايير القانونية الحالية. يشكل وجود (glycomacropeptide, GMP) علامة على الغش. يتم إطلاقه في المصل بسبب التحلل المائي لببتيد الكازين المحفز بواسطة وضع الرينين. في هذا العمل ، تم توحيد كشف GMP كمؤشر غش الحليب المبستر ، عن طريق عزل GMP مع الترسيب المتسلسل في حمض ثلاثي كلورو أسيتيك بنسبة 24% و 50% ، والمعالجة بإيثانول إيثر وإعادة التعليق في داريء Tris-HCl -EDTA ، من الشرش الحلو ، والشرش الحمضي ، والحليب الخام المسحوب حديثاً ، وخليط شرش الحليب ؛ الحليب المبستر. تم تحليل الرواسب بواسطة SDS-PAGE و GMP تم إثباته على أنه 20.8 كيلو دالتون في عينات الشرش الحلو ، وخليط شرش الحليب (1 ، 5 ، 10 ، و 50%) ، ولكنها غائبة في عينات شرش الحليب الحمضي والمسحوب الخام. حليب. توضح النتائج التي تم الحصول عليها أن فحص GMP بواسطة SDS-PAGE في الحليب ، مهم للكشف عن غش الحليب مع شرش الحليب ، إلى مستويات منخفضة تصل إلى 1% ، وهو أمر لا يمكن الكشف عنه إلا من خلال تقييم الحالة الفيزيائية الكيميائية للحليب.

الكلمات المفتاحية: الكاسيين، GMP ، غش الحليب، الرحلان الكهربائي للهلام بولي أكريلاميد، الشرش.



Abstract

The polyacrylamide gel electrophoresis sodium dodecylsulfate (SDS-PAGE) has been extensively used to separate native and denaturalized proteins. In various countries this has been useful to detect milk adulteration with whey, which is a fraud according to the current legal standards. The presence of glycomacropeptide (GMP) constitutes a marker of adulteration. It is released to the serum due to the hydrolysis of κ -casein peptide catalyzed by rennin in cheese elaboration. In this work, the GMP detection was standardized as a pasteurized milk adulteration index, by means of GMP isolation with sequential precipitation in trichloroacetic acid at 24% and 50%, treatment with ethanol-ether and resuspension in buffer Tris-HCl 0.05 M, EDTA 1 mM, from sweet whey, acid whey, recently drawn raw milk, mixtures of whey milk; pasteurized milk and powder milk locally commercialized. Precipitates were analyzed by SDS-PAGE and GMP was evidenced as a trimer of 20.8 kDa in samples of sweet whey, and mixtures of whey: milk (1, 5, 10 and 50%), but absent in samples of acid whey and drawn raw milk.. The results obtained demonstrate that investigation of GMP by SDS-PAGE in milk, constitute a sensible and specific parameter to detect milk adulteration with whey, to levels as low as 1%, something that can not be revealed only with the evaluation of the physical-chemical parameters of milk.

Keywords: Casein, GMP, Milk adulteration, SDS-PAGE, Whey.



Oldentification of Milk and Milk Product Adulteration by Molecular Methods / Original article

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الكشف عن غش الحليب ومنتجاته بالطرق الجزيئية

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Conclusion

The obtained results indicate that the polymer (ethylene – glycol-phthalic anhydride) grow between the layer of the crystal plane (001) of the modified bentonite minerals due to the closure of active sites as a result of polymerization process.

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that silica or quartz were not affected by the polymerization process [20]. The basic units of montmorillonite are shown in Table No. 1.

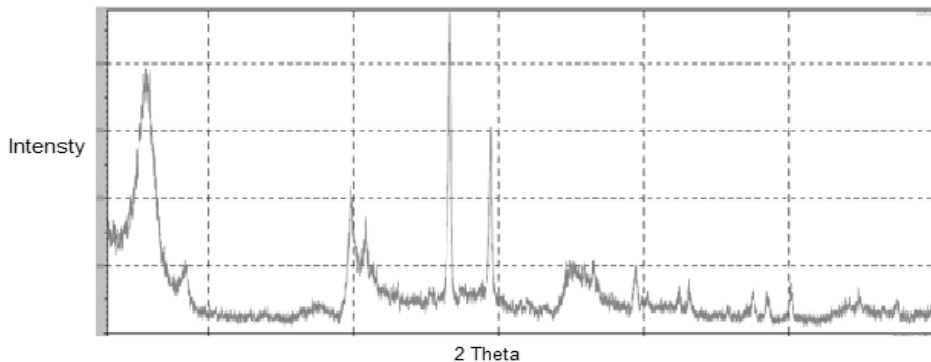


Figure (8) XRD pattern of the Bentonite

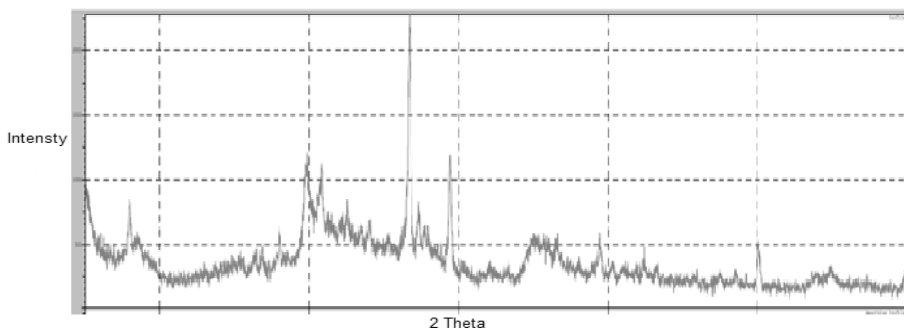


Figure (9) XRD pattern of the Bentonite-polymer composite

Table (1) Shows the basic units of montmorillonite mineral.

No		d-spacing	Hkl	I %
1	4.64-6.62	19-13.34	(001)	55
2	17.32	5.12	(003)	3
3	19.81	4.48	(100)	40
4	26.59	3.35	Q	100
5	29.40	3.04	CaCO ₃	60
6	39.38	2.29	(007)	14



XRD Diagnosis

Montmorillonite constitutes the largest proportion in the composition of bentonite, as its crystal pattern is of the type (Mono clinic) and its crystal unit dimensions are ($A = 5.717\text{Å}$, $B = 8.94\text{ Å}$, $C = 9.95\text{ Å}$) and the value of C varies according to the moisture and mineral content in the (001) plane [16]. Figure (8) shows that the (001) plane exists in the form of a wide band extending from the value of $2\theta = 4.640$ to 6.620 , as the thickness of this plane ranges from 17.66 Å to 13.34 Å . The reason for this dispersion is due to the lack of homogeneity of the bentonite sample in the content of cations and interlayer water content in the (001) plane [17]. The bentonite sample contained sand impurities (Q) with characteristic band at $2\theta=20.59$, $d=3.35\text{ Å}$ [18]. In the modified bentonite as shown in Figure (9) the band of the crystal plane (001) disappears due to the shift of this level to a higher (d) value below the detection limits of the device due to the growth of the polymer between the layers of this plane, which worked to increase the value of the expansion of this crystal level and thus its appearance at much lower values of 2θ . When reviewing the rest of the reflection multiples at this level, we note that they are not present, which means that the value of (d) expanded to infinity, meaning that the crystal peeled off at this level in a way that isolated the layers of the montmorillonite mineral in the form of two-dimensional sheet flakes [19]. It is also noted that the location of the crystal plane (100) was not affected as a result of polymerization, meaning that the polymerization process did not destroy the structure (octahedral and tetrahedra) in the single layers of aluminosilica, but rather worked to peel these layers and make them two-dimensional crystals with a length and width that did not change, but the arrangement of these layers along the height axis was destroyed. We also note



SEM Diagnosis

Figures (6) and (7) show the scanning electron microscope images of the bentonite sample before and after modification, which clearly shows what was previously indicated about increasing the homogeneity of the sample in the AFM diagnosis in terms of increasing the size of the small microscopic crystals through the growth of the polymer within the (100) plane, in addition to the destruction of very large crystals and their re-formation to reduce the variation in the sizes of these crystals, as the polymer appears as a fabric that increases the cohesion of the bentonite particles with each other.

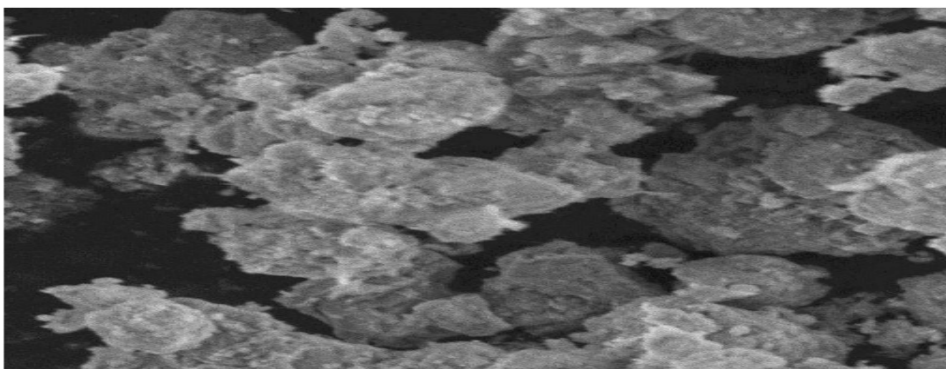


Figure (6) SEM image for the Bentonite

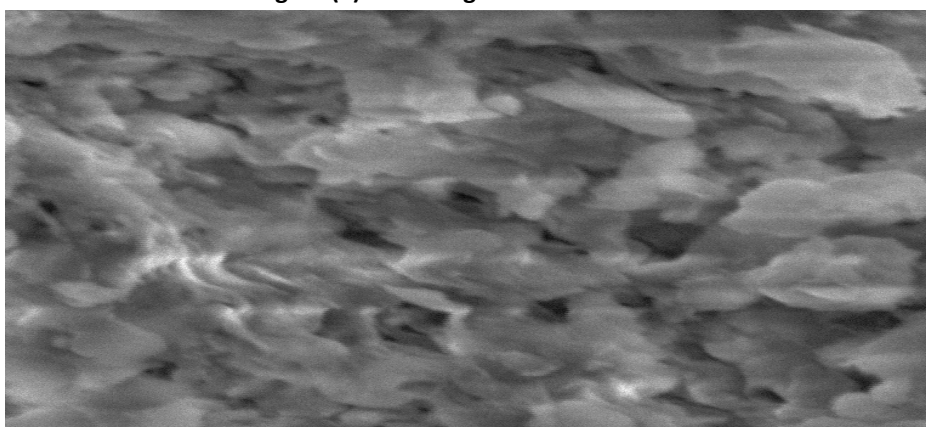


Figure (7) SEM image for the Bentonite-polymer composite

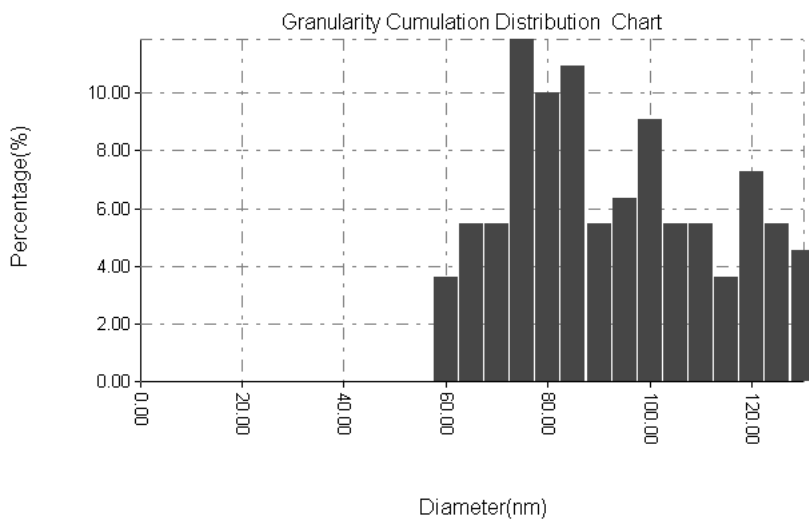
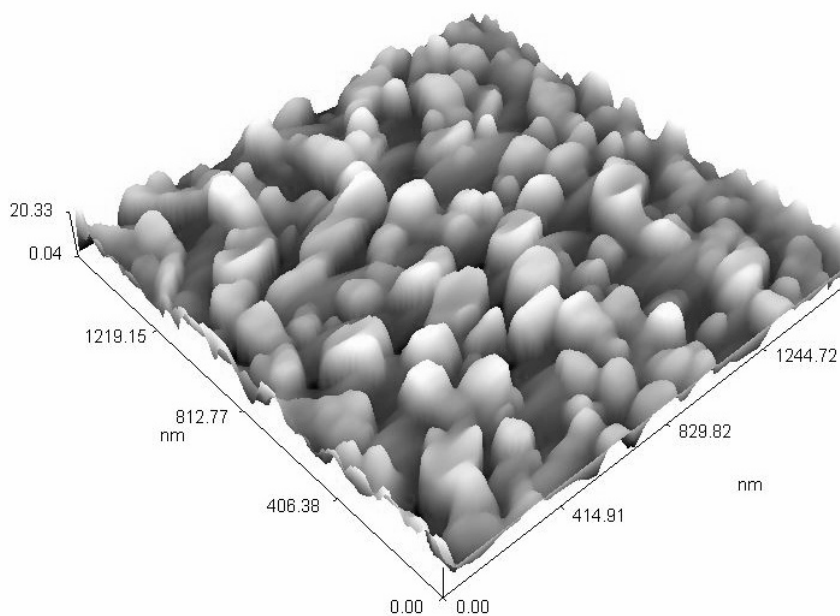


Figure (5) AFM picture and particle size distribution of the Bentonite-polymer composite

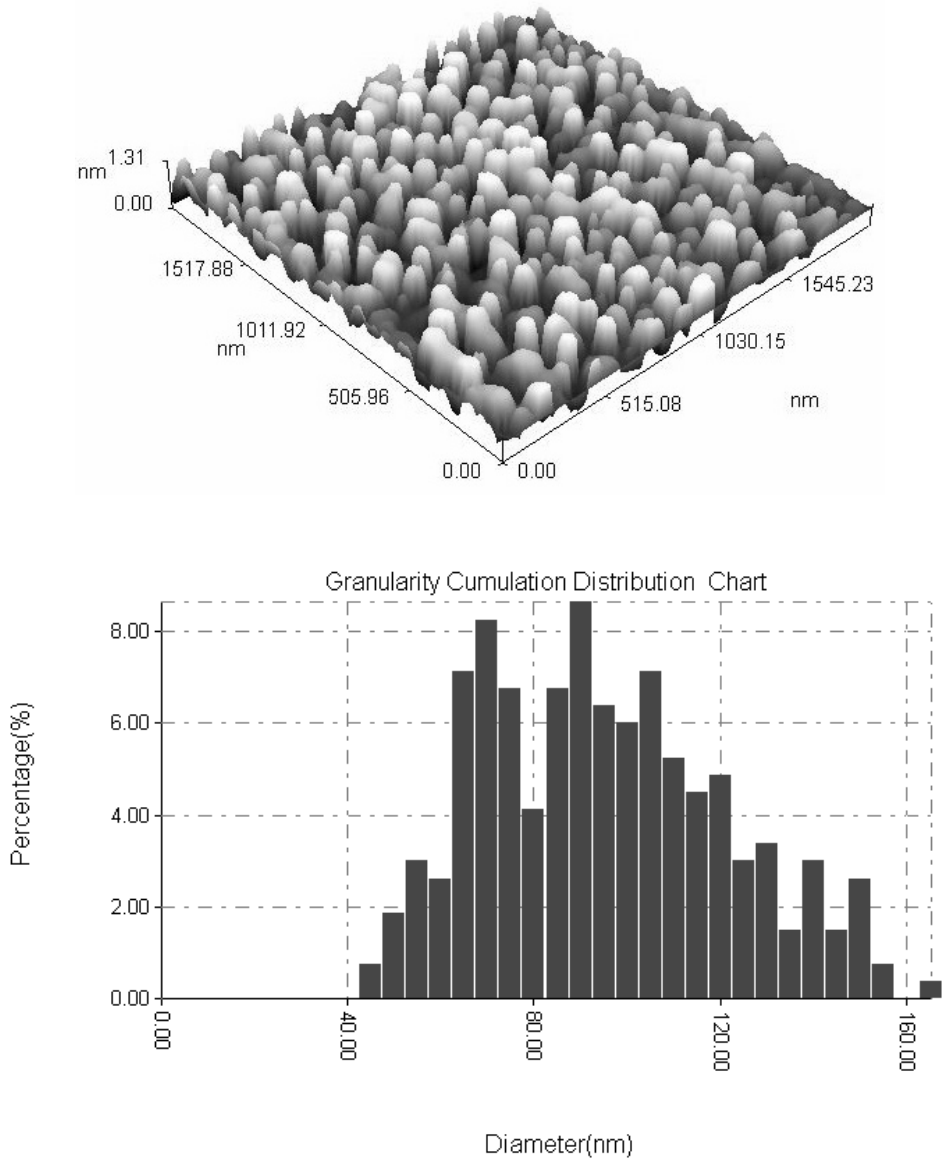


Figure (4) AFM picture and particle size distribution of the Bentonite

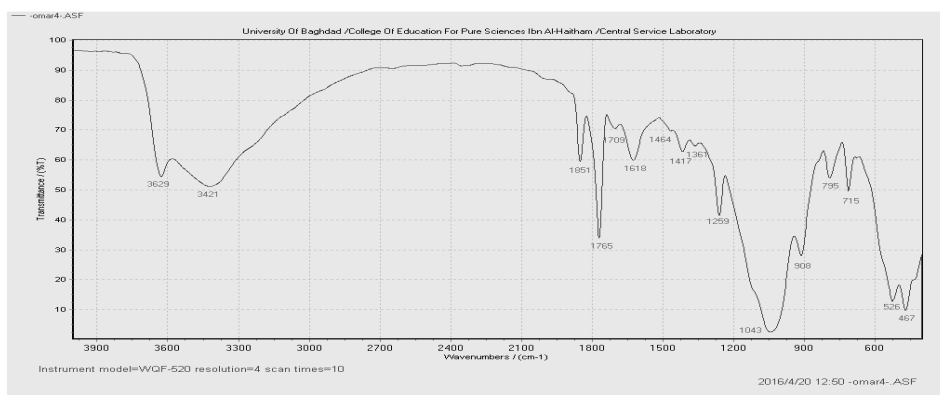


Figure (3) FTIR spectra of Bentonite-polyethylenglycol phthalate interlayer complex.

AFM Diagnosis

The results of the AFM for the bentonite model in Figures (4) and (5) show that the particle size of the particles ranges between (40-60nm) and the dominant diameter is at (90 nm) and the maximum height of the particles is (1.131 nm). The results for the modified bentonite mineral showed that the size distribution of the bentonite particles ranges from (60-130 nm) and the dominant diameter is at (75 nm) and the maximum height of the particles is (20.33 nm). After modification, it becomes clear that the polymerization process worked to increase the size of the bentonite particles through its positioning between its polymeric layers, in addition to its work to reduce the dispersion (poly dispersity) in the bentonite model. This means that the polymer worked to increase the homogeneity of the size distribution of the clay micro crystals. Increasing the homogeneity of the size distribution of the bentonite particles gives the surface desirable mechanical properties or electrical or thermal insulation properties similar to What is the case for the concept of (poly dispersity) for polymers.



As for the stretching vibrations of the carbonyl groups, they are present at 1618 cm^{-1} , as they shifted to a frequency lower than that of free bentonite due to the lack of opportunity for hydrogen bonding as a result of the competition of the nucleophilic polymer centers for hydrogen bonding. It is noted that the frequency of the building block (Si-O-(Al)Si) remains stable at 1043 cm^{-1} , which indicates that it has not entered into new bonds other than those that occurred during the formation of the bentonite-ethylene glycol (B-EG) complex, but the width of that band increases as a result of the increased randomness resulting from the positioning of the polymer between the clay layers.

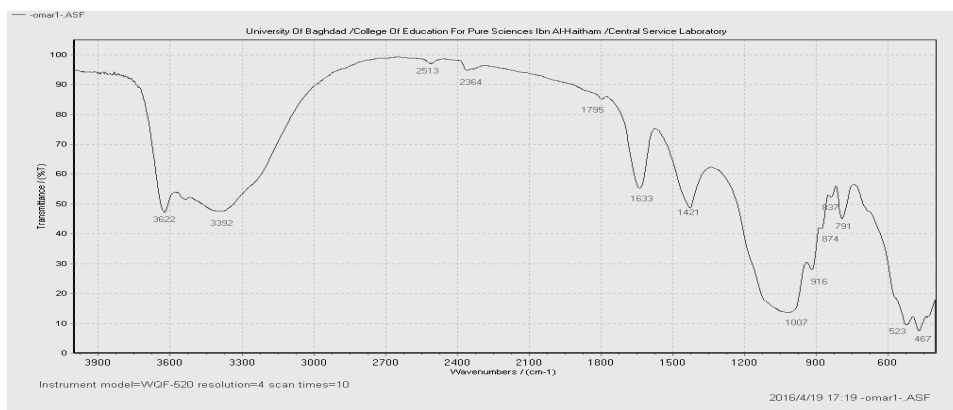


Figure (1) FTIR spectra of Bentonite

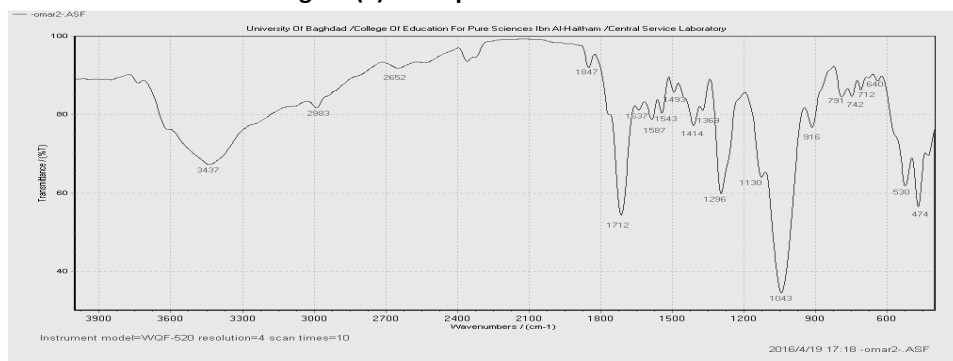


Figure (2) FTIR spectra of Bentonite-Ethylene glycol complex



bending vibration of the water molecule was shifted towards the higher frequency to reach 1847 cm^{-1} , which indicates that the (H_2O) molecule within the clay structure worked as a centers of receive ethylene glycol protons, which led to a decrease in the electronic density on the water oxygen atom and the appearance of a semi-positive molecular charge on it, which made the frequency to shift towards the higher frequency [15]. Also, the absorption band of the carbonyl group of (CO_3^{-2}) radical is shifted towards the higher frequency for the same reason. Also, the structural frame unit (Si-O-(Al)Si) band has shifted towards the higher frequency 1043 cm^{-1} combined by decrease band broadening, which means that the structural units (Si-O-(Al)Si) becomes more isotropic through the action of ethylene glycol molecules to increase the distances between the clay layers and thus reduce the molecular interactions between those clay layers which was the dominant case characterized by bentonite crystalline anisotropy. After the polymerization of ethylene glycol molecules present between the clay layers in the bentonite mineral, as noticed in Figure (3) the return of the free (OH) group, but with a higher frequency than that of bentonite, which indicates the disengagement of the hydrogen bonding between the (OH) group and the ethylene glycol molecules. Also, a slight decrease in the frequency of the hydrogen-bonded (wide) (OH) group is observed due to the increase in hydrophobicity through the growth of the polymer between the bentonite mineral layers. As for the bending frequency band of the (H_2O) molecule, it remained the same, which indicates that it remains a center for receiving the few remaining protons that cause hydrogen bonding. We note that the stretching vibration of the new ester carbonyl group formed at 1765 cm^{-1} indicates that these groups act as centers for bonding with the protons that belong to the (OH) groups in the clay structure and thus have this high value.



Results and Discussion

FT-IR Diagnosis

Figure (1) shows the FTIR spectrum of bentonite, the band appears at 3622 cm^{-1} is due to the stretching vibration of the (OH) group associated with the aluminosilicate structure in the clay. What distinguishes this (OH) group its presence in an environment with few hydrogen bonds, which make it appeared as a sharp band with a high frequency [10]. There is also another band at 3392 cm^{-1} due to the stretching vibration of the hydrogen-bonded (OH) groups, characterized by the shift to lower frequency and by an increase in the broadening of the absorption band [11] which due to the weakening of the (OH). At 1795 cm^{-1} , the bending vibration of the interlayer water molecules present between the clay sheets is noted [12]. At 1633 cm^{-1} , the stretching vibration of the carbonyl group in the carbonate radical is observed due to the presence of calcite mineral (calcium carbonate) impurities in the bentonite sample [13]. At 1007 cm^{-1} , a strong and broad band is observed due to the symmetric and asymmetric stretching vibrations of the aluminosilicate unit (Si-O-(Al)Si) [14]. Figure (2) shows the FTIR spectrum of the bentonite-ethylene glycol complex, where the free (OH) band is almost completely absent due to the hydrogen bonding between bentonite and ethylene glycol, at the same time, it is noticed a shift in the stretching frequency of the hydrogen-bonded (OH) group towards the higher frequency (blue shift) due to the contribution of the free (OH) group in the bentonite material, by hydrogen bonding, but this time with ethylene glycol molecules, this led to shifting the previous frequency of the hydrogen bonded of (OH) group towards the higher frequency. The weak band at 2983 cm^{-1} belongs to the stretching vibration of the aliphatic (C-H) bonds of ethylene glycol. The



removal of all ions present in the clay. The clay was dried using a laboratory oven at a temperature of 80 °C for two days until it was completely dry. Then, the bentonite was ground using a mortar mill and then the ground clay was sieved using a sieve with a diameter of 150 µm.

Preparation of Bentonite-Ethylene Glycol Complex B-EG

A (15 gm) of bentonite was placed in a beaker and gradually add (7 gm) of ethylene glycol in a mortar. The sample was ground continuously while adding ethylene glycol. The grinding process continued after the ethylene glycol was added for half an hour. The sample was covered tightly and left for 10 days to complete the diffusion process.

Preparation of Bentonite-Polyethylene Glycol Phthalate Compound

A (5 gm) of the previous complex (B-EG) was mixed with (5.5 gm) of freshly prepared phthalic anhydride (obtained from the thermal decomposition of phthalic acid under vacuum) in a mortar and the materials were ground until a homogeneous mixture was formed. This mixture was placed in the microwave for activation the polymerization condensation reaction for half a minute. After that, the mixture was taken out and ground again and returned to the microwave. This process was repeated until the sample hardened, then the time period was doubled to insure complete polymerization process. The obtained material was a homogeneous mass stable with microwave heating, does not dissolve in water and does not leave any trace of dissolved materials in water. This is confirmed by comparing the absorbance of the sample washing solution above with the absorbance of distilled water by UV/VIS spectrophotometer.



Materials and Methods

Equipment uses

In this study, the raw materials and prepared materials were characterized using an X-ray Diffraction device, model 6000 shimaduz, Japan. Atomic force microscopy (AFM) type spam AA300 USA2008. FT-IR-600, Bio Tech U.K., England. SEM in the service laboratory of the College of Education for Pure Sciences / Ibn Al-Haitham. The work also included the use of the following devices during the preparation stage of the new composite material: a sensitive balance type Sartorius Lab. BL 210 S, Germany with $\pm 0.0001\text{g}$ accuracy, laboratory furnace Green Equipment LTD, Melting Point Apparatus Galincamp England. Single Stage vacuum pump, China. Hyundai home microwave, 150 μm sieve, Saxohlet device, and finally UV/VIS spectrometer, Double beam, shimadzu.1800, Japan.

Materials

The following chemicals were used in this research: Ethylene Glycol and Phthalic acid from BDH

Clay

Bentonite was obtained from the General Geological Survey Company. The the oxides percentages were (SiO_2 : 54.66, CaO : 4.77, Al_2O_3 : 14.65, MgO : 6.00, Fe_2O_3 : 4.88, SO_3 : 1.20, Na_2O : 0.65, loss on ignition: 12.56)

Bentonite preparation

The bentonite clay was washed with distilled water several times, then filtered and washed again using the Saxohlet device for a week to ensure the



Introduction

Composite materials are formed from the union of two or more components has different properties from the other, those come together to form a new material differs in its properties from the parent materials, and a stable structure due to homogeneity of mixing at microscopic scale [1]. The composite consists of two basic elements, the binding medium or matrix and dispersed materials or additive. The binding material is the largest one which surrounds the other components and works to held all elements together producing a compact system. Additives are materials added to polymeric materials to give them specific properties and improving some other properties. These materials are added in the form of small granules or spheres. Additives to enhance the properties of polymeric materials by improving electrical conductivity [2], reducing or increasing porosity [3], maintaining dimensional stability, increasing the polymer's resistance to shocks [4], improving friction properties [5], and obtaining some magnetic properties [6-8]. The development of manufacturing clay minerals which are used as fillers allows obtaining thermoplastic materials with high mechanical strength. The approved method that was developed includes dispersing the filler granules in the polymer molten material after covering them with a thick layer of resin in which a very strong binding with the mold is achieved. The efficiency of clays in filling polymer systems also depends on the degree of their dispersion in the matrix medium, because well dispersion of the filler gives a higher surface area able to interact with the polymer frame [9].

In this work we amid to modify the surface of Iraq clay (Bentonite) by developing ester polymers and studying its morphology by scanning electron microscopy (SEM), Atomic force microscopy (AFM), diagnosing it by X- RD and FTIR.



Abstract

In this study, a new composite material was characterized and prepared by polymerizing ethylene glycol located among bentonite layers with phthalic anhydride. The results showed that the polymer bonds with the clay structure through hydrogen bonding. The polymerization process led to the destruction of the three-dimensional crystal structure of the clay and the isolation of its layers in the form of two-dimensional nanoscale shells. The growth of the polymer around the isolated clay layers increased the total particle size at the microscopic scale.

Keywords: Composite materials, Bentonite, Polyethylene glycol phthalate

المستخلص

حضرت و شخصت في هذه الدراسة مادة متراكبة جديدة من خلال بلمرة الاثيلين كلايكول الموجود بين طبقات معدن البنتونايت مع مادة الفثالك انهايديرأيد. بينت النتائج ان البوليمر يترابط مع هيكل الطيف من خلال التآصر الهيدروجيني. ادت عملية البلمرة الى تحطيم التركيب البلوري ثلاثي الابعاد للطين وعزل طبقاته على شكل قشور نانوية ثنائية البعد، كما ان نمو البوليمر حول طبقات الطين المنعزلة عمل على زيادة الحجم الحبيبي الكلي للجسيمات على المستوى المجهرى.

الكلمات المفتاحية: مواد متراكبة، بنتونيت، فتالات بولي إيثيلين جلايكول



Synthesis of a New Composite Nanomaterial Consisting of Bentonite- Polyethylene Glycolphthalate / Original article

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**تخليق مادة نانوية متراكبة جديدة تتكون من
البننتونيت - بولي إيثيلين جلايكول فثالات**

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lymphomas) are examples of cancers that change red cell antigens, which reduces their reactivity when cells gather together. The patient's true blood group may be confirmed through saliva testing if the patient is a secretor. Saliva and other secretions containing ABH compounds have some advantages. Additionally, non-secretors are more likely to suffer some disorders [1, 14].

The nature of different amino acid substitutes present on the surface of red blood cells determines the nature of Rh systems, which have significant clinical significance. The percentage distribution of the Rh factor in all study groups revealed that the majority of secretors and non-secretors have Rh-positive blood groups, while the minority has Rh-negative blood groups and this agrees with Jaffa [7] and Saboor [14]. Additionally, there is no link between BMI and secretor status as a show in table-2 and the BMI is not affected.

Conclusion

According to these findings, it can be concluded that secretor status as genetic trait is not gender-specific, and BMI is not affected by this trait. However, individuals who have non-O blood groups (A, B, and AB) are more likely to be non-secretors than those with O blood group, which may indicate an association in the expression of these two genetic traits that needs further investigation.

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**Table-2: Comparison between secretors and non-secretors according to their BMI categories**

BMI		Secretors (n=128)	Non-secretors (n=39)	P value
M ± SD (kg/m ²)		26 ± 4.9	26.1 ± 4.3	0.920
Category (n, %)	Normal	120 (48%)	41 (44.5%)	0.845
	Overweight	88 (35.2%)	34 (37%)	
	Obese	42 (16.8%)	17 (18.5%)	

Discussion

Comparing these findings with those attained by other researchers, the majority of previous studies found no link between gender and secretor status [8, 7, 11].

On the other hand, Figure-2 shows non-secretor status is far more common in individuals who are not in the O blood type; in contrast, secretor status is higher in O blood group individuals. These findings are consistent with those of Jaffa [7] research refers to Secretor status is substantially more common in blood group O persons than in non-O blood group individuals. This could account for the low prevalence of some diseases in people with blood group O.

In contrast, Saboor [14] discovered that B blood group has highest secretor frequency, while has the lowest in AB blood group. Secretor positivity is found in blood groups A and O, respectively. While, Imoru [6] discovered that the differences in the frequencies of secretors and non-secretors in the ABO blood group system were not statistically significant. Furthermore, Fakorede [4] found an incidence of secretor status was more prevalent among blood group A individuals compared to any other blood group but the difference was not statistically significant.

Secretor status and (ABO) blood types have an impact on clinical and forensic medicine as well as specific disorders. (Leukemia's and non-Hodgkin



Furthermore, there is no significant difference ($P= 0.785$) in the distribution of blood groups based on Rh system. Figure-3 shows that the majority of secretors and non-secretors have Rh positive blood group (89.2% and 90.2% respectively), and the minority of them have Rh negative group (10.8% and 9.8% respectively).

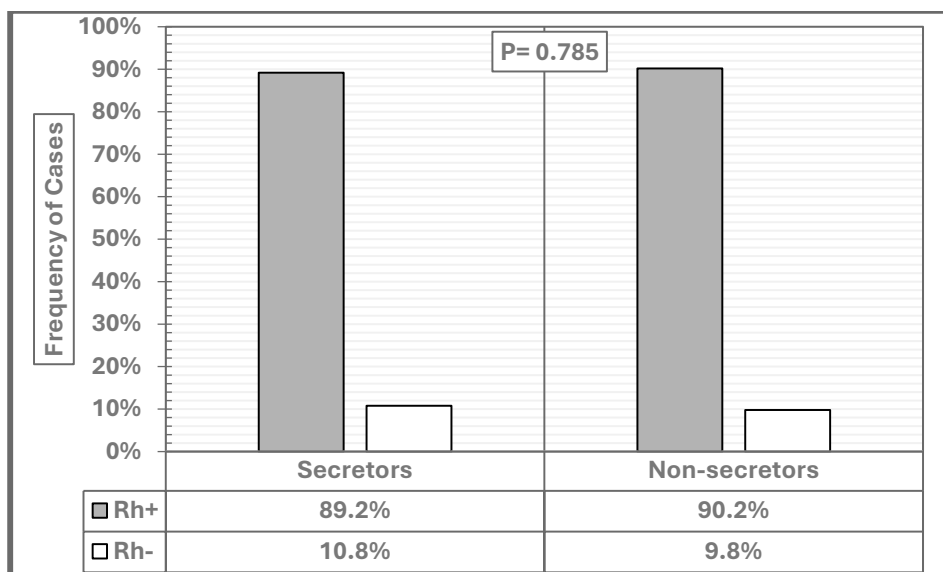


Figure-3: Distribution of Rh blood groups in secretors and non-secretors

To identify whether secretors have different BMI from non-secretors, Table-2 reveals non-significant difference ($P= 0.920$) between two groups (26 ± 4.9 vs 26.1 ± 4.3 kg/m² respectively). Also, this table shows no significant difference ($P= 0.845$) in the frequency of normal, overweight, and obese cases between secretors (48%, 35.2%, and 16.8% respectively) and non-secretors (44.5%, 37%, and 18.5% respectively).

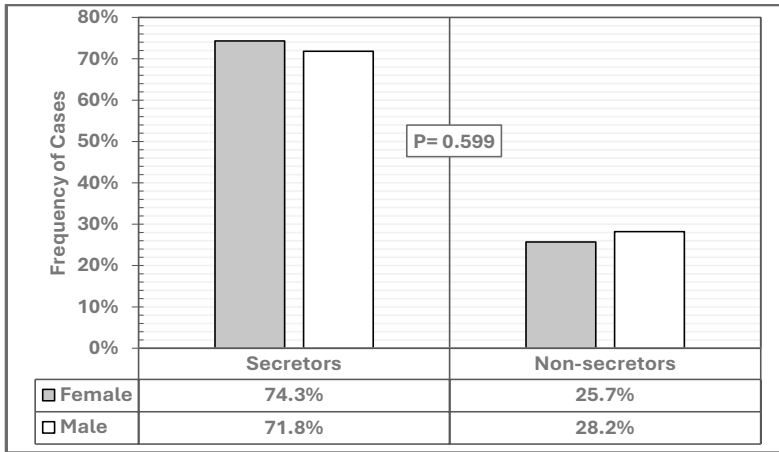


Figure-1: Frequency of secretors and non-secretors in male and female individuals

However, Figure-2 reveals that about three quarter (73.9%) of non-secretors are those who have non-O blood groups (A, B, and AB), which is significantly ($P= 0.0003$) higher than 52.4% of those in secretors' group. In contrast, about half (47.6%) of secretors have O blood group, which is significantly higher than only quarter (26.1%) of non-secretors.

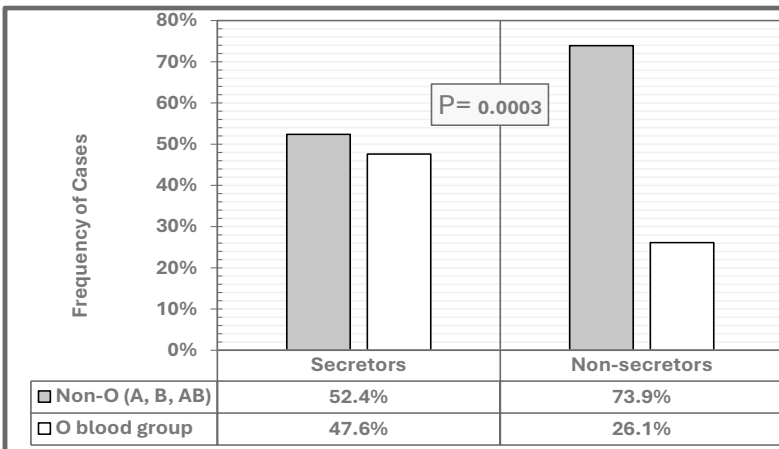


Figure-2: Distribution of O and non-O blood groups in secretors and non-secretors



Table-1: Characterization of individuals enrolled in this study

Character		Values
Gender (n, %)	Male	163 (47.9%)
	Female	179 (52.1%)
	Total	342 (100%)
Age (years)	Range	18 – 63
	M ± SD	27.5 ± 9.1
Secretor status (n, %)	Secretors	250 (73.1%)
	Non-secretors	92 (26.9%)
ABO blood group (n, %)	A	85 (24.9%)
	B	80 (23.4%)
	AB	34 (9.9%)
	O	143 (41.8%)
Rh blood group (n, %)	+ve	306 (89.5%)
	-ve	36 (10.5%)
BMI (kg/m ²)	M ± SD	26.1 ± 4.7
BMI category	Normal	161 (47.1%)
	Overweight	122 (35.7%)
	Obese	59 (17.2%)
(M ± SD: mean ± standard deviation; BMI: body mass index)		

To estimate the frequency of non-secretors among those people based on their gender, Figure-1 shows that 25.7% of females are non-secretors which is lower than 28.2% in males but this difference is not significant (P= 0.599).



proteomics, and bioinformatics, saliva contains hundreds of components that can be used to diagnose systemic disorders, as proof of exposure to numerous dangerous agents, and as health and disease biomarkers [15, 12].

Materials and Methods

A case-control study was conducted on 342 Iraqi adults over the course of five months, from October 2022 to February 2023. Individuals (both males and females) were recruited from educational institutions. Several data were recorded for all individuals including: gender, age and Body mass index (BMI). BMI was calculated by dividing body weight (in kg) by square height (in meters), with kg/m^2 as the unit. All subjects have saliva samples taken to determine their secretor status. In addition, blood groups based on the ABO and Rh systems were determined using the hemagglutination slide method.

Results

Three hundred and forty-two Iraqi adults were enrolled in this study at average age of 27.5 ± 9.1 years ranging from 18 to 63 years, 163 (47.9%) of them are males and the rest 179 (52.1%) are females (Table-1). This table shows that 250 (73.1%) of them are secretors and only 92 (26.9%) of them are non-secretors. According to ABO system, 143 (41.8%) of those people are presented with blood group "O", 85 (24.9%) and 80 (23.4%) of them have group A and B respectively, while only 34 (9.9%) have blood group AB. However, 306 (89.5%) of individuals have positive Rh blood group, and only 36 (10.5%) of them have negative Rh group. Moreover, this table reveals that the average body mass index in those people is $26.1 \pm 4.7 \text{ kg}/\text{m}^2$, 161 (47.1%) of them with normal BMI, 122 (35.7%) with overweight BMI, and only 59 (17.2%) of them are obese.



Introduction

Depending on their ability to secrete ABO blood group antigens in body fluids, people are classified as "secretors" or "non-secretors" [10]. In 1930 Putkonen reported that a person's hereditary proclivity to emit ABH blood group chemicals in secretions could be a non-secretor or secretor [3]. Karl Landsteiner, an Austrian, discovered the ABH blood group system in 1901 [5, 14]. The Blood group (ABH) antigens (B, A, and H) have been found on lymphocytes, red blood cells, platelets, secretions, body tissue cells, and body fluids (except cerebrospinal fluid) [14]. In 1926, it was discovered that antigens A and B were present not only on red cells but also in saliva in the form of soluble antigens [3]. The ABO system categorizes blood types as A, B, AB, and O based on the presence or absence of A, B, and H antigens on the surface of RBCs [17]. In fluids, blood group "O" secretes only H element, blood group "A" secretes both A and H substances, and blood group "B" secretes both B and H substances [10, 17]. The qualitative and quantitative aspects of secretors' mucus, saliva, and other secretion bodies are the primary distinctions between secretors and non-secretors [2]. Approximately 80% of the population secretes H-substance regardless of ABO blood group [13]. The fucosyltransferase-2 secretor gene, which is located on the short arm of chromosome 19, has two alleles: dominant "Se" and recessive "se." In their inheritance pattern, (Se Se) and (Se se) have a dominant secretor phenotype, whereas (se se) has a recessive non-secretor phenotype. As a result, blood group "O" secretes only material H in fluids, while blood group "A" secretes both substances A and H, and blood group "B" secretes both substances B and H [16, 9]. Saliva research is now rapidly progressing. Because of the use of novel methods such as metabolomics, genomics,



المستخلص

تم تصنيف نظام فصائل الدم ABO إلى أربعة أنماط ظاهرية وهي A و B و AB و O بعد اكتشافه من قبل كارل لاندشتاينر في عام 1990. حيث يمكن العثور على مستضدات فصيلة الدم ABO في سوائل الجسم مثل السوائل اللعابية والهضمية. علاوة على ذلك، يتم تصنيف الأشخاص إلى "مفرزين" أو "غير مفرزين" بناءً على قدرتهم على إفراز مستضدات (فصيلة الدم ABO) في سوائل الجسم. أجريت الدراسة القائمة على الضبط على 342 بالغاً عراقياً تم تقديمهم في المؤسسات التعليمية. استمرت الدراسة من أكتوبر 2022 إلى فبراير 2023 وهدفت إلى تحديد العلاقة بين حالة الإفراز و (الجنس وفصائل الدم ومؤشر كتلة الجسم). أظهرت النتائج أن الإناث غير مفرزات وهي أقل من الذكور ولكن هذا الفرق ليس ذا دلالة إحصائية. بينما غير المفرزين هم أولئك الذين لديهم فصائل دم غير O (A و B و AB)، وهي أعلى بكثير من أولئك في مجموعة المفرزين. في المقابل، يمتلك حوالي نصف مفرزي الدم فصيلة دم O، وهي نسبة أعلى بكثير من ربع غير المفرزين. علاوة على ذلك، لا يوجد فرق جوهري في توزيع فصائل الدم بناءً على نظام Rh. كما لا توجد فروق جوهريّة في تواتر الحالات الطبيعية، وزيادة الوزن، والسمنة بين مفرزي الدم وغير المفرزين.

الكلمات المفتاحية: فصائل الدم، مؤشر كتلة الجسم، اللعاب، المفرزين



Abstract

The ABO blood group system was divided into four phenotypes i.e., A, B, AB, and O following its discovery by Karl Landsteiner in 1990. Antigens of the ABO blood group can be found in bodily fluids such as the salivary and digestive fluids. Furthermore, People are classified as "secretors" or "non-secretors" based on their ability to secrete (ABO blood group) antigens in body fluids. The control-based study was conducted on 342 Iraqi adults who were presented at educational institutions. The study lasted from October 2022 to February 2023 and aimed to determine the relationship between secretor status with (gender, blood groups, and body mass index). The results showed that the females are non-secretors which are lower than in males but this difference is not significant. While, non-secretors are those who have non-O blood groups (A, B, and AB), which is significantly higher than of those in the secretors' group. In contrast, about half of secretors have O blood group, which is significantly higher than only a quarter of non-secretors. Furthermore, there is no significant difference in the distribution of blood groups based on the Rh system. In addition, there are no significant differences in the frequency of normal, overweight, and obese cases between secretors and non-secretors.

Keywords: Blood groups, Body mass index, Saliva, Secretor



Distribution of Secretor Status in a Sample of Iraqi Population Based on Their Gender, Blood Groups and Body Mass Index / Original article

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تصنيف حالة الإفراز في عينة من السكان العراقيين
حسب الجنس وفصيلة الدم ومؤشر كتلة الجسم

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associated molecular pattern molecule, influencing immunological and inflammatory responses via RAGE and toll-like receptors (Chen *et al.*, 2022). HMGB1 functions as a pro-inflammatory cytokine upon extracellular release, initiating inflammation and immunological responses (Wei *et al.*, 2022). HMGB1 stimulates NF- κ B to enhance the expression of pro-inflammatory factors (Lotze & Tracey, 2005). HMGB1 has a role in the pathophysiology of several immune-mediated and inflammatory diseases (Chen *et al.*, 2022). Elevated serum ferritin levels are posited to be associated with diminished Vitamin D levels, perhaps resulting from impaired calcium metabolism and increased iron absorption (Pishgahi *et al.*, 2020), which adversely impacts calcium absorption and bone metabolism (Amelia *et al.*, 2020).

Conclusion

Serum levels of DKK1, HMGB1, C-reactive protein, and ferritin are high in both TDT patient cohorts relative to the control groups. Only serum DKK1 exhibits a substantial elevation in the TDT+Hypocalcemia group compared to the TDT+Normocalcemia group. The results, along with elevated CRP levels, an inflammatory biomarker, indicate the involvement of DKK1 and inflammation in the hypocalcemic condition of TDT patients. Serum DKK1 serves as a predictor for hypocalcaemia in TDT patients, demonstrating adequate sensitivity and specificity. Consequently, DKK1 protein may serve as a therapeutic target for the bone issues of thalassaemia patients.



et al., 2020). The inflammatory state and the positive connection with hsCRP may explain the increase in HMGB1 (Wang *et al.*, 2015; Yan *et al.*, 2009). HMGB1 may be released into the extracellular environment and may act as proinflammatory mediators (Czura & Tracey, 2003). HMGB1 may associate with toll-like receptors to activate NF- κ B, consequently influencing total immune-inflammatory responses (Yang *et al.*, 2005) by promoting the production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF α (Park *et al.*, 2003). Upon the binding of HMGB1 to RAGE, the production of IL-6 may occur, hence modulating inflammation (Nativel *et al.*, 2013). The ROC analysis findings in Figure 3 and Table 4 indicated that the elevation of DKK1, followed by an increase in CRP, serves as the most effective predictor for hypocalcaemia in TDT patients. DKK1 serum levels were elevated in individuals with TDT (Voskaridou *et al.*, 2009). At baseline, all patients had elevated blood levels of DKK1 in comparison to controls (Voskaridou *et al.*, 2008). The rise of DKK1 may contribute to osteoblast dysfunction in thalassaemia and indicate a potential target for developing new treatments for bone loss in thalassaemia patients (Voskaridou *et al.*, 2008). DKK1 may have a significant role in certain essential facets of bone biology (Daoussis *et al.* 2010). DKK1 is involved in the aetiology of osteoporosis and may serve as a serological marker for assessing bone mass in adults (Butler *et al.*, 2011). DKK1 does not correlate with illness and inflammation (Giordano *et al.*, 2022). Patients with TDT exhibited elevated blood DKK1 levels and heightened bone turnover (Voskaridou *et al.*, 2012). DKK1 has an inverse correlation with whole-body BMD, indicating its potential use as a bone biomarker in this patient demographic (Wiromrat *et al.*, 2023). HMGB1, whether actively or passively released into the extracellular compartment, serves as a damage-



infection (Zhang *et al.*, 2008) and inflammation (Tian *et al.*, 2020) are facilitated by calcium-mediated signal transduction. Upon release, extracellular HMGB1 interacts with the RAGE receptor on macrophages, subsequently activating the stimulator of the interferon response pathway to generate pro-inflammatory cytokines, including TNF and IL-6 (Fang *et al.*, 2021). The activation of Ca^{2+} -dependent protein kinases, namely Ca^{2+} /calmodulin-dependent protein kinase kinase (Li *et al.*, 2020), governs the phosphorylation and release of HMGB1. Calcium ions function as a universal second messenger by participating in many physiological processes via their allosteric regulatory effects on intracellular enzymes or proteins (Zhao *et al.*, 2017). Cellular demise or significant injury may result from disruption of intracellular calcium signalling (Criddle, 2016).

Table (3) indicates that the levels of the analysed biomarkers are unaltered by the confounding variables. Consequently, thalassaemia and its sequelae, which include increased ferritin, anaemia, and inflammation, account for the alterations in the levels of the evaluated biomarkers. The correlation matrix in Table 4 indicates that DKK1 exhibits a substantial association with CRP and HMGB1, and most notably, it has a significant negative correlation with Adj.T.Ca. These results underscored the importance of inflammation and calcium in the levels of DKK1 and HMGB1. Furthermore, elevated concentrations of the master inflammatory protein (HMGB1) and the pro-inflammatory glycoprotein (DKK1) may impair the blood-brain barrier (BBB) and contribute to neurodegenerative processes (Al-Dujaili *et al.*, 2020; Menet *et al.*, 2020). The second is an intrinsic inhibitor of the canonical Wnt pathway that may influence neuro-repair mechanisms and may lead to excitatory synaptic disintegration (Al-Dujaili *et al.*, 2020; Menet



(about 500 ng/l), there is no significant change in blood calcium and albumin concentrations between the control group and thalassaemia patients (Al-Hakeim *et al.*, 2017). Nevertheless, our results, along with those of other studies, indicate that blood calcium levels decrease in people with elevated ferritin levels (Al-Dujaili *et al.*, 2019; Al-Hakeim & Alhillawi, 2018; Ridha *et al.*, 2024; Ridha *et al.*, 2018; Moustafa *et al.*, 2023). Individuals with raised ferritin levels have a higher prevalence of low calcium levels compared to those with normal ferritin levels (Iskandar *et al.*, 2020). In TDT patients, increased ferritin levels diminish calcium levels (Iskandar *et al.*, 2020). Insufficient blood calcium levels may signify poor bone mineral density, especially in areas with prevalent hypovitaminosis D (Ali *et al.*, 2023). Reduced parathyroid hormone levels correlate with declining calcium levels in these people (Ansaf *et al.*, 2024). Potential reasons for this variation across studies include the compensatory mechanisms of the body that keep calcium levels within a normal range despite possible underlying problems. Occasionally, these mechanisms may partially mitigate the hypocalcaemia effects, preventing the emergence of unconcealed symptoms (Lertsuwan *et al.*, 2018b). TDT patients had elevated blood HMGB1 levels in comparison to healthy people. Individuals with an intact spleen had lower levels of HMGB1 than those who underwent splenectomy (Chirico *et al.*, 2015). Patients with thalassaemia had significantly elevated HMGB1 levels compared to healthy controls (Ghalwash *et al.*, 2024).

Patients with hypocalcemia have higher C-reactive protein levels relative to the TDT and control groups, offering further evidence of inflammation. An elevation in DKK1 and HMGB1 may indicate the presence of an inflammatory disorder. The translocation and release of HMGB1 during



Table 5: Receiver operating characteristic-area under curve (ROC-AUC) analysis of the measured biomarkers for diagnosis of hypocalcemia in TDT patients.

Variable	Cut-off	Sensitivity %	Specificity %	Youdin's J statistic	AUC(95% CI)	P
DKK1 pg/ml	901.44	73.2	72.2	0.454	0.83(0.75-0.91)	<0.001
CRP mg/l	7.90	53.4	52.6	0.060	0.63(0.51-0.74)	0.034
HMGB1 ng/ml	9.71	56.1	55.6	0.117	0.56(0.44-0.68)	0.340
Ferritin ng/ml	2457.65	51.2	50.0	0.012	0.55(0.43-0.66)	0.452

Table (5) data reveal that an elevation in DKK1 over the threshold value (901.44 pg/ml) suggests that the participants may substantially exhibit hypocalcemia ($p < 0.001$), with a sensitivity of 73.2% and specificity of 72.2% (Youden's J statistic=0.454). The findings indicated that a CRP rise beyond the cut-off value (7.90 mg/l) suggests the participants may have hypocalcemia, exhibiting low sensitivity (56.1%) and specificity (58.6%), along with a minimal Youden's J statistic (0.117). HMGB1 and ferritin have no significant predictive value for hypocalcemia ($p > 0.05$).

Discussion

The primary findings of this research include increased levels of DKK1 and HMGB1 in TDT patients with hypocalcaemia compared to those without hypocalcaemia. This may be attributable to the influence of these markers on intracellular or plasma calcium concentrations. Prior research indicates that calcium levels are reduced in people with TDT relative to healthy individuals (Karim *et al.*, 2016).

Individuals with beta-thalassemia had reduced blood levels of vitamin D and calcium (Bashir *et al.*, 2023). In Iraqi persons with low ferritin levels



Receiver operating characteristic analysis for prediction of hypocalcemia

A study of receiver operating characteristics (ROC) was conducted to ascertain the diagnostic sensitivity and specificity of the observed biomarkers for predicting hypocalcemia in TDT patients. Figure (3) displays the ROC curves of the study.

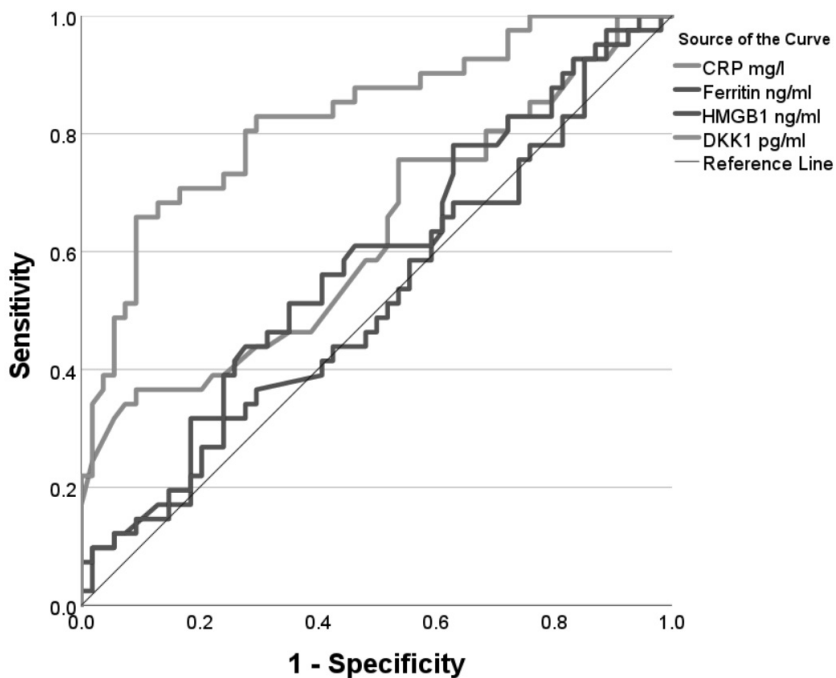


Figure 3. Receiver operating characteristic curves (ROC) of serum CRP, ferritin, HMGB1, and DKK1 for differentiation between TDT patients with and without hypocalcemia.

Table (5) presents the coordinates of the ROC values and the concentration cut-off that yields optimal sensitivities and specificities.



Table 4: Correlation matrix of the measured biomarkers with demographic, clinical, and biochemical parameters in transfusion-dependent thalassemia

Parameters	LnCRP	LnHMGB1	LnDKK1	LnFerritin
Sex	0.006	-0.196	-0.254*	0.020
Splenomegaly	-0.331**	-0.018	-0.156	-0.057
Adj.T.Ca	-0.260*	-0.133	-0.355**	-0.119
Adj.I.Ca	-0.260*	-0.138	-0.352**	-0.116
LnCRP	1	0.045	0.209*	0.399**
LnHMGB1	0.045	1	0.472**	-0.015
LnDKK1	0.209*	0.472**	1	0.069
LnFerritin	0.399**	-0.015	0.069	1
Ln(No.of transfusion)	0.169	0.000	0.025	0.245*
Ln(Days after transfusion)	-0.125	0.137	0.085	-0.205*

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level. The following parameters are not mentioned in the correlation matrix because they have no significant correlation with any of the measured biomarkers: family history, splenectomy, age, weight, height, PCV, haemoglobin, albumin, T.Ca/Mg, IonizedCa/Mg, iron, T. & I.Mg, T.Ca/Mg, IonizedCa/Mg, duration of disease, and age of onset,

The following parameters are not mentioned in the table because they have no significant correlation with the measured biomarkers (LnHMGB1, LnFerritin, LnCRP, and LnDKK1): family history, splenectomy, haemoglobin, age, weight, height, PCV, albumin, T.Ca/Mg, IonizedCa/Mg, iron, T. & I.Mg, T.Ca/Mg, IonizedCa/Mg, duration of disease, and age of onset. The results show LnCRP has a significant correlation with DKK1 and ferritin, in addition to a significant inverse correlation with splenomegaly, and total and ionized adj.Ca. There is also a significant correlation between HMGB1 and DKK1. DKK1 also has a significant inverse correlation with sex, splenomegaly, and Adj.T.Ca. Serum ferritin has a significant correlation with the number of transfusions and a significant inverse correlation with the number of days after transfusion.



The findings indicate that the diagnosis of TDT and hypocalcemia in a patient has a significant effect on the levels of the measured biomarkers ($F=50.914$, $p<0.001$) with the highest size effect (partial $\eta^2=0.851$). The cofounders (age, sex, residency, weight, and height) have no significant impact ($p>0.05$) on the biomarker levels. Based on these data, it appears that having TDT or being a patient is the main factor impacting the serum levels of the biomarkers. While other confounding variables included in the investigation do not significantly affect changes in the biomarker levels. The between-subject effects tests demonstrated that the inclusion of the disease had a substantial impact on multiple parameters. Out of all the factors examined, the diagnosis can explain 64.9% and 63.7% of the variation in adjusted total and ionized calcium, respectively. Other biomarkers that were significantly affected were haemoglobin (partial $\eta^2=0.556$), PCV (partial $\eta^2=0.555$), ferritin (partial $\eta^2=0.430$), CRP (partial $\eta^2=0.315$), iron (partial $\eta^2=0.310$), and DKK1 (partial $\eta^2=0.258$), HMGB1 (partial $\eta^2=0.097$), total and ionized magnesium (both partial $\eta^2=0.054$).

Correlation between the biomarkers and other parameters

The correlation coefficients and their significance between the measured biomarkers and all parameters are presented in Table (4).



has significantly higher albumin and total and ionized Ca/Mg ratios than the TDT+Hypocalcemia and the control groups. As expected from the classification of groups, the control group has a significantly higher Adj.T.Ca and Adj.I.Ca than both TDT groups with the the lowest level in the TDT+Hypocalcemia group.

Results of multivariate generalized linear model (GLM) analysis

Table (3) shows the results of multivariate GLM analysis which examined the association between the measured biomarkers and diagnosis (control versus TDT+Normocalcemia and TDT+Hypocalcemia) while adjusting for covariates (age, sex, residency, weight, and height).

Table 3: Results of multivariate GLM analysis with the biomarkers as dependent variables and diagnosis as explanatory variables while adjusting for extraneous variables.

Type	Dependent Variables	Explanatory Variables	F	P	Partial η^2
Multivariate	PCV, Hb, CRP, Albumin, T.Mg, I.Mg, Adj.T.Ca, Adj.I.Ca, T.Ca/Mg, IonizedCa/Mg, Iron, Ferritin, HMGB1, and DKK1	Diagnosis	50.914	<0.001	0.851
		Sex	0.759	0.701	0.078
		Residency	1.090	0.374	0.109
		Age	1.027	0.431	0.103
		Weight	0.937	0.517	0.095
		Height	1.073	0.389	0.107
Between-subjects effect	Diagnosis	Adj.T.Ca	236.547	<0.001	0.649
		Adj.I.Ca	224.288	<0.001	0.637
		Hb	159.740	<0.001	0.556
		PCV	159.676	<0.001	0.555
		Ferritin	96.639	<0.001	0.430
		CRP	58.791	<0.001	0.315
		Iron	57.407	<0.001	0.310
		DKK1	44.404	<0.001	0.258
		HMGB1	13.762	<0.001	0.097
		T. & I.Mg	7.336	0.008	0.054
		IonizedCa/Mg	1.319	0.253	0.010
		Albumin	1.227	0.270	0.009
T.Ca/Mg	0.227	0.635	0.002		

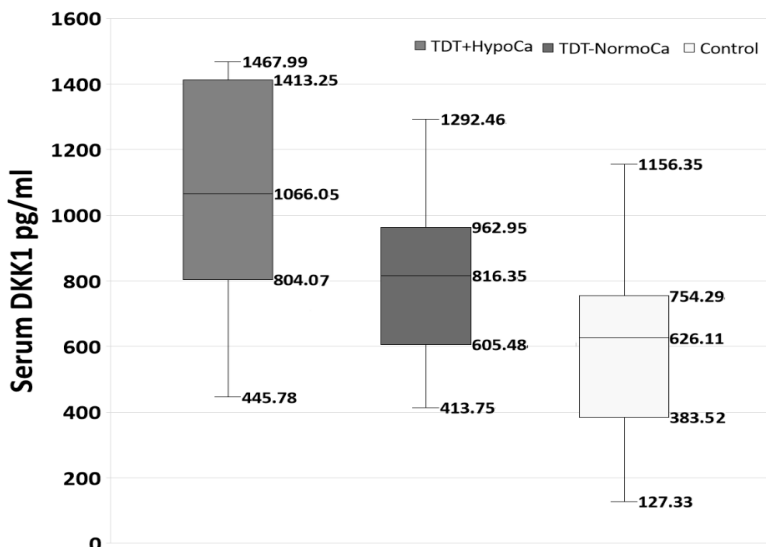


Figure 2. Serum DKK1 of the transfusion-dependent thalassemia (TDT) patients with and without hypocalcemia, and the control groups. The medians, interquartiles, and ranges of data are presented in this Figure too.

The findings indicated that both the TDT+Normocalcemia group (816.34 (603.77-962.95) pg/ml) and the TDT+Hypocalcemia group (1066.05 (804.07-1413.25) pg/ml) exhibited significantly elevated DKK1 levels ($p < 0.001$) compared to the control group (626.11 (383.52-754.29) pg/ml). Also, the TDT+Hypocalcemia group has a significantly higher ($p < 0.001$) serum DKK1 than the TDT+Normocalcemia group.

Comparison of the routinely measured biomarkers among groups

Table (2) shows the comparison of the routinely measured biomarkers among groups. Hemoglobin, PCV, total and ionized magnesium, have significant decreases in both TDT groups in comparison with the control group. While, iron, CRP, and ferritin levels are higher in both TDT groups compared with the control group. While no significant difference between TDT+Normocalcemia and the TDT+Hypocalcemia groups. TDT+Normocalcemia



Serum HMGB1 of the TDT patients with and without hypocalcemia and the control groups are presented in Figure (1).

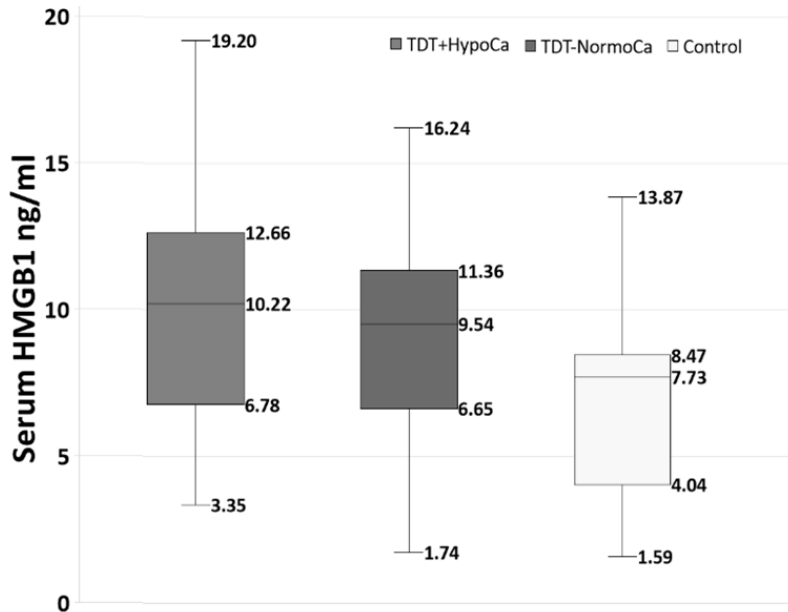


Figure 1. Serum HMGB1 of the transfusion-dependent thalassemia (TDT) patients with and without hypocalcemia, and the control groups. The medians, interquartiles, and the ranges of data are presented in this Figure too.

The results showed that both TDT+Normocalcemia group (9.54(6.65-11.36) ng/ml) and TDT+Hypocalcemia group (10.22(6.78-12.66) ng/ml) have a significantly higher HMGB1 ($p < 0.001$) than the control group (7.73(4.04-8.47) ng/ml). While there is no significant difference between TDT groups.

Figure (2) illustrates the serum DKK1 levels in TDT patients with and without hypocalcemia alongside the control groups.

**thalassemia (TDT) patients with and without hypocalcemia, and the control groups**

Parameters	Controls ^A n=40	TDT+ Normocalcemia ^B n=54	TDT+ Hypocalcemia ^C n=41	F	p
PCV %	40.98±4.16 ^{B,C}	23.11±3.51 ^A	24.29±3.42 ^A	312.58	<0.001
Hemoglobin g/dl	13.85±1.35 ^{B,C}	8.08±1.13 ^A	8.46±1.11 ^A	312.92	<0.001
Albumin g/dl	4.47±0.6 ^B	3.98±0.51 ^{A,C}	4.61±0.51 ^B	17.74	<0.001
T.Mg mM	1.27±0.30 ^{B,C}	1.05±0.24 ^A	1.11±0.31 ^A	7.89	0.001
I.Mg mM	0.88±0.2 ^{B,C}	0.73±0.16 ^A	0.77±0.2 ^A	7.89	0.001
Adj.T.Ca mM	2.37±0.14 ^{B,C}	2.24±0.08 ^{A,C}	1.99±0.1 ^{A,B}	132.49	<0.001
Adj.I.Ca mM	1.31±0.04 ^{B,C}	1.28±0.02 ^{A,C}	1.21±0.03 ^{A,B}	130.72	<0.001
T.(Ca/Mg)	1.97±0.51 ^B	2.22±0.42 ^{A,C}	1.91±0.46 ^B	6.18	0.003
Ionized (Ca/Mg)	1.57±0.37 ^B	1.81±0.32 ^{A,C}	1.66±0.37 ^B	5.82	0.004
Iron µg/dl	116.91±20.56 ^{B,C}	211.05±58.34 ^A	208.33±56.35 ^A	49.61	<0.001
CRP mg/l	4.01(2.03-4.99) ^{B,C}	7.98(5.61-9.48) ^A	8.22(6-14.6) ^A	KWT	<0.001
Ferritin ng/ml	84.00(64.25- 124.75) ^{B,C}	2505.15(1497.35- 3242.13) ^A	2449.40(1741.05- 3486.45) ^A	KWT	<0.001

^{A, B, C}: pairwise comparison. PCV: packed cell volume, T.Mg: total magnesium, I.Mg: ionized magnesium, Adj.T.Ca: adjusted total calcium, Adj.I.Ca: adjusted ionized calcium, CRP: C-reactive protein.

There is no significant difference in the age, sex ratio, residency, weight, height, disease duration, age of onset, splenectomy, number of transfusions, 1-Alpha taken, and days after the last transfusion have no significant difference between TDT patients with and without hypocalcemia. TDT patient groups have a substantial increase in the family history, splenomegaly, and drugs taken (folic acid, vitamin B12, Desferral, and Exjade) compared with the control groups with no significant difference between TDT groups.

Comparison of the DKK1 and HMGB1 among groups



Table 1: Demographic and clinical characteristics of the transfusion-dependent thalassemia (TDT) patients with and without hypocalcemia, and the control groups

Parameters	Controls ^A n=40	TDT+ Normocalcemia ^B n=54	TDT+ Hypocalcemia ^C n=41	F	p
Age year				0.26	0.768
Sex male/female				0.42	0.810
Rural/Urban				1.52	0.469
Family history No/ Yes				100.42	<0.001
Weight kg				0.06	0.939
Height cm				0.13	0.883
Duration of Dis. Years	9.73±1.92	9.56±1.73	9.83±1.95	MWUT	0.701
Age of onset, months	18/22	24/30	17/24	MWUT	0.226
No. of transfusion	13/27	16/38	17/24	MWUT	0.315
Days after last transfusion	40/0 ^{B,C}	7/47 ^A	2/39 ^A	2.25	0.325
Splenectomy No/ Yes	26.42±9.68	27.01±8.19	26.58±7.53	13.335	0.001
Splenomegaly No/Yes	126.05±16.19	127.11±12.29	125.71±15.14	126.02	<0.001
Folic acid No/Yes	-	8.8(7-10.9)	9(7.45-10.85)	23.79	<0.001
Vitamin B12 No/ Yes	-	8(5.75-12)	6(4.5-9)	12.98	0.002
Desferral No/Yes	-	144(96-219)	164(121.5-240)	63.38	<0.001
Exjade No/Yes	-	23(15-30)	20(15-29)	5.21	0.074
1 Alpha No/Yes	40/0	51/3	38/3		
	40/0 ^{B,C}	41/13 ^A	29/12 ^A		
	40/0 ^{B,C}	2/52 ^A	0/41 ^A		
	40/0 ^{B,C}	30/24 ^A	26/15 ^A		
	40/0 ^{B,C}	43/11 ^{A,C}	29/12 ^{A,B}		
	40/0 ^{B,C}	15/39 ^{A,C}	9/32 ^{A,B}		
	40/0	48/6	39/2		

^{A, B, C}: pairwise comparison. Results are expressed as mean ± standard deviation for normally distributed data. Binomial data were expressed as ratios and analyzed by Chi-squared test. p: probability value.

Table 2: Comparison of the Biomarkers levels among the transfusion-dependent



Biostatistical analysis

Analysis of variance (ANOVA) was used to evaluate variations in continuous variables among groups, whereas contingency table analysis (χ^2 -test) was performed to examine comparison in the categorical variables. Relationships between variables were calculated using Pearson's correlation coefficients. We used multivariate general-linear model (GLM) analysis to elucidate the impacts of hypocalcemia across three groups: TDT+Normocalcemia, TDT+Hypocalcemia, and healthy controls while adjusting for confounders such as age, sex, weight, and height. To normalise the distribution of the detected biomarkers, which were evaluated using the Kolmogorov-Smirnov test, we used a natural logarithm (Ln) transformation to their data. Receiver-operating characteristic (ROC) analysis was used to assess the hypocalcemia effectiveness of the discovered biomarkers. The statistics, cut-off points, sensitivities, and specificities of Youdin are computed variables. The statistical analyses were conducted with SPSS software version 27.

Results

Comparison of the characteristics among groups

The comparison of the demographic and clinical characteristics of TDT patients with and without hypocalcemia and the control groups is shown in Table (1).



Exjade (Deferasirox) is twenty mg/kg/day of body weight each day. The control group included forty adolescents, with ages comparable to the patients (17 males and 23 females), all of whom seemed to be in fair health. The subjects had no indications of systemic or inflammatory illnesses, and none were anemic. Systemic illnesses such as diabetes mellitus, hypertension, or renal issues were excluded, along with patients or controls who had splenectomy. Both parents or other first-degree relatives of the patient were asked to sign an informed consent form. Approval number 214/2024 was given by the University of Kufa's IRB for the project.

Assays

The blood samples were collected in the morning hours. Iron in serum was measured by using Ferrozine method (Linear[®], Spain). The VIDAS ferritin test, an automated equipment supplied by BioMérieux Co., France, measures serum ferritin levels using an enzyme-linked fluorescence immunoassay (ELFA). The ferritin intra-assay precision within-assay coefficients of variation (CV) was less than 5.70 per cent and the iron CV% was less than 2.19%. Commercial enzyme-linked immunosorbent assay (ELISA) sandwich kits were used to measure serum DKK1 and HMGB1 (Pars Biochem Co, Nanjing, China). We diluted samples as needed for those containing very intense biomarkers. The intra-assay CV% were less than 10%. The C-Reactive Protein (CRP) latex slide test is used for measuring CRP in human serum semi-quantitatively (Spinreact[®], Barcelona, Spain). The titer of serum is equal to the positive control concentration times reciprocal of the highest dilution showing positive reactivity. The approximate formula for calculating the CRP concentration (in mg/l) in the patient sample is 6 multiplied by the CRP titer.



diagnostic sensitivity and specificity of the observed biomarkers for predicting hypocalcemia in TDT patients.

Materials and Methods

Participants

Ninety-five Iraqi TDT children (41 males and 54 females) aged 7-12 years were collected at the Thalassemia Unit at Al-Zahra'a-Teaching Hospital, Najaf, Iraq. Diagnosis of thalassemia was performed according to the Diagnosis Code number D56.1 from the ICD-10-CM (2019). The diagnosis was based on the clinical symptoms, haematological parameters (primarily; $Hb < 7g/dl$, and in blood smear there is microcytic-hypochromic RBCs with anisopoikilocytosis and increased reticulocyte percentage), and by haemoglobin HPLC which assayed using HPLC (VARIANTTM β -Thalassemia Program). The patients were classified according to the results of the adjusted serum calcium (Adj.T.Ca) into the TDT group with normal levels of the Adj.T.Ca (TDT+Normocalcemia) and TDT group with hypocalcemia (TDT+Hypocalcemia). Hypocalcemia is defined as the serum level of Adj.T.Ca below the cut-off value of 2.12 mmol/L (8 mg/dl) (Pepe *et al.*, 2020).

Blood transfusions were a fundamental component of the therapeutic regimen for each patient. To maintain haemoglobin levels over 90 g/L before transfusions, all patients received packed red blood cell transfusions every two to four weeks. Based on the ferritin level, patients received an iron-chelating therapy with an infusion of Desferal® (deferroxamine mesylate) at a dose of 25-50 mg/kg/day over eight hours daily. The recommended dose for



2018) and this protein may play a role in inflammatory reactions by stimulating the release of cytokines that promote inflammation (Jaschke *et al.*, 2022). Atherosclerosis (Al-Dujaili *et al.*, 2021), cardiovascular disorders (Garcia-Martín *et al.*, 2014), and other related pathologies are influenced by proteins whose expression DKK1 directly controls. There is a negative correlation between serum DKK1 and PTH and alkaline phosphatase concentrations and a positive correlation with serum calcium concentrations (Wang *et al.*, 2024).

Low levels of calcium and vitamin D are common in TDT patients who get frequent transfusions despite receiving chelation therapy (Li *et al.*, 2024). Some studies have shown that β -TM contains small amounts of calcium (Ananvutisombat *et al.*, 2024; Mirhosseini *et al.*, 2013b). Calcium and vitamin D levels are lower in thalassemic children compared to healthy children of the same age group, according to much research conducted around the globe (Ara *et al.*, 2023; Ridha *et al.*, 2022). Compared to healthy children, patients undergoing TDT had substantially lower levels of blood total calcium, greater levels of serum ferritin, and considerably lesser levels of serum inorganic phosphate (Urmi *et al.*, 2023). In children suffering from β -thalassemia, there is a significant reduction in blood calcium, vitamin D, and parathyroid hormone levels, which may lead to growth failure. These abnormalities might be caused by excessive iron and poor nutritional support, which brings attention to the need for therapeutic techniques (Meshram *et al.*, 2024). The primary aim of the current investigation is the study of DKK1 and HMGB1 levels in TDT patients with hypocalcemia (TDT+Hypocalcemia) and normocalcemia (TDT+Normocalcemia) as a function of inflammation. The secondary aim of the study is to ascertain the



biomarkers of other associated pathways is less well understood. Specifically, we aimed to compare the levels of Dickkopf-related protein 1 (DKK1) and high mobility group box 1 (HMGB1) in TDT patients who had hypocalcaemia and those who did not (Wulandari *et al.*, 2023; Yuan *et al.*, 2020). Active monocytes, natural killer cells, macrophages, and mature dendritic cells, may leak the nonhistone chromosomal binding protein HMGB1, whereas necrotic and injured cells can passively release it (Chi *et al.*, 2024; Yuan *et al.*, 2024). When the body detects an inflammatory stimulus, such as an injury or infection, it secretes HMGB1, a cytokine that promotes further inflammation by binding to complexes of the receptors for Toll-like receptors (TLR) 2 and 4 and advanced glycation end-products (RAGE) (Fan *et al.*, 2024; Ostrand-Rosenberg *et al.*, 2023).

The extracellular HMGB1 regulates inflammation and immunological responses via various receptors or direct absorption after its active secretion or passive release (Chen *et al.*, 2022). More so, HMGB1 may maintain the chronic inflammatory state linked to diabetes (Ngcobo & Sibiyi, 2024), and interaction with TLR-4 increases insulin resistance by phosphorylating the peripheral insulin-receptor substrate (Ren *et al.*, 2023). After being secreted or released passively from cells, HMGB1 normally acts as a damage-associated molecular pattern molecule outside of cells, influencing immune responses and inflammation via different receptors or direct absorption (Chen, Kang *et al.*, 2022).

Inflammation is often accompanied by an increase in the levels of a pro-inflammatory glycoprotein called DKK1, which is released by several cell types including endothelial cells and platelets (Chae & Bothwell, 2019). Acute infections are associated with a spike in systemic DKK1 levels (Mazon *et al.*,



Introduction

Beta-thalassemia (β -TM) is a family of genetic diseases that manifests as anaemia and erythrocyte membrane disintegration due to a lack of the β -globin chain in haemoglobin A (Raguram *et al.*, 2022). This condition is associated with significant morbidity and death (Sanchez-Villalobos *et al.*, 2022). Among the world's most common hereditary diseases, thalassaemia is especially common in Mediterranean nations like Iraq (Al-Hakeim *et al.*, 2020). Red blood cell (RBC) depletion is addressed by continuously transfusing blood into these individuals (Shah *et al.*, 2019). Blood transfusions are necessary for β -TM patients throughout their lives in order to raise haemoglobin levels and reduce the negative impacts of poor erythropoiesis (Aydinok, 2020). Because of the high frequency of blood transfusions, patients with transfusion-dependent thalassaemia (TDT) are at increased risk for a variety of problems (Shah, Sayani *et al.*, 2019). Iron overload (IO) may be caused by chronic blood transfusions and can cause toxicity in several organs, such as the endocrine glands, heart, and liver (Daher *et al.*, 2017).

Hypoparathyroidism and hypovitaminosis D are symptoms that TDT patients may have due to the parathyroid gland being one of the glands impacted by iron excess. Consequently, hypocalcaemia, or low blood calcium levels, will occur in TDT patients due to impaired or absent parathyroid hormone (PTH) synthesis, which in turn reduces calcium absorption from the intestines (Ansaf *et al.*, 2024; Lertsuwan *et al.*, 2018a). Among those diagnosed with beta major thalassaemia, 22% had hypocalcaemia (Mirhosseini *et al.*, 2013a). Hypocalcaemia is recognised to cause bone abnormalities (Merchant & Gafni, 2024), but its impact on

المستخلص

المقدمة: يرتبط الثلاثسيما المعتمد على نقل الدم (TDT) بأعراض مختلفة بما في ذلك فقر الدم ومشاكل العظام. هناك حاجة لمزيد من المؤشرات الحيوية لاضطرابات الكالسيوم في مرضى الثلاثسيما المعتمد على نقل الدم. في الدراسة الحالية، تمت دراسة DKK 1 و HMGB1 في مرضى الثلاثسيما المعتمد على نقل الدم الذين يعانون من نقص كالسيوم الدم (نقص كالسيوم TDT+والمرضى الذين لديهم مستوى كالسيوم الدم الطبيعي) كالسيوم طبيعي (TDT+ كدالة للالتهاب).

طرق العمل: تم تصنيف مرضى الثلاثسيما المعتمد على نقل الدم وفقاً لقيمة القطع (2.12 مليمول) من الكالسيوم في المصل المعدل (Adj.T.Ca) إلى مجموعتين (نقص كالسيوم TDT+ وعددهم 54 ومجموعة تحوي 41 مريضاً ولديهم كالسيوم طبيعي) كالسيوم طبيعي (TDT+ كما شارك أربعون شخصاً سليماً في الدراسة الحالية. تم قياس DKK1 و HMGB1 باستخدام تقنية الـإيلايزا.

النتائج: لوحظ ارتفاع معنوي في مستويات DKK1 و HMGB1 و بروتين سي التفاعلي و الفيريتين في كلتا مجموعتي المرضى مقارنة مع مجموعات التحكم. أظهرت مجموعة (نقص كالسيوم TDT+ مستوى DKK1 في المصل أعلى بشكل ملحوظ من مجموعة) كالسيوم طبيعي (TDT+ أظهرت النتائج أن البروتين التفاعلي سي له ارتباط كبير مع DKK1 والفيريتين، بالإضافة إلى ارتباط عكسي كبير مع تضخم الطحال، و Adj.T.Ca. كما يوجد ارتباط كبير بين HMGB1 و DKK1. كما أن DKK1 له ارتباط عكسي كبير مع الجنس، وتضخم الطحال، و Adj.T.Ca. كشفت نتائج خصائص تشغيل المستقبل (ROC) عن زيادة في DKK1 أكثر من تركيز القطع (901.44 بيكو جرام/مل) مما يشير إلى أن الأشخاص الذين خضعوا لـ TDT قد يعانون من نقص كالسيوم الدم بشكل ملحوظ ($p < 0.001$) بحساسية 73.2% وخصوصية 72.2% (Youdin's J=0.454). في حين أن HMGB1 والفيريتين ليس ليهما قابلية للتنبؤ بنقص كالسيوم الدم بشكل ملحوظ. الاستنتاج. يعد DKK1 عاملاً مهماً في نقص كالسيوم الدم لدى مرضى الثلاثسيما وقد يعمل كهدف دوائي لعلاج اضطرابات العظام لدى مرضى الثلاثسيما.

الكلمات المفتاحية: الثلاثسيما ، نقص الكالسيوم ، بروتين مرتبط ديكوبف

مرتبط 1، الكالسيوم ، البروتين HMGB1



thalassemia patients.

Keywords: Thalassemia, hypocalcemia, DKK 1, calcium, and HMGB1.

Abbreviations

HMGB1: high mobility group box 1

TDT: Transfusion-dependent thalassemia

TDT+Hypocalcemia: TDT patients with hypocalcemia

TDT+Normocalcemia: TDT patients with normocalcemia

Adj.T.Ca: adjusted serum calcium into

DKK1: Dickkopf-related protein 1

PTH: parathyroid hormone

β-TM: Beta-thalassemia

RBC: Red blood cell

IO: Iron overload

TLR: Toll-like receptors

ELFA: enzyme-linked fluorescence immunoassay

CV: coefficients of variation

ELISA: enzyme-linked immunosorbent assay

CRP: C-Reactive Protein

ANOVA: Analysis of variance

GLM: general-linear model

Ln: natural logarithm

ROC: Receiver-operating characteristic



Abstract

Background. Transfusion-dependent thalassemia (TDT) is associated with various symptoms including anaemia and bone problems. There is a need for more biomarkers of calcium disorders in TDT patients. In the present study, DKK 1 and HMGB1 are studied in TDT patients with hypocalcemia (TDT+Hypocalcemia) and normocalcemia (TDT+Normocalcemia) as a function of inflammation.

Methods. TDT patients were classified according to the cut-off value (2.12 mM) of the adjusted serum calcium (Adj.T.Ca) into TDT+Normocalcemia (n=54) and TDT+Hypocalcemia (n=41) groups. Forty healthy controls have participated in the present study. DKK1 and HMGB1 were measured using the ELISA technique.

Results. Both patient groups have significantly higher DKK1, HMGB1, C-reactive protein, and ferritin than the control groups. The TDT+Hypocalcemia group has a significantly higher serum DKK1 level than the TDT+Normocalcemia. The results show CRP has a significant correlation with DKK1 and ferritin, in addition to a significant inverse correlation with splenomegaly, and adj.T.Ca. There is also a significant correlation between HMGB1 and DKK1. DKK1 also has a significant inverse correlation with sex, splenomegaly, and Adj.T.Ca. The results of receiver operating characteristics (ROC) revealed an increase in DKK1 more than the cut-off concentration (901.44 pg/ml) indicating that the TDT subjects may have hypocalcemia significantly ($p < 0.001$) in a sensitivity of 73.2% and specificity of 72.2% (Youdin's J statistic=0.454). While HMGB1 and ferritin have no significant predictability of hypocalcemia.

Conclusion. DKK1 is an important factor in hypocalcemia in TDT patients and may act as a drug target for the treatment of bone disorders in



Impact of Hypocalcemia on Dickkopf-Related Protein 1 and HMGB1 in Transfusion-Dependent Thalassemia Patients / Original study

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**تأثير نقص الكالسيوم في الدم على بروتين ديكوف المرتبط
1 و HMGB1 في مرضى الثلاسيميا المعتمدين على نقل الدم**

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considered a primary defense mechanism. Furthermore, various investigations have demonstrated the impact of iron on the enzymatic activities of two specific enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), as well as on the survival of the feeding phase of the parasite. The objective of reducing or eliminating iron is to explore its potential as a treatment option in the future (Espinosa *et al.*, 2004; Espinosa *et al.*, 2009). The reticuloendothelial system (RES) cells play a crucial role in the metabolism of iron. The primary storage locations for iron include aged red blood cells and the liver parenchyma (Edison *et al.*, 2008).

Entamoeba histolytica is recognized as an intestinal parasite that necessitates iron for its metabolic activities. Consequently, it utilizes the iron found in the liver and the lining of the large intestine, where the parasite initially manifests. Research conducted by López-Soto *et al.*, (2009) revealed that *E. histolytica*, during its trophozoite stage, attaches to ferritin via specific proteins, thereby utilizing it as a vital iron source during this phase. This interaction leads to a notable reduction in the iron reserves of individuals infected with this parasite.

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the parasite when compared to healthy individuals (Weinberg, 1999). A study conducted by Oguntibeju (2003) indicated a correlation between parasitic infestations and alterations in hematological parameters, which can lead to anemia. Additional evidence from community research has highlighted the involvement of *E. histolytica* and *G. lamblia* infections in cases of iron deficiency anemia, as reported in other studies (Juomaa, 2006; Al-Naemi *et al.*, 2011). The liver contains many iron sources, so the parasite's ability to use these sources may be related to its survival in the liver and the formation of liver abscesses, The parasite *Entamoeba histolytica* uses iron as a cofactor for its enzyme alcohol dehydrogenase 2 (EhADH2) (Hernandez-Cuevas *et al.*, 2014). This enzyme serves a dual function, operating as both Alcohol dehydrogenase (ADH), which removes hydrogen atoms from alcohol molecules, and Aldehyde dehydrogenase (ALDH), which extracts hydrogen atoms from aldehyde molecules within the glycolysis pathway of *Entamoeba histolytica* (Espinosa *et al.*, 2001).

Since *Entamoeba histolytica* does not possess mitochondria, it derives the energy required for essential functions through fermentation. The glucose fermentation pathway culminates in the conversion of Acetyl-coA to Ethanol, facilitated by the activation of Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes. Consequently, the enzyme EhADH2 is essential for the survival of this parasite (Espinosa *et al.*, 2004). Numerous studies have indicated that iron plays a crucial role in the growth of parasites and the activation of enzymes. These studies have also underscored the significance of iron in the pathogenesis of parasitic infections and proposed the use of iron chelation as a potential chemotherapy strategy, contingent upon the immune response of mammals. The isolation of iron is



The findings of this study indicated an increase in Total Iron Binding Capacity (TIBC) among patients with β -thalassemia when compared to the control group. This observation aligns with the results reported by Hesham *et al.*, (2018) and Guimaraes *et al.*, (2015), who also identified elevated TIBC levels alongside increased ferritin and iron concentrations. Furthermore, the present study corroborates the conclusions of Karim *et al.*, (2016), who demonstrated a significant rise in TIBC levels in individuals diagnosed with β -thalassemia. Furthermore, Ali (2018) indicated a positive correlation between Total Iron Binding Capacity (TIBC) and increased serum ferritin and iron levels. The quantity of iron stored in the body is influenced by the levels of TIBC and ferritin; as the iron content rises, so do the levels of TIBC and ferritin (Badiee *et al.*, 2015).

the current investigation, a statistically significant decrease in iron levels and total iron-binding capacity (TIBC) was observed ($P < 0.0001$) when comparing patients with β -thalassemia to those infected with *Entamoeba histolytica*. The reduction of iron levels in patients infected with *E. histolytica* is linked to the pathogenicity of this organism, which is influenced by the relationship between iron concentration and the adhesion of *E. histolytica* to epithelial cells (Al-Hadraawy *et al.*, 2015). The findings align with those of Hernandez-Cuevas *et al.*, (2014), which demonstrated a reduction in total iron-binding capacity (T.I.B.C) among individuals infected with the *E. histolytica* parasite. This suggests that the parasite utilizes ferritin, the protein responsible for iron storage, to support its survival and reproductive processes. Also, this finding aligns with Weinberg's research, which indicated that *E. histolytica* employs serum ferritin as a source of iron. Consequently, this interaction results in decreased ferritin levels in humans infected with



(2011). Thalassemia management is primarily concerned with iron levels and issues related to excess or scarcity (Needs *et al.*, 2018). Iraqi patients had significantly elevated serum iron levels, indicating an existing iron excess, with an elevated iron level being a significant risk factor for myocardial infarction. Frequent blood transfusions in β -thalassemia without chelation therapy can cause iron overload, leading to splenomegaly and other complications.

The liver's functionality is also influenced, leading to an increased likelihood of additional complications (Abdulla, 2018). According to **Mishra and Tiwari** (2013), all patients with β -thalassemia major exhibited elevated serum iron, ferritin levels, and total iron-binding capacity (TIBC), which could suggest iron overload. This phenomenon may occur due to enhanced iron absorption stemming from disrupted erythropoiesis or continuous blood transfusions. The accumulation of iron in patients with thalassemia can surpass the storage and detoxification capacity of ferritin, resulting in the complete saturation of transferrin and the presence of free iron in the bloodstream and tissues. This highly reactive free iron can generate hydroxyl radicals that target lipids, leading to the formation of lipid peroxides, which in turn contribute to oxidative stress. Consequently, this process results in the production of extremely harmful compounds, such as hydroxyl radicals (Prabhu, *et al.*, 2009).

Additionally, iron overload and tissue injury resulting from excessive iron due to frequent blood transfusions or heightened intestinal iron absorption, along with early hemolysis and ineffective erythropoiesis, represent two prevalent and serious complications of thalassemia. Consequently, iron chelators have been employed to prevent iron accumulation in patients with thalassemia (Saeidnia, *et al.*, 2022).



amine groups in amino acids (Al-Khashli and Al-Shawi, 2013). ALT and AST are common in many tissues of the human body. AST enzymes are more effective than ALT enzymes. It is more prevalent in the heart, liver, skeletal muscles, and kidneys. The liver contains a large amount of ALT, as do the kidney, heart, and skeletal muscles. Increased enzyme effectiveness in patients may be due to elevated iron levels in their serum, which can lead to fat breakdown in certain organ cells as a result of the free radical's formation that attacks all vital molecules such as lipids, proteins, and cell DNA. (Hashim, *et al.*, 2020; Bushra, *et al.*, 2013, Ellis, 2010). Sateriale and his colleagues (2011) discovered that approximately 50 million infections of the *E. histolytica* parasite affect the liver each year. Amoebic liver abscesses are caused by eating food or water contaminated with human feces. However, the most common symptomatic infection is amoebic dysentery. In rare cases, trophozoites can invade the intestinal mucosa and spread blood, resulting in extra-intestinal disease. Aside from the intestine, the most common symptom is liver abscess. The trophozoites enter the liver through the portal venous circulation and cause significant damage (Tharmaratnam *et al.*, 2020). The current findings align with previous research indicating increased levels of liver enzymes in the serum of individuals diagnosed with amebiasis (Morán, *et al.*, 2023; Pluta and Pluta, 2008). Moreover, ALT and AST were good predictors of the disease with AUC 0.7946 and 7595 respectively.

In the present study the patients with β -thalassemia exhibited elevated iron levels in comparison to the control group. This observation aligns with the findings of Abdulla (2022), who similarly reported increased iron levels in cases of β -thalassemia major. Furthermore, the results of the current investigation corroborate the conclusions drawn by Kuppusamy and Tan



Hepcidin, a hormone synthesized in the liver, regulates iron absorption in the intestine, influences iron release from the reticuloendothelial system, and is also involved in placental iron transfer. The extracellular parasite *E. histolytica*, which causes intestinal and hepatic diseases known as amoebiasis, has a significant impact on iron levels in the body. Its presence leads to increased iron consumption and a subsequent reduction in overall iron levels. Given that the liver is the site of hepcidin production, the infection results in markedly decreased levels of this hormone, indicating that the parasitic infection adversely affects the regulation of iron within the body. In β -thalassemia, higher erythropoiesis suppresses hepcidin synthesis, leading to elevated iron absorption through the gastrointestinal tract. Blood transfusions provide additional iron for β -thalassemia patients (Jones *et al.*, 2015).

The findings of the present study indicated a statistically significant elevation in the levels of AST and ALT enzymes among thalassemia patients, both those uninfected and infected with the *Entamoeba histolytica*, compared to the control group as shown in in table 3.2 and figure 3.2. Iron overload, indicated by high serum ferritin levels, is known as a cause of liver dysfunction which is reflected by elevated ALT and AST levels. Our findings regarding highly liver enzymes among thalassemia patients were agreement with previous reports from Iraq (Mohammad, 2012). According to Hashim *et al.*, reported in Maysan research, the difference in the concentration of Transferases compared to healthy individuals is due to excessive hemolysis (Hashim *et al.*, 2020).

Excessive red blood cell breakage (hemolysis) or the need for peptide chain synthesis may cause a difference in transportable enzyme concentrations compared to healthy individuals. These enzymes are effective in transmitting



inadequate erythropoiesis leads to a compensatory reduction in hepcidin levels, which in turn enhances the absorption of iron in the intestines. Furthermore, thalassemia major is characterized by a disruption in globin chain production, resulting in ineffective erythropoiesis, anemia, and an elevated production of erythropoietin. This, in turn, stimulates the synthesis of erythroferrone, a hormone derived from erythroid cells that inhibits the liver's capacity to produce hepcidin. Furthermore, **Ali** (2018) indicates that following blood transfusions, levels of hepcidin-25 decrease, facilitating iron absorption and resulting in an excess of iron. Dysregulation of ferroportin has been associated with hepcidin (HAMP) or related genetic disorders, such as hemochromatosis (HFE), transferrin receptor 2 (TFR2), and HJV, as well as conditions like intermediate β -thalassemia. These disorders are often correlated with heightened iron absorption and export, which can lead to elevated levels of free systemic iron and excessive iron accumulation in tissues, ultimately resulting in tissue damage (Liu *et al.*, 2016). In hereditary hemochromatosis, the accumulation of iron is associated with inadequate production of hepcidin due to mutations in either the hepcidin or HJV gene. Similarly, beta-thalassemia, marked by ineffective erythropoiesis and impaired beta-globin production, is also associated with increased absorption of dietary iron. This phenomenon is connected to the presence of hepcidin inhibitors such as ERFE (Agarwal and Yee, 2019).

Hepcidin is an important regulator of iron homeostasis and participates in a variety of iron metabolism pathways. Specific polymorphisms in the HAMP promoter region have been shown to reduce this hormone's expression, resulting in increased serum iron (Zarghamian *et al.*, 2020).



4. Discussion

The age of the patients did not exhibit a statistically significant difference when compared to the control group. This outcome may be attributed to the sample selection process. Various studies have indicated differing average ages, with **Chirico et al.**, (2013) noting that the mean age of the patients was 34.4 years. **Ang et al.**, (2014) indicated that the average age of patients with β -thalassemia was 36 years, with a range from 18 to 59 years. Furthermore, **Poggi et al.**, (2016) reported a mean age of 39.9 years among β -thalassemia patients. The discrepancies in these age figures may stem from variations in selection criteria, patient demographics, and lifestyle factors. Additionally, it is conceivable that age may not play a crucial role in the onset of β -thalassemia, as suggested by **Rai & Stuart**, (2024). Further investigation is warranted to better understand the connection between age and β -thalassemia.

Hepcidin concentration showed a highly significant decrease ($P < 0.0001$) in β -thalassemia patients in both negative and positive for *Entamoeba histolytica* and no statistically significant difference ($P < 0.9042$) was observed in the levels of hepcidin patients with β -thalassemia and those infected with *Entamoeba histolytica*. Additionally, the ROC curve analysis illustrated in figure 3-4 demonstrated that serum hepcidin possess remarkable prognostic accuracy in patients with β -thalassemia major, regardless of their *Entamoeba histolytica* status, whether negative or positive.

The present research aligns with the findings of **Mohammadi et al.**, (2018), who demonstrated that the hepatic peptide hepcidin plays a crucial role in regulating the body's iron homeostasis by preventing iron from entering the plasma through various mechanisms. In cases of thalassemia,



To evaluate the effectiveness of various biochemical markers in predicting β -thalassemia among patients both positive and negative for *Entamoeba histolytica*, Receiver Operating Characteristics (ROC) Curve analysis was utilized. This method involves plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity). The area under the ROC curve (AUC) was computed, serving as an indicator of the test's validity in forecasting the outcome. As interpretation, AUC of less than 0.600 indicates failure of a test as predictor, 0.600 to 0.700 is sufficient predictor, 0.700-0.800 good, 0.800 -0.900 very good and > 0.900-1.00 indicates excellent predictor test, according to this cutoff value of AUC, S. ALT and S. AST were good predictors of the disease with AUC 0.7946 and 7595 respectively, , while hepcidin, iron and TIBC were excellent predictors of disease with AUC 0.9481, 0.9969, and 0.9973 respectively Figure (4).

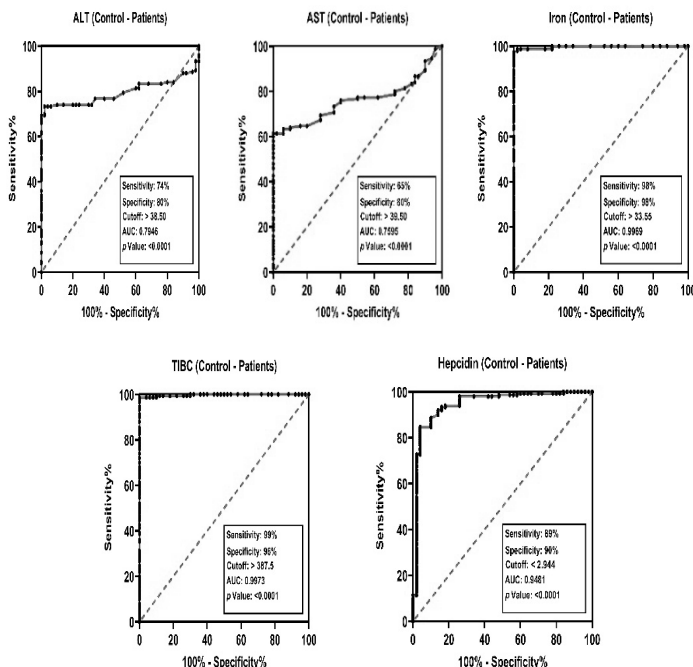


Figure (4). Receiver Operating Characteristics (ROC) curve for the validity of Hepcidin S. ALT, S. AST, S. Iron and S. TIBC in prediction of severity of disease.



Concentration of Hepcidin in β - thalassemia major patients with or without *Entamoeba histolytica* and Control.

The concentration of Hepcidin showed a highly significant ($P < 0.0001$) decrease in patients with β -thalassemia (1.57 ± 1.12) as compared with control (4.16 ± 1.34) as shown in Figure (3). In the present study, no statistically significant difference ($P < 0.9042$) was observed in the levels of Hepcidin between patients with β -thalassemia and those infected with *Entamoeba histolytica*, as illustrated in the Table (3).

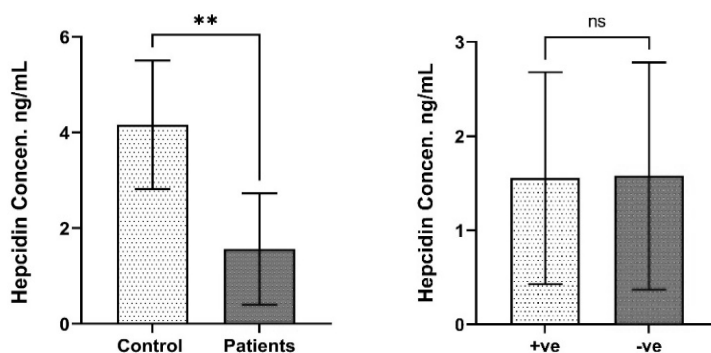


Figure (3). Hepcidin levels in β -thalassemia patients and β -thalassemia major patients with negative and positive *Entamoeba histolytica* were compared to controls.

Table (3). Concentration of GSH in β -thalassemia major patients and with negative and positive *Entamoeba histolytica*

Parameter	Mean \pm SD		Sig.	p Value
	Control	Patients		
Hepcidin	4.16 ± 1.34	1.57 ± 1.12	HS	<0.0001
Parameter	Mean \pm SD		Sig.	p Value
	+ve	-ve		
Hepcidin	1.553 ± 1.126	1.576 ± 1.208	NS	0.9042

Concentration of GSH in β -thalassemia major patients with negative and positive *Entamoeba histolytica*.



Table (2). Concentration of ALT, AST and LDH in β -thalassemia major patients with negative and positive *Entamoeba histolytica*

Parameter	Mean \pm SD		Sig.	p Value
	+ve	-ve		
ALT	118.3 \pm 51.9	74.74 \pm 64.43	HS	<0.0001**
AST	107.0 \pm 52.77	49.17 \pm 42.83	HS	<0.0001**
Iron	77.99 \pm 27.01	109.0 \pm 37.05	HS	<0.0001**
TIBC	450.3 \pm 50.30	552.2 \pm 101.9	HS	<0.0001**

By using independent T-test S: Significant, HS: Highly significant ,NS: Not significant

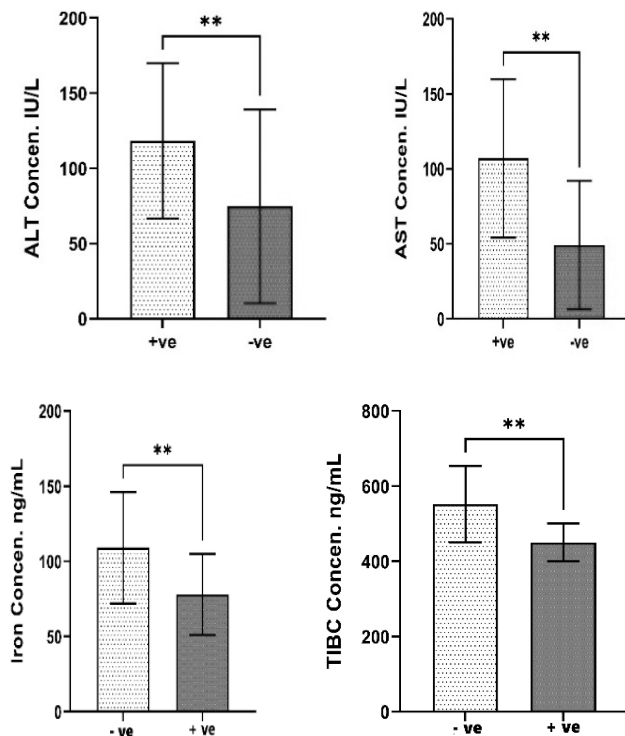


Figure (2). ALT, AST, iron and TIBC in β -thalassemia major patients with negative and positive *Entamoeba histolytica*



By using independent T-test S: Significant , HS: Highly significant ,NS: Not significant.

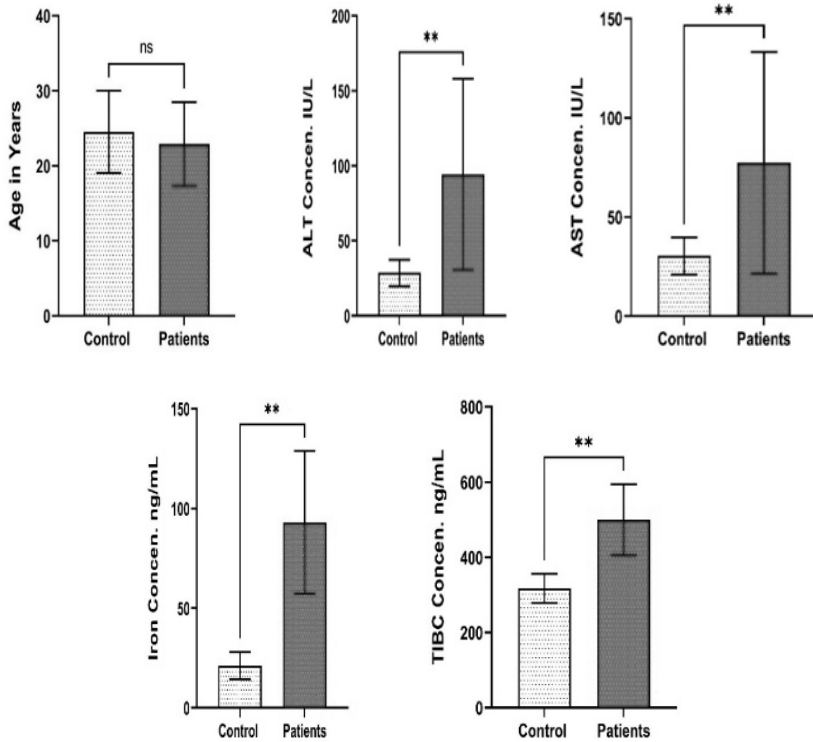


Figure (1). Age and some biochemical markers of β -thalassemia major in patients and control

The current study found significant decreases ($P < 0.0001$) in iron and TIBC levels, but a significant increases ($P < 0.0001$) in ALT and AST was observed when comparing patients with β -thalassemia to those infected with *Entamoeba histolytica*, as shown in the Table (2) and Figure (2).



3. Results

3.1 Age and Biochemical Parameters Value in β -Thalassemia Major Patients with or without *Entamoeba histolytica* and Control

The result of this study showed that there was no significant (P value = 0.0737) difference in the age between the patients with β -thalassemia major (22.91 ± 5.6) years and controls (24.54 ± 5.5) years as shown in Table (1) and Figure (1). From the same Table and the accompanying Figure indicate a highly significant increase in serum ALT, AST, and iron and TIBC levels in the patient group when compared to the control group. The mean values for ALT, AST, iron and TIBC in patients with β -thalassemia were found to be significantly elevated ($P < 0.0001$), with values of 94.28 ± 63.7 , 77.33 ± 55.9 , 93.08 ± 35.7 ng/mL and 499.9 ± 94.4 ng/mL, respectively, in contrast to the control group, which exhibited mean values of 28.58 ± 8.8 , 30.34 ± 9.3 , 21.22 ± 6.84 ng/mL and 317.7 ± 38.9 ng/L respectively.

Table (1). Age and Biochemical Parameters Value in β -Thalassemia Major Patients with or without *Entamoeba histolytica* *E. histolytica* and Control

Parameter	Mean \pm SD		Sig.	p Value
	Control	Patients		
Age	24.54 ± 5.5	22.91 ± 5.6	NS	0.0737
ALT	28.58 ± 8.8	94.28 ± 63.7	HS	<0.0001**
AST	30.34 ± 9.3	77.33 ± 55.9	HS	<0.0001**
Iron	21.22 ± 6.84	93.08 ± 35.7	HS	<0.0001**
TIBC	317.7 ± 38.9	499.9 ± 94.4	HS	<0.0001**



several 0.5 ml aliquots and immediately frozen at -20°C until used for detection of: Hepcidin and biochemical indicators (ALT, AST, iron and TIBC).

The blood samples (*Gel tube*) were centrifuged for 10 minutes at 6000 rpm to obtain serum which is used for the determination of alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Serum concentrations of Hepcidin were evaluated by using the technique enzyme-linked immunosorbent assay (ELISA). While, the ALT, AST and iron were evaluated by using electro-chemiluminescence immunoassay, Roche Cobas Integra 400 plus (Roche Diagnostics GmbH, Mannheim, Germany) serum TIBC were evaluated by spectrophotometer, Serum samples for all subjects were applied to the instrument then the concentration of biochemical parameters were automatically calculated.

2.3. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA). Student's *t*-test was used to determine whether group variance was significant or not. Pearson coefficient *r* value was employed to assess correlation. Receiver operating characteristic (ROC) curve analysis was performed to determine area under curve (AUC) and the optimum cut-off value of markers best prediction. Quantitative parametric data were subjected to Shapiro-Wilk test to confirm the normal distribution and were expressed as mean \pm SD and statistical differences were defined as $p < 0.05$ and $p < 0.01$ iss significant .



some biochemical indicators and changes in hepcidin level in patients suffering from β -thalassemia major. Therefore, the current study was conducted to guess cut off values of hepcidin and biochemical indicators (ALT, AST, iron and TIBC) and clarify their correlation with beta-thalassemia who positive and negative for *Entamoeba histolytica*.

2. Materials and methods

2.1. Populations studied

The current study involved 200 (150 with β -thalassemia patients and 50 healthy subjects) during their attendance at AL-Karama hospital and Ibn AL-Baladi hospital in Baghdad. The study was conducted during the period from January 2024 to May 2024. The patients had suffered from β -thalassemia major from different period of time. The age range of all patients were from (15–35) years and the necessary information's were taken from all patients after taking their permission depending on the letter of approved by the Research Ethical Committee and Scientific Committee designated by Middle Technical University, the College of Health and Medical Techniques, and the Department of Medical Laboratory Techniques (No. 172/3) on January 08, 2024.

2.2. Laboratory tests

Stool specimens were collected from patients with blood and /or mucus diarrhea and five ml disposable syringe was used to collect venous blood by vein puncture. blood was transferred to 10 ml sterile serum separator tubes (gel tube), centrifuged, and the serum was then divided into



/ dispar was the most commonly found intestinal protozoan, followed by *Blastocystis hominis* and, *Ascaris lumbricoides* infestation, and *Ancylostoma duodenale* infestation were all revealed to be helminthic infections (Gupta et al., 2016). Furthermore, parasite infection stimulates catecholamine release, which can mobilize red blood cells from the spleen or cause red blood cell enlargement due to fluid shifting into the intracellular compartment (Thachil & Bates 2017). Hecpidin, identified as the liver-expressed antimicrobial peptide, is primarily produced by hepatocytes located near portal veins (which transport dietary iron) and Kupffer cells (which sense bacteria and recycle erythrocytes). Furthermore, adipocytes and macrophages both produce a small amount of hepcidin (Ganz and Nemeth, 2012). Hepatocytes produce more hepcidin when iron is abundant because iron regulates this process; when iron is scarce, hepatocytes produce little or no hepcidin, allowing more iron to reach the plasma (Ramos et al., 2011). During beta-thalassemia, the suppressive and stimulating effects of erythropoiesis work together to regulate hepcidin production. Prior to transfusion, erythropoietic activity lowers hepcidin. It causes a low hepcidin level, which boosts dietary iron absorption. After the transfusion, inefficient erythropoiesis partly subsides, increasing hepcidin levels (Nemeth, 2013). Parasite protozoa require iron for survival and it is necessary for *E. histolytica* growth and enzymatic activities because iron enters the synthesis of parasite enzymes (Frederick et al., 2011, Nevitt, 2011). Increased hepcidin may be a host defense mechanism to reduce iron availability for microbes during inflammation, but this may not be effective in non-infection inflammatory disorders that cause anemia (Elzen et al., 2009, Hernandez et al., 2014). Infection with *Entamoeba histolytica* has been linked to increased levels of



1. Introduction

Thalassemia syndromes encompass a wide range of hemoglobin disorders resulting from the absence or diminished production of normal globin chains. It is estimated that between 1% and 5% of the global population carries a hereditary thalassemia mutation, making it the most common recessive disorder (Brancaleoni *et al.*, 2016). Thalassemia is characterized by several molecular irregularities, such as early termination of the protein chain, instability of mRNA, substitutions or deletions in the gene, and complications in the initiation of chain synthesis (Nang, 2016; Sharma *et al.*, 2017). The word "thalassemia" originates from the Greek terms "thalassa," meaning sea, and "haema," meaning blood. This condition arises from the abnormal production of hemoglobin (Hb) subunits, which are inherited as pathological alleles of one or more globin genes located on chromosomes 11 (β) and 16 (α) (Rachmilewitz *et al.*, 2011). Thalassemia occurs in two types: α -thalassemia and β -thalassemia (Minor, Intermediate, and Major) (Moafi *et al.*, 2010). Similar to sickle cell anemia and β -thalassemia, α -thalassemia is more common in tropical and subtropical regions (Goh *et al.*, 2020). Beta-thalassemia is an autosomal-recessive hereditary anemia with a lack or reduction in β globin chain synthesis. The excess of unbound α globin chains in the bone marrow leads to early mortality of erythroid precursors and inefficient erythropoiesis. The severity of β -thalassemia phenotypes ranges from severe, transfusion-dependent thalassemia major to moderate, intermedial forms (Danjou *et al.*, 2011). In a previous study, Gupta *et al.*, discovered that patients with beta-thalassemia major had an intestinal parasite infection rate of 21.9%. This study shows that individuals with beta-thalassemia major frequently have intestinal parasites. *Entamoeba histolytica*



اسبارتيت ترانسفيريز) و (الحديد و اجمالي سعة ارتباط الحديد) مقارنة مع السيطرة ($P < 0.0001$). وفقاً لتركيز الهيبسيدين في المصل فقد أظهر انخفاضاً في مجموعة مرضى الثلاسيميا بيتا (80.83 ± 77.7) ($P < 0.0001$) مقارنة مع مجموعة السيطرة (88.1 ± 320.9). في هذه الدراسة لم يلاحظ أي فرق ذي دلالة إحصائية ($P < 0.9042$) في مستويات الهيبسيدين بين المرضى الذين يعانون من الثلاسيميا بيتا والمصابين بالمتحولة الحالة للنسج. كما لوحظ وجود انخفاض معنوي ($P < 0.0001$) في مستويات الحديد و اجمالي سعة ارتباط الحديد ، بينما لوحظ زيادة معنوية ($P < 0.0001$) في الالنين ترانس امينيز و اسبارتيت ترانسفيريز عند المقارنة مع مرضى الثلاسيميا بيتا المصابين بالزحار الاميبي **الاستنتاجات** : أظهر المرضى الذين يعانون من الثلاسيميا بيتا والمصابين بالزحار الاميبي انخفاضاً ملحوظاً في مستويات الهيبسيدين، إلى جانب اختلاف كبير في بعض المؤشرات البيوكيميائية.

الكلمات المفتاحية : بيتا الثلاسيميا ، الزحار الاميبي ؛ هيبسيدين ، الالنين

ترانس امينيز ، اسبارتيت ترانسفيريز ، الحديد و اجمالي سعة ارتباط الحديد .

المستخلص

المقدمة: تشمل متلازمات الثلاسيميا مجموعة واسعة من اضطرابات الهيموجلوبين التي تتميز إما بغياب أو انخفاض إنتاج سلاسل الجلوبين الطبيعية. الهيبسيدين، وهو ببتيد ينتجه الكبد وله خصائص مضادة للميكروبات، يتم تصنيعه بشكل أساسي بواسطة خلايا الكبد الموجودة بالقرب من الأوردة البابية، المسؤولة عن نقل الحديد الغذائي، وكذلك بواسطة خلايا كوبفر. قبل عمليات نقل الدم، تؤدي زيادة نشاط تكوين الكريات الحمر إلى انخفاض الهيبسيدين، مما يؤدي إلى انخفاض مستويات الهيبسيدين التي تعزز امتصاص الحديد الغذائي. تعتمد الأوليات الطفيلية على الحديد من أجل بقائها على قيد الحياة، لأنه يلعب دورًا حاسمًا في النمو والوظائف الأنزيمية للزحار الأميبي من خلال المشاركة في تخليق إنزيمات الطفيلي. قد يكون الارتفاع في مستويات الهيبسيدين بمثابة آلية دفاع مضيقة تهدف إلى الحد من توافر الحديد للميكروبات أثناء الاستجابات الالتهابية. ومع ذلك، قد تكون هذه الآلية غير فعالة في الحالات الالتهابية غير المعدية التي تؤدي إلى فقر الدم. **الهدف:** للتعرف على هرمون الهيبسيدين مع العديد من المؤشرات البيوكيميائية التي يمكنها التنبؤ بالنتائج لدى المرضى العراقيين المصابين بالثلاسيميا الكبرى، وتحديد أولئك المصابين أو غير المصابين بمرض الزحار الأميبي. **طريقة العمل:** شارك في هذه الدراسة 200 شخص (150 مريض بمرض الثلاسيميا الكبرى و 50 من الأصحاء) أثناء حضورهم إلى مستشفى الكرامة و مستشفى ابن بلدي في بغداد. أجريت الدراسة خلال الفترة من يناير 2024 إلى مايو 2024. وتراوحت الفئة العمرية لجميع المرضى من (15-35) سنة. أخذت المعلومات اللازمة من جميع المرضى فضلًا عن جمع عينات البراز من المرضى الذين يعانون من الإسهال الدموي و/أو المخاط وتم استخدام حقنة سعة 5 مل لجمع الدم الوريدي عن طريق ثقب الوريد. نقل الدم إلى أنابيب فاصل مصل معقمة سعة 10 مل (أنبوب هلامي يستخدم للكشف عن : الهبسيدين بواسطة الاليزا ، الالنين ترانس امينيز ، اسبارتيت ترانسفيريز ، الحديد ، اجمالي سعة ارتباط الحديد) باستخدام جهاز التحليل الذاتي المخصص للتحاليل البيوكيميائية المحددة. **النتائج :** أظهرت نتائج المؤشرات الكيموحيوية في الكبد ارتفاع مستويات إنزيمات (الالنين ترانس امينيز ،



tube used for detection of: Hepcidin by ELISA ALT, AST, Iron and TIBC by using biochemical specific auto-analyzer instrument. **Results:** The results of biochemical indicators showed an elevated levels in liver the enzymes (ALT, AST) and (Iron and TIBC) was significantly higher than that observed in the control ($P < 0.0001$). According to serum concentration of Hepcidin showed a lower for β -thalassemia patients' group ($P < 0.0001$) (80.83 ± 77.7) as compared with control (320.9 ± 88.1). In the present study, no statistically significant difference ($P < 0.9042$) was observed in the levels of Hepcidin between patients with β -thalassemia and those infected with *Entamoeba histolytica*. Also, the results showed significant decreases ($P < 0.0001$) in Iron and TIBC levels, but a significant increases ($P < 0.0001$) in ALT and AST was observed when comparing to patients with β -thalassemia those infected with *Entamoeba histolytica*. **Conclusion:** Patients with β -thalassemia who are infected with *Entamoeba histolytica* exhibited a notable reduction in hepcidin levels, along with a significant difference in certain biochemical indicators.

Keywords: β -thalassemia, *Entamoeba histolytica*; Hepcidin, ALT, AST, Iron and TIBC.

Abbreviations

ALT: Alanine transaminase

AST: Aspartate aminotransferase

TIBC: Total Iron Biding Capacity

ELISA: Enzyme Linked Immune Sorbent Assay

BTM: B Thalassemia Major



Abstract

Thalassemia syndromes encompass a wide range of hemoglobin disorders characterized by either the absence or diminished production of normal globin chains. Hepcidin, a peptide produced by the liver with antimicrobial properties, is mainly synthesized by hepatocytes situated near the portal veins, which are responsible for transporting dietary iron, as well as by Kupffer cells. Before blood transfusions, increased erythropoietic activity leads to a reduction in hepcidin, resulting in lower hepcidin levels that enhance the absorption of dietary iron. Parasite protozoa depend on iron for their survival, as it plays a crucial role in the growth and enzymatic functions of *E. histolytica* by participating in the synthesis of the parasite's enzymes. An elevation in hepcidin levels may serve as a host defense mechanism aimed at limiting iron availability to microbes during inflammatory responses; however, this mechanism may prove ineffective in non-infectious inflammatory conditions that lead to anemia. **Aims:** to identify the hepcidin hormone along with various biochemical indicators that can predict outcomes in Iraqi patients with β -thalassemia major, specifically those who have tested either positive or negative for *Entamoeba histolytica*. **Methods:** A total of 200 (150 with β -thalassemia patients and 50 healthy subjects) were involved in this study during their attendance at AL-Karama hospital and Ibn AL-Baladi hospital in Baghdad. The study was conducted during the period from January 2024 to May 2024. The age range of all patients were from (15–35) years and the necessary information's were taken from all patients. Stool specimens were collected from patients with blood and /or mucus diarrhea and five ml disposable syringe was used to collect venous blood by vein puncture. blood was transferred to 10 ml sterile serum separator tubes (gel



Exploring the Role of Heparin and Some Biochemical Indicators in β -thalassemia Major Patients with Negative and Positive *Entamoeba histolytica* / Original study

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استكشاف دور الهيبسيدين وبعض المؤشرات البيوكيميائية لدى مرضى
الثلاسيميا الكبرى المصابين وغير المصابين بطفيلي الزحار الاميبي

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Second, the number of patients was relatively small and may have introduced statistical bias. It is worth noting that a personalized protocol, similar to the approach used in treating other medical conditions, may lead to better outcomes. Therefore, future research should focus on conducting additional studies with longer follow-up periods.

5. Conclusion

The factors under study might be crucial for assessing the therapy response of PCOS women in Baghdad City.

Remarkably, this study discovered a strong positive correlation between the LH and insulin resistance levels in PCOS-afflicted women.



Our result in Table (4) the mean levels of FSH were normal in range when compared with normal range (3-11 mg/dl) with little differences, statistically these differences were highly significant (P-value ≤ 0.0001). The normal gonadotrophin axis is disturbed in women with PCOS, thus increasing LH levels and decreasing FSH levels, leading to an inversion of the LH/FSH ratio (Balen, *et al.*, 2003).

However, Saadia's study failed to show any significant association between BMI and serum hormone levels in women with PCOS including LH, FSH, and LH/FSH ratio. (Saadia, 2022). Obesity, insulin resistance, and dyslipidemia are comorbidities associated with PCOS and have been found to be associated with the LH/FSH ratio in a nationally representative sample of postmenopausal women in the United States (Beydoun, *et al.*, 2012).

At level of LH and FSH our result agreed with Malini and Roy study that said there is relationship of insulin to LH and testosterone was positive and significant ($p < 0.05$) within the whole PCOS bunch and in five PCOS subcategories with expended LH rise (i.e. 1.3, 2, 3, 4 & 5 times of LH rise in connection to FSH levels in each gather individually) (Malini & Roy, 2021).

This consider found noteworthy affiliations between affront levels or HOMA-IR and the LH proportion as appeared in Table (3). This contrasts with a ponder by Pratama, *et al.*, which appeared a negative relationship between affront resistance and LH levels or the LH/FSH proportion (Pratama, *et al.*, 2024).

About the result shown in table (5) we can say there are certainly some limitations to this study. First, when considering the outcomes for overweight/ obese patients, it should be noted that these were exploratory analyses of subsequent subgroups of the original trial and were not pre-specified in the study designs, and therefore, the results should be treated with caution.



Different clinical phenotypes of PCOS carry diverse metabolic dangers, and the classic and ovulatory phenotypes are characterized by IR. Hyperandrogenemia is caused by IR and hyperinsulinemia, which have the impact of boosting LH's effect on ovarian androgen union. Insulin and androgens both block Sex hormone-binding globulin (SHBG) secretion, which raises free and bioactive androgen levels and exacerbates clinical androgen excess. Additionally, IR plays a significant role in the development of metabolic syndrome and cardiovascular disease in PCOS women (Polak, *et al.*, 2016).

In women with PCOS, IR stimulates the release of insulin from pancreatic cells and encourages adipose tissue mobilization alongside an elevation of hepatic production. All these actions cause a rise in plasma-free fatty acid (FFA) which in turn impairs glucose transport function by the inactivation of major enzymes such as dehydrogenase pyruvate (PDH) (Ding, *et al.*, 2021).

Sex hormone-binding globulin (SHBG) is the most official protein for testosterone, and it is the unbound testosterone (moreover called free testosterone) that acts on target tissues. Affront is accepted to cause a rise in circulating levels of free testosterone by two correct components: to begin with, by empowering ovarian biosynthesis and emission of testosterone, and, moment, by straightforwardly inhibiting hepatic production of SHBG (Mayer, *et al.*, 2015).

Higher levels of circulating insulin, contribute to hyperandrogenemia in women with PCOS which in turn promotes the production of ovarian androgen and suppresses the development of sex hormone-binding globulin. This scenario of events could be one mechanism underpinning the increased risk of T2D in women with hyperandrogenemia in PCOS. An alternative mechanism may involve elevated levels of free testosterone, which could exacerbate insulin resistance (Persson, *et al.*, 2021).



proteins such as CRP (Peña, *et al.*, 2020). Polycystic ovarian disorder (PCOS) is one of the promptly perceived endocrine organ sicknesses in ladies, being overweight, and feebleness. Long-term, low-grade irritation has risen as a vital figure driving to PCOS. In Indian ponder they found a rise in glucose levels may invigorate oxidative stretch and alarming response from mononuclear cells (MNC) of females with PCOS, which regularly don't rely on fat. This is often required since MNC-derived macrophages are the major source of cytokine blend in enormous fat tissue and additionally energize adipocyte cytokine generation. In outline, information un cover the significant dangers of affront resistance in stout individuals who are enduring from PCOS. The discoveries of this particular lesson shown that people with the customary PCOS phenotype had weight and higher affront levels and affront resistance, ignoring the nonappearance of BMI contrasts from other phenotypes (Purwar & Nagpure, 2022).

Besides, smothered levels of serum adiponectin in ladies with PCOS compared with BMI-matched control ladies (illustrated in a huge meta-analysis on >3400 subjects) may assist contribute towards the foundation of affront resistance in PCOS. The part of tall atomic weight adiponectin in PCOS remains not completely caught on and ought to shape a center for future investigate as there may be suggestions for treatment, as proposed by a later consider in a rat show of PCO (Barber & Franks, 2020).

An inadequate cellular response to insulin activity is referred to as insulin resistance, which is considered an important aspect of PCOS multisystem pathophysiology. Insulin resistance exacerbates hyperandrogenism and the resulting hyperinsulinemia increase ovarian androgen synthesis while decreasing hepatic sex hormone-binding globulin (Greenwood & Huddleston, 2019).



The negative effects of PCOS on metabolism and reproduction are further exacerbated by obesity. Adipogenesis and lipolysis are both boosted by obesity's increased insulin resistance and compensatory hyperinsulinemia, which also boosts adipogenesis. Obesity upregulates the synthesis of ovarian androgen, which in turn makes thecal cells more sensitive to LH activation and intensifies functional ovarian hyperandrogenism. There is a vicious feedback cycle between obesity and inflammatory adipokines, which promote hyperinsulinemia and, in turn, obesity (Glueck & Goldenberg, 2019).

Table (1) showed comparison between treated and untreated PCOS groups showed a slight, but not statistically significant, increase in mean age in women participating in the study, respectively (84 (100.0%), 40 (100.0%), 6 (100.0%). This marginal difference, as indicated by the p-value of 0.66, suggests that while there may be a trend towards higher levels of age (19–28) in PCOS female, it is not large enough to draw definitive conclusions. This finding is consistent with some studies suggesting that environmental factors such as endocrine disruptors may be associated with PCOS, but more robust and larger studies are needed to confirm any significant associations (Kshetrimayum, *et al.*, 2019). This results agreed with (Jozkowiak, *et al.*, 2022) polycystic ovary disorder (PCOS) is the foremost common heterogeneous endocrine clutter among ladies of regenerative age. The pathogenesis of PCOS remains tricky; in any case, there's prove proposing the potential commitment of hereditary intelligent or inclinations combined with natural components (Jozkowiak, *et al.*, 2022).

As shown in Table (2) the results can explained as Jozkowiak, said in their study that the prognosis of IR is commonly associated with the excessive production of inflammatory cytokines such as (IL-8, IL-10, IL-12) and acute-phase



direction amid metformin treatment was essentially more articulated in PCOS ladies with metabolic disorder at standard, compared with those without metabolic disorder ($p = 0.0369$ for interaction). In conclusion, metformin progressed the metabolic profile of PCOS ladies over 36.1 months, especially in BMI (Akhtar, *et al.*, 2021). Other studies observed that metformin with way of life alteration driven to the lessening of BMI. Most of the considers found comparative comes about and few considers moreover found that metformin diminished affront resistance indeed without decreasing BMI in PCOS (Melin, *et al.*, 2023).

The results were similar to those of the two Iraqi researchers (Alsaadi and Mohamad, 2019). There was a statistically significant difference ($P < 0.05$) in body mass index between women with polycystic ovary syndrome and control ladies. The predominance of overweight and corpulence was also significantly higher in women with polycystic ovary syndrome than in matched control women.

Being the most prevalent endocrine disorder in women of reproductive age.

It is commonly acknowledged that adipose tissue functions as an active endocrine organ, generating and secreting "adipokines," which are biologically active chemicals. Adipokines play a role in the control of several homeostatic processes, including the regulation of insulin sensitivity, atherosclerosis, inflammation, and energy metabolism. Adipose tissue alters structurally and functionally with obesity, causing high blood sugar, high cholesterol, high leptin levels, and persistent low-grade inflammation. It appears that women with PCOS experience similar changes in their adipose tissue (Calcaterra, *et al.*, 2021).



4. Discussion

PCOS is one of the most causes of infertility. PCOs, oligo-anovulation, and hyperandrogenism are among the diagnostic criteria, but a lot of PCOS-afflicted women are also overweight or obese. The management of symptoms and reproductive results are both impacted by adiposity, which has a significant impact on specific PCOS phenotypes (Cena, *et al.*, 2020).

The results shown highly significant (P -value ≤ 0.0001) by observed the most cases of PCOs were obese and overweight BMI (Kg/m^2) among the untreated (not taken metformin treatment) groups than (taken metformin treatment) with 18/50 (51.4%), 21/50 (34.4%) 00/11 (26.2%), 16/40 (45.7%) respectively. Thus agreed with (Jensterle, *et al.*, 2020) who metformin made strides the metabolic profile of ladies with PCOs over a cruel follow-up of 36.1 months, especially in HDL cholesterol, diastolic blood weight and BMI. In expansion, ladies with PCOs who too had the metabolic clutter at standard showed up to gather more metabolic benefits from metformin.

Findings suggest that affront resistance could be a key highlight of polycystic ovary disorder (PCOS) and may increment the hazard of cardiovascular disease. Because of insulin resistance, metabolic disorder is more predominant in ladies with PCOS than in ordinary ladies. Metformin progresses the metabolic profile in PCOS in short-term thinks about. Within the Indian consider assessed the long-term impact of metformin on metabolic parameters in ladies with PCOS amid schedule care without a controlled count calorie. Metabolic chance variables were compared some time recently and after metformin treatment (Shahebrahimi, *et al.*, 2016). Improvements in body mass file were watched. The predominance of metabolic disorder diminished from 34.3% at first to 21.4% ($p = 0.0495$). The BMI lessening



Also there was a non-significant difference (P-value ≥ 0.05) in mean levels of insulin between the female groups who were treated with metformin when classified according to BMI scores.

Table (5): Comparative the mean levels of FBS, Insulin, IR, FSH and LH between cases and control according to BMI (Kg/m^2) score.

Test	Groups	BMI (Kg/m^2) score			P-value	Sig.
		Normal weight	Overweight	Obese		
		M \pm SD	M \pm SD	M \pm SD		
FBS (mg/dl)	Treated	99 \pm 7.48	94.75 \pm 8.72	96.56 \pm 10.89	0.58	N.S
	Untreated	108.90 \pm 5.61	108.04 \pm 6.35	110.72 \pm 7.48	0.46	N.S
	Control	78.93 \pm 6.30	80.16 \pm 6.01	81.0 \pm 0	0.81	N.S
Insulin	Treated	6.43 \pm 3.27	7.17 \pm 3.60	7.60 \pm 3.28	0.73	N.S
	Untreated	6.82 \pm 2.94	9.81 \pm 3.72	12.01 \pm 3.31	0.001	H.S
	Control	2.85 \pm 0.69	3.38 \pm 1.09	3.0 \pm 0	0.25	N.S
IR	Treated	1.58 \pm 0.81	1.67 \pm 0.86	1.82 \pm 0.85	0.77	N.S
	Untreated	1.84 \pm 0.82	2.63 \pm 1.04	3.30 \pm 0.99	0.001	H.S
	Control	0.55 \pm 0.15	0.67 \pm 0.23	0.60 \pm 0	0.25	N.S
FSH	Treated	6.13 \pm 1.82	5.93 \pm 1.62	5.27 \pm 1.0	0.29	N.S
	Untreated	5.99 \pm 1.26	6.50 \pm 1.30	5.99 \pm 1.19	0.38	N.S
	Control	7.56 \pm 1.22	7.19 \pm 1.48	8.50 \pm 0	0.57	N.S
LH	Treated	8.52 \pm 1.90	9.54 \pm 3.05	10.33 \pm 2.32	0.27	N.S
	Untreated	9.83 \pm 2.26	10.87 \pm 2.85	12.06 \pm 3.34	0.14	N.S
	Control	8.83 \pm 1.50	8.65 \pm 1.98	5.70 \pm 0	0.26	N.S

**Table (4): Comparative the mean levels of FSH and LH between cases (n=90) and Control (n=40)**

Test	Groups	M±SD	G1&G3		G2&G3		G1&G2	
			P-value	Sig.	P-value	Sig.	P- value	Sig.
FSH mg/dl (3-11)	G1-Treated (n=40)	5.71±1.46	≤0.0001	H.S	≤0.0001	H.S	0.089	N.S
	G2-Untreated (n=50)	6.20±1.25						
	G3- Control (n=40)	7.36±1.38						
ANOVA P-value		≤0.0001 (H.S)						
LH mg/dl (1-12)	G1-Treated (n=40)	9.65±2.60	P-value	Sig.	P-value	Sig.	P- value	Sig.
	G2-Untreated (n=50)	11.07±2.99	0.09	N.S	≤0.0001	H.S	0.01	S
	G3- Control (n=40)	8.64±1.83						
ANOVA P-value		≤0.0001 (H.S)						

3.4 Comparative the mean levels of FSH and LH between cases and control according to BMI (Kg/m²) score.

The results of this study (Table 5) showed that there was a highly significant differences (P-value = 0.001) in the levels of Insulin among female groups whose untreated with metformin with high levels among obese groups (12.01±3.31) than overweight and normal weight groups with (9.81±3.72, 6.82±2.94) when comparing with the levels with control groups. While there was a non-significant difference (P-value ≥0.05) in mean levels of FBS (mg/dl) between the female groups whose treated and those whose untreated with metformin in comparing with control groups when classified according to BMI scores.



3.3 Comparative the mean levels of FSH and LH between case.

The results of this study showed that the mean levels of FSH were normal in range when compared with normal range (3-11 mg/dl) with little differences, statistically these differences were highly significant (P-value ≤ 0.0001) as arranged in Table (4). The results of this study also showed there were a highly differences (P-value ≤ 0.0001) in the levels of FSH between treated and control groups (G1&G3) and between untreated and control (G2&G3) with (P-value ≤ 0.0001) , While there was a non-significant differences (P-value=0.089) in the levels of FSH between treated and untreated groups (G1&G2).

The mean levels result of LH showed that G1 compared to G3: There is a p-value of 0.09, showing that there is no significant distinction (N.S.) between the treated and control groups.

- In the comparison of G2 and G3, the p-value of ≤ 0.0001 shows a highly significant difference (H.S.) between the untreated and control groups, with the untreated group showing higher LH levels.

G1 compared to G2: A p-value of 0.01 shows a significant distinction between the treated and untreated groups, with the untreated group showing elevated LH levels.



resistance (IR) when compared to the control group. However, the untreated group displays the highest levels for all these parameters.

Table (3): Comparative the mean levels of F.B.S(mg/dl) Insulin (MI/UL) and IR among cases (Treated, Untreated) and control

Test	Groups	M±SD	G1&G3		G2&G3		G1&G2	
			P-value	Sig.	P-value	Sig.	P-value	Sig.
FBS (70-120)	G1-Treated (n=40)	96.32±9.35	≤0.0001	H.S	≤0.0001	H.S	≤0.0001	H.S
	G2-Untreated (n=50)	109.20±6.61						
	G3- Control (n=40)	79.72±6						
ANOVA P-value		≤0.0001 (H.S)						
Insulin (2-25)	G1-Treated (n=40)	7.20±3.35	≤0.0001	H.S	≤0.0001	H.S	≤0.0001	H.S
	G2-Untreated (n=50)	9.95±3.87						
	G3- Control (n=40)	3.17±0.97						
ANOVA P-value		≤0.0001 (H.S)						
IR (0-1.2)	G1-Treated (n=40)	1.71±0.83	≤0.0001	H.S	≤0.0001	H.S	≤0.0001	H.S
	G2-Untreated (n=50)	2.70±1.10						
	G3- Control (n=40)	0.6279±0.20						
ANOVA P-value		≤0.0001 (H.S)						



Table (2): Comparative the mean levels of Age (Years) and BMI (Kg/m²) among cases (Treated, Untreated) and Control groups (n=40)

Test	Groups	M±SD	SE	P-value
Age (Years)	G1-Treated (n=40)	28.20±4.45	0.70	0.45
	G2-Untreated (n=50)	26.76±5.94	0.84	
	G3- Control (n=40)	27.57±4.70	0.74	
BMI (Kg/m ²)	G1-Treated (n=40)	30.40±5.68	0.89	≤0.0001
	G2-Untreated (n=50)	29.67±6.26	0.88	
	G3- Control (n=40)	25.17±2.45	0.38	

3.2 Comparative analysis of the average levels of fasting blood sugar (F.B.S), insulin, and Insulin Resistance (IR) among three groups.

Table (3) showed a comparative analysis of the average levels of fasting blood sugar (F.B.S), insulin, and insulin resistance (IR) across three groups: treated (G1), untreated (G2), and control (G3).

The p-values for comparisons between groups (G1&G3, G2&G3, G1&G2) are all ≤0.0001, indicating highly significant differences (H.S) in F.B.S levels among the groups. The ANOVA p value is also ≤0.0001, confirming that at least one group differs significantly from the others. Similar to F.B.S, the p-values for comparisons between groups are all ≤0.0001, indicating significant differences in insulin levels. The ANOVA p-value is also ≤0.0001. as well as, the p-values for comparisons are ≤0.0001, indicating significant differences in IR levels among the groups. The ANOVA p-value is ≤0.0001. The data reveals that both the untreated and treated groups exhibit significantly elevated levels of fasting blood sugar (F.B.S), insulin, and insulin



cases which analyzed with PCOs were 12 out of 50 (30%) versus 13 out of 40 cases of treated (taken metformin treatment) were at the age bunches (29-38) a long time, Whereas the less cases of PCOs of both untreated and treated cases were recorded at the age bunches $38 <$ were 2 (33.3%),3 (50%) separately, measurably these contrasts were non-significant (P -value=0.66) as illustrated in Table (1).

Table (1): Characterization of Age range (years) and BMI (Kg/m²) among cases (treated, untreated) and control group

Variable	Category	Treated (n=40)	Untreated (n=50)	Control (n=40)	Total	P value	Sig.
Age range (Years)	(19-28)	25 (29.8%)	35 (41.7%)	24 (28.6%)	84 (100.0%)	0.66	N.S
	(29-38)	13 (32.5 %)	12 (30.0%)	15 (37.5%)	40 (100.0%)		
	<38	2 (33.3%)	3 (50.0%)	1 (16.7%)	6 (100.0%)		
Total		40 (30.8%)	50 (38.5%)	40 (30.8%)	130(100.0%)		
BMI (Kg/m ²)	Normal weight	8 (23.5%)	11 (32.4%)	15 (44.1%)	34 (100.0%)	0.001	H.S
	Overweight	16 (26.2%)	21 (34.4%)	24 (39.3%)	61 (100.0%)		
	Obese	16 (45.7%)	18 (51.4%)	1 (2.9%)	35 (100.0%)		
Total		40 (30.8%)	50 (38.5%)	40 (30.8%)	130(100.0%)		

The results of this study showed the mean age of PCOs female whose treated and untreated were 28.20 ± 4.45 , 26.76 ± 5.94 respectively in compared with control groups were 27.57 ± 4.70 , These differences had P -value=0.45.

The results of the current study observed the mean of BMI (Kg/m²) of treated groups were higher than untreated group with 30.40 ± 5.68 , 29.67 ± 6.26 respectively in compared with control groups were 25.17 ± 2.45 , These differences had P -value ≤ 0.0001 as arranged in Table (2).



2.5 Measure Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH):

Levels were determined on cycle day 2 to secure normality of ovarian functions. Hormonal measurements were done by using Mini Vidas (bio Merieux, France and U.S.A.) (Zhao, *et al.*, 2023).

2.6 The statistical analysis:

Performed using IBM SPSS statistics software (version 26.0). Results analyzed using Variation-ANOVA and expressed as mean \pm standard error. The p-value was used to determine level of statistical significance, with $p \leq 0.05$ or $p \leq 0.0001$ being statistically significant.

3. Results

3.1 Demographical characteristic of the study groups

The results shown highly significant (P-value ≤ 0.0001) by observed the most cases of PCOs were obese and overweight BMI (Kg/m^2) among the untreated (not taken metformin treatment) groups than (taken metformin treatment) with 18/50 (51.4%), 21/50 (34.4%), 00/11 (26.2%), 16/40 (45.7%) respectively.

The results of the current study recorded the number of untreated (not taken metformin treatment) cases which diagnosed with PCOs were 35 out of 50 (41.7%) at the age groups (19-28) years, while the number of treated (taken metformin treatment) cases which diagnosed with PCOs were 25 out of 40 (29.8%) at the age groups (19-28) years. The results about moreover archived the number of untreated (not taken metformin treatment)



2.2 Determination of Fasting Blood Sugar (FBS) (mg/dL):

The blood sample was prepared after the centrifugation process, and only the serum was taken. An amount of 0.5 ml from serum was placed in the cuvette to read the absorption rate. The absorbance was measured at 505 nm. Serum fasting blood sugar (FBS) ≥ 120 mg/dl was decided by utilizing the Mindray BA-88A machine, a semi-automated organic chemistry analyzer outlined to perform a wide extend of biochemical tests. FBS: (75_126 mg/dl), (4.1_6.1 mmol/l). (Ambade, *et al.*, 1998).

2.3 Determination of Fasting Insulin (μ U/mL):

Using the Roche Kit of the Cobas equipment 0.5 ml of serum was taken and placed in the device. COBAS INTEGRA 400 plus analyzer, a fully automated device that uses more than 140 applications for all types of sample matrices, was used to measure serum fasting insulin (Dahman, *et al.*, 2021).

2.4 Determination of HOMA-IR:

A measurement of blood sugar is made. Together with the insulin level test, the woman's degree of insulin resistance is then determined using the equation.

Insulin Resistance = [(fasting glucose mg/dL) x (blood insulin $\hat{1}$ /₄U/mL)] / 405 or
Insulin Resistance = [(fasting glucose mmol/L) x (blood insulin $\hat{1}$ /₄U/mL)] / 22.5. The optimal insulin sensitivity of HOMA-IR is less than 1, while levels above 1.9 signal early insulin resistance, and a value of 2.5 or above is taken as an indicator of IR in females (Cheang, *et al.*, 2019).



enroll a few follicles from each ovary. These follicles will total development, and as it were one Graafian follicle will go through ovulation amid that cycle. The increment in FSH too fortifies the emission of Inhibin B by the granulosa cells. Inhibin B will in the long run limit the emission of FSH toward the conclusion of the follicular stage. Inhibin B levels will be most elevated amid the LH surge some time recently ovulation, and will rapidly decrease off after (Reed & Carr, 2018). The goals of the study are focuses on evaluating the hormonal markers of polycystic ovary syndrome and insulin resistance and determining the effect of insulin resistance on these hormones.

2. Materials and methods

2.1 Patients and samples

This study included 130 female aged 19 to 38 years, categorized into three cohorts: 40 women with PCOs treated (metformin) and 50 women with PCOs untreated, and 40 control. Female data encompassed various factors, including age, length, weight.

Samples collection: collected 5 ml blood from each woman attended Kamal Al-Samarrai Hospital for Infertility and Baghdad Medical City with PCOs, and the same volume of blood from the control group, then transferred into gel tubes, all samples were appropriately labeled, sent to the laboratory using dry-ice packs to maintain low temperatures.

Inclusion criteria: Based on the gynecologist's diagnosis, a serum sample from polycystic ovary syndrome female was collected.

Exclusion criteria: pregnant woman



incompletely understood, the underlying defect is reported to occur within the post-receptor phosphatidylinositol 3-kinase (PI3K) insulin pathway that mediates the metabolic effects of insulin. Other factors may also contribute towards the establishment of insulin resistance in women with PCOS.

Follicle stimulating hormone, commonly abbreviated to FSH, belongs to the 'gonadotropin' group of hormones (Orlowski & Sarao, 2022).

The treatment of PCOS is primarily symptomatic rather than pathophysiological. The central component of the disorder is thought to be a neuroendocrine clutter, as prove by raised LH/FSH proportions in PCOS ladies, particularly in ladies with destitute PCOS (Pratama, *et al.*, 2022).

As a reaction to the rise in FSH levels amid the early follicular stage, there will be a expansion of granulosa cells. This rise within the number of granulosa cells will cause a concurrent rise in FSH receptors on the cells. The expanded FSH levels permit the granulosa cells to deliver estradiol, which initiates LH receptors on granulosa cells as well. With LH receptors presently display, the granulosa cells will deliver little sums of progesterone and 17hydroxyprogesterone. The progesterone discharged by the granulosa cells controls granulosa cell expansion and eventually moderates follicular development (Monis & Tetrokalashvili, 2022).

Based on a 28-day cycle, the follicular stage measures from the primary day of feminine cycle (day 0) until the starting of ovulation (day 14). When the past menstrual cycle completes, and the corpus luteum breaks down, the levels of estrogen, progesterone, and inhibin A will diminish. This chain of occasions will cause positive criticism to the hypothalamus and front pituitary, and a ensuing pulsatile discharge of GnRH and FSH into circulation. This increment in FSH will invigorate the granulosa cells of the ovaries to



Introduction

Polycystic ovary syndrome (PCOS) an endocrinologic disorder among women of reproductive age with a predominance of 4-18% (Ding, et al., 2018), although infertility is a major problem in females with PCOS, hormone disorders including menstrual abnormalities (oligomenorrhea or amenorrhea), chronic anovulation and hyperandrogenism (resulting in androgenic alopecia, acne, and hirsutism) are also common problems in these women (Zhang, et al., 2018).

Polycystic Ovary Disorder (PCOS) could be a heterogeneous clutter characterized by constant ovulatory dysfunction and hyperandrogenism. It is considered as the foremost common endocrinological issue influencing ladies, and the foremost predominant cause of their menstrual abnormalities amid the regenerative age (Saadia, et al., 2020).

Polycystic Ovary Disorder could be a therapeutic condition characterized by a collection of symptoms that negatively impact a woman ovulatory function and ovarian morphology (Singh, et al., 2023). The present condition was initially characterized in 1935 (Witchel, et al., 2019), and it is typified by persistent anovulation, a polycystic ovary, female sterility, menstrual irregularities, hirsutism, hyperandrogenism, and excessive weight gain (Singh, et al., 2023). The emergence of pathological or atypical cysts, including polycystic ovary syndrome (PCOS) cysts, may lead to distressing and uncomfortable symptoms. A diagnosis of Polycystic Ovary Syndrome requires that a woman manifest at least two of the previously delineated symptoms (Rasquin, et al., 2022).

Insulin resistance is present in many, if not most, women with PCOS (Amisi, 2022). Although the mechanism of insulin resistance in PCOS remains



الاستنتاجات: تظهر هذه الدراسة ارتباطاً مهماً بين مستويات مقاومة الأنسولين ونسبة LH لدى النساء المصابات بمتلازمة تكيس المبايض. قد تلعب المعايير المدروسة دوراً مهماً في تقييم الاستجابة العلاجية لنساء POCS في مدينة بغداد. هذا البحث يكتشف ارتباطاً إيجابياً جديراً بالملاحظة بين مستويات مقاومة الأنسولين ونسبة LH لدى النساء المصابات بمتلازمة تكيس المبايض.

الكلمات المفتاحية: مقاومة الأنسولين، متلازمة تكيس المبايض، LH، FSH، سكر الدم الصائم، الميتفورمين، مؤشر كتلة الجسم.



Abbreviations

PCOS: Polycystic ovary syndrome

LH: luteinizing hormone

FSH: Follicle-Stimulating Hormone

FBS: fasting blood sugar

IR: insulin resistance

BMI: body mass index

SHBG: sex hormone-binding globulin

المستخلص

الخلفية: متلازمة تكيس المبايض هي اضطراب غدي يصيب النساء وهو السبب الأكثر شيوعاً لاضطرابات الدورة الشهرية في سن الإنجاب. متلازمة تكيس المبايض هي في الواقع شكل مزمن من انقطاع التبويض والذي يُلاحظ عادةً إلى جانب مجموعة كبيرة من الأعراض والعلامات السريرية. **الأهداف:** تركز الدراسة على تقييم العلامات الهرمونية لمتلازمة تكيس المبايض ومقاومة الانسولين ومعرفة تأثير مقاومة الانسولين على هذه الهرمونات. **المواد والطرائق العمل:** شملت هذه الدراسة 130 امرأة تتراوح أعمارهن بين 19 و38 عاماً، تم تقسيمهن إلى ثلاث مجموعات: 40 امرأة مصابة بمتلازمة تكيس المبايض تم علاجهن بـ (الميتفورمين) و50 امرأة مصابة بمتلازمة تكيس المبايض لم يتم علاجهن بـ (الميتفورمين). بالإضافة إلى ذلك، مثلت المجموعة الضابطة 40 امرأة. قيست مستويات الهرمونات و مقاومة الأنسولين و سكر الدم الصائم و LH.FSH **النتائج:** أظهر نمط الارتباط اتجاهًا متزايدًا من ارتفاع منخفض إلى متزايد في LH مقارنة بـ FSH تكشف البيانات أن كل من المجموعتين غير المعالجة والمعالجة تظهر مستويات مرتفعة بشكل ملحوظ في سكر الدم الصائم (FBS) والأنسولين ومقاومة الأنسولين (IR) عند مقارنتها بمجموعة التحكم. ومع ذلك، فإن المجموعة غير المعالجة تظهر أعلى المستويات لجميع هذه المعايير.



Abstract

Background: Polycystic ovarian syndrome is an endocrinological disorder influencing ladies, and it is the foremost common cause of menstrual unsettling influence amid the regenerative age. Polycystic ovary disorder (PCOS) is in fact a chronic form of anovulation that is usually observed besides a large spectrum of clinical symptoms and signs. **Objectives:** The study's main goal is to evaluate the hormonal indicators of "PCOS" and insulin resistance and determine how insulin resistance affects these hormones. **Materials and methods:** This study included 130 women, aged 19–38, who were separated into three bunches: 40 women with "PCOs" who were treated with metformin and 50 women with "PCOs" who were not treated with. In expansion, 40 from the control gather. Hormone levels: luteinizing hormone (LH), follicle-stimulating hormone (FSH), insulin resistance, and fasting blood sugar were measured. **Results:** The correlation pattern showed an increasing trend from lower to increased rise of LH compared to FSH. The data reveals that both the untreated and treated groups exhibit significantly elevated levels of fasting blood sugar (FBS), insulin, and insulin resistance (IR) when compared to the control group. However, the untreated group displays the highest levels for all these parameters. **Conclusions:** This study shows a significant association between insulin resistance levels and the LH proportion in ladies with PCOS. The studied parameters may play an important role in the evaluation of therapeutic responses in POCS women in Baghdad City.

Keywords: Insulin Resistance, PCOS, LH, FSH, Fasting Blood Sugar, Metformin, BMI.



Impact of Insulin Resistance in PCOS (LH / FSH) in A Group of Woman in Baghdad City / Original study

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تأثير مقاومة الأنسولين في متلازمة تكيس المبايض (LH/FSH) لدى مجموعة من النساء في مدينة بغداد

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Variable Outcomes:

The success of PRF in socket preservation can vary. Some studies show significant improvements in bone preservation and implant success with PRF, while others show less pronounced or no significant differences compared to control groups.

Potential for Infection:

Though generally considered safe, PRF, like any injectable material, carries a small risk of infection at the injection site.

Cost and Availability:

The cost of PRF preparation and the time required for its preparation can be a limiting factor for some patients and practices(80).

10. Conclusion

Combining injectable platelet-rich fibrin (I-PRF) with collagen plugs provides an effective and minimally invasive method for socket preservation after tooth extraction. This technique enhances soft and hard tissue healing by delivering growth factors and creating a bioactive scaffold that supports angiogenesis and regeneration. It reduces postoperative complications, preserves alveolar bone structure, and simplifies clinical procedures without the need for graft harvesting or flap elevation. Due to its biocompatibility, ease of use, and regenerative potential, this approach is a promising option in oral surgery and implantology.



cells. Additionally, I-PRF differs from L-PRF in cytokine composition, being richer in interleukin-10 (IL-10), which helps reduce inflammatory mediators and supports tissue regeneration(76). Its practical clinical use allows physicians to easily apply this liquid platelet concentrate alone or combined with other biomaterials to promote bone regeneration(77).

3. Anti-bacterial capability:

I-PRF has been shown to exhibit strong antibacterial properties against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Consequently, it is proposed that, in addition to its osteoinductive potential, I-PRF may also exert antimicrobial effects(78).

9. Limitations of Injectable PRF in Socket Preservation:

The disadvantages of PRF include, but are not limited to:

Resorption:

PRF, including injectable PRF (I-PRF), is susceptible to resorption over time. This means that the material breaks down and is absorbed by the body, potentially reducing its effectiveness in maintaining socket volume.

Limited Duration of Action:

While PRF can stimulate initial healing and release growth factors, its effects on bone regeneration may not be sustained in the long term. Studies suggest that PRF is most effective in the early healing period after tooth extraction, but longer-term benefits may be limited(79).



I-PRF's slower centrifugation results in a higher concentration of regenerative cells and growth factors compared to other PRF types. Applying PRF to titanium implants improves early implant stability and promotes thicker fibrin networks on implant surfaces, enhancing cell migration and differentiation around the implant(73).

8. Effect of I-PRF on bone tissue engineering:

I-PRF is a recently introduced biomaterial in bone tissue engineering that has gained significant attention. It is recognized as a substantial reservoir of bioactive compounds with diverse functional properties.

1. Regenerative capability:

Research evaluating the osteoinductive properties of I-PRF has demonstrated favorable outcomes in promoting osteoblast attachment, maturation, mobility, and proliferation, as well as enhancing the mineralization capacity of stem cells.(74). Furthermore, Fernández-Medina *et al.* found that at low concentrations, I-PRF outperformed A-PRF, L-PRP, P-PRP, and blood clot in enhancing mineralization, making it a preferred option for tissue engineering that uses autologous factors to stimulate bone regeneration(64). Its injectable nature and ease of application have made I-PRF suitable for various clinical procedures in oral and maxillofacial bone restoration, including procedures like alveolar ridge reconstruction, maxillary sinus elevation, and surgical correction of cleft palate defects(75).

2. Anti-inflammatory capability:

Due to its slower centrifugation speed compared to traditional counterparts, I-PRF contains higher levels of growth factors and regenerative



Dohan Ehrenfest *et al.* (2010) reported that histological analysis of all PRF-based matrices shows three main components(67):

1. Blood cells: PRF matrices contain platelets, leukocytes, and red blood cells. Platelets release growth factors crucial for tissue repair, while leukocytes support wound healing and stimulate angiogenesis through immune regulation. Macrophages also aid angiogenesis by secreting growth-promoting factors. Injectable PRF (I-PRF) has the highest levels of platelets and leukocytes among solid PRF types(68-70).
2. The three-dimensional fibrin matrix: is crucial for tissue repair, even though most research has focused mainly on growth factors. It functions as a biological scaffold that supports the gathering of adherent cells at the healing site. Furthermore, the fibrin matrix serves as a carrier for growth factors, providing a controlled release that preserves their bioactivity throughout the healing process(71).
3. Growth factors of PRF:
Key growth factors found in PRF include the following(72):
 1. Platelet-Derived Growth Factor (PDGF) regulates the growth and movement of mesenchymal cells.
 2. Transforming Growth Factor-beta (TGF- β) promotes the proliferation of mesenchymal cells and matrix production.
 3. Vascular Endothelial Growth Factor (VEGF) strongly stimulates tissue angiogenesis.
 4. Insulin-like growth factor (IGF) supports the differentiation, proliferation, and protection of mesenchymal cells.



protocol—1300 rpm for 8 minutes—resulting in a dense fibrin network that enhances healing(64).

C. Injectable platelet-rich fibrin (I-PRF):

An Injectable platelet-rich fibrin (I-PRF), developed by Choukroun and Ghanaati in 2018, is a liquid form prepared without anticoagulants using a gentle centrifugation of 700 rpm for 3 minutes(65). It separates into two layers, with the upper I-PRF layer used for regenerative procedures. This injectable form, “Fig. 1”, can be applied directly to defect sites or mixed with bone grafts to enhance graft stability and handling(66).

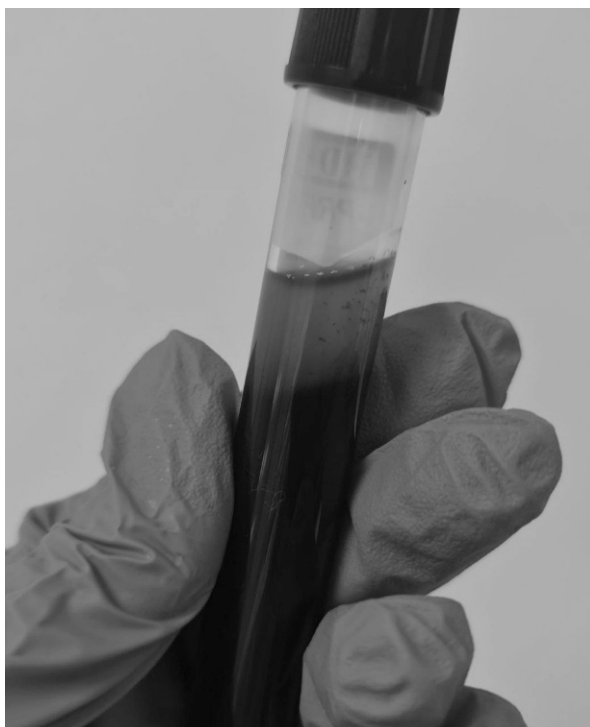


Fig. 1: Upper I-PRF layer.



7. PLATELET CONCENTRATES

Platelet concentrates were introduced over 20 years ago to support tissue healing by using blood-derived growth factors that enhance revascularization and cell proliferation(57).

Platelet concentrates are classified into four main types based on their leukocyte content and fibrin structure (58):

- Pure platelet-rich plasma (P-PRP) or leukocyte-poor platelet-rich plasma (LP-PRP).
- Leukocyte- and platelet-rich plasma (L-PRP).
- Pure PRF (P-PRF)—or leukocyte-poor platelet-rich fibrin.
- Leukocyte- and platelet-rich fibrin (L-PRF).

A. Platelet-rich plasma (PRP):

As a first-generation platelet concentrate, it is produced from the patient's blood and activated using calcium chloride and thrombin(59). Once activated, platelets rapidly begin releasing growth factors, with most being fully released within the first hour(60).

B. Platelet-rich fibrin (PRF):

Leukocyte-PRF (Choukroun's PRF), introduced in 2000, is a second-generation platelet concentrate prepared without anticoagulants, aiming to promote wound healing and tissue regeneration(61). It is prepared by centrifuging venous blood at 3000 rpm for 10 minutes, forming a PRF clot that is then compressed into a strong membrane(62). The absence of additives reduces the risk of postoperative complications(63). A more recent version, Advanced PRF (A-PRF), is produced using a softer centrifugation



D. Collagen plug:

Collagen plugs are cylindrical collagen sponges designed to fit into extraction sockets, acting as scaffolds that support healing by attracting fibroblasts and aiding in hemostasis(52). As components of the extracellular matrix, they stabilize the blood clot, support osteoblast migration, promote soft tissue repair, and contribute to bone regeneration and ridge preservation(3).

These plugs are made from highly purified porcine or bovine collagen, offering excellent biocompatibility and seamless integration with host tissues. Their porous structure creates a favorable environment for the infiltration of key cells like fibroblasts and endothelial cells, essential for new connective tissue and blood vessel formation(53). One major advantage of collagen plugs is that they eliminate the need for flap elevation or graft harvesting, thus reducing postoperative morbidity(52). They also help minimize complications after extraction, such as bleeding and infection(54). Their flexibility and adaptability make them easy to shape and apply to sockets of various sizes(55). In addition, they can be used in different surgical procedures, enhancing soft tissue healing and reducing postoperative discomfort(56). However, while collagen plugs offer many benefits, a potential limitation is their lack of mechanical strength compared to other graft materials, which may affect their ability to support large defects. Nonabsorbable types include titanium mesh and various forms of polytetrafluoroethylene (ePTFE, high-density PTFE, titanium-reinforced PTFE), while absorbable membranes include polymeric, collagen, pericardium membranes, platelet-rich fibrin, and acellular dermal matrix(51).



B. Bone graft and bone substitute:

Bone grafts are widely used to prevent alveolar bone loss after tooth extraction and can be combined with materials like barrier membranes (45).

There are four main types:

- **Autogenous grafts** (from the patient) are the gold standard due to their ability to promote bone growth and biocompatibility, with common donor sites inside or outside the mouth (46).
- **Allografts** come from donors of the same species and have good bone-regenerating properties, but carry risks of immune response and disease transmission (47).
- **Xenografts** are animal-derived, mainly bovine or porcine, and are biocompatible but more immunogenic and riskier for disease transmission (48).
- **Synthetic substitutes** like hydroxyapatite and tricalcium phosphate mimic natural bone, provide consistent resorption rates, and avoid disease risks linked to biological grafts (49).

C. Barrier membrane in socket preservation:

Barrier membranes play a key role in guided tissue and bone regeneration by protecting the bone site from surrounding soft tissues, promoting bone cell growth, and preventing infiltration by undesired cells. This helps manage bone defects and accelerates healing in a controlled way(50). These membranes are classified as absorbable or nonabsorbable based on their degradation properties. Nonabsorbable types include titanium mesh and various forms of polytetrafluoroethylene (ePTFE, high-density PTFE, titanium-reinforced PTFE), while absorbable membranes include polymeric, collagen, pericardium membranes, platelet-rich fibrin, and acellular dermal matrix(51).



6. Socket preservation techniques:

To minimize alveolar bone loss to an acceptable extent, various surgical approaches have been suggested, including minimizing extraction-related trauma and restricting flap elevation(38). Socket preservation typically involves the following procedures:

A. Immediate implants:

Dental implant placement is typically scheduled between 6 and 12 months following tooth extraction to ensure full maturation and healing of the alveolar socket. Nevertheless, during this period, alterations in the size and morphology of the alveolar bone can occur, potentially complicating implant placement and affecting treatment outcomes (39). To avoid these changes, Paolantonio *et al.* (40) proposed immediate implantation. Despite this, studies have reported inconsistent results. For instance, Velasco *et al.* (41) found an estimated reduction of about 2.6 mm in bone three months after immediate implant placement, and also observed initial bone-to-implant contact one month post-extraction, followed by buccal wall resorption and partial loss of contact by the third month (42). Mao *et al.* (43) confirmed that immediate loading does not alter the extent of buccal bone resorption. Nevertheless, using bone graft materials—either with or without immediate implantation—has been shown to reduce horizontal bone changes, especially within the coronal segment of the buccal alveolar plate, when contrasted with spontaneous or natural healing processes. Therefore, combining immediate implant placement with alveolar ridge preservation techniques, such as bone grafting, may be an effective strategy to limit modifications in osseous tissue structure and shorten the total duration of the therapeutic process(44).



for this effect not yet being fully elucidated(32). Liao *et al.* (33). Demonstrated that hyperbaric oxygen therapy not only enhances the management of jaw osteoradionecrosis but also contributes to extraction socket healing and preservation of the alveolar ridge by reducing post-extraction alveolar bone resorption. This effect is achieved by promoting osteoblast formation and suppressing osteoclast activity. Therefore, they suggest that this approach could be applied clinically to accelerate alveolar socket healing and support ridge preservation in human patients.

The extent of tissue trauma caused by the surgeon during tooth extraction and the size of the resulting bone defect should also be considered, as these variables have the potential to significantly impact the regenerative outcome of alveolar socket healing. Under these circumstances, bone resorption tends to be more pronounced compared to less invasive cases (34).

5. Socket preservation

Extraction socket preservation (ESP) is a technique performed immediately after tooth extraction to minimize soft tissue and bone loss. Studies have shown that ESP helps maintain greater bone dimensions compared to sites where no preservation is done (35). Without restoration, extraction can lead to disuse ridge atrophy and significant vertical and horizontal bone loss, often requiring additional grafting procedures for successful implant placement (36). ESP allows for the placement of wider and longer implant fixtures and reduces the need for bone augmentation during implantation (37). However, ESP may not be necessary in all cases, and the decision should be based on the condition of the socket and surrounding tissues at the time of extraction(36).



prevalence rate of alveolar osteitis was 13.2% among smokers, compared to just 3.2% in non-smokers (25). Research on the harmful components of cigarette smoke, especially nicotine and carbon monoxide, indicates possible ways in which smoking could hinder wound healing (26). Nicotine causes vasoconstriction, which results in hypoxia, thereby obstructing tissue repair by limiting the availability of oxygen that is vital for cell movement, collagen formation, and immune function(25). Likewise, carbon monoxide worsens hypoxia by binding to hemoglobin more effectively than oxygen, which further diminishes oxygen supply to tissues(26). There is evidence indicating that osteoporosis in postmenopausal women may lead to a reduction in the residual ridge after tooth extraction, which can result in a mandibular ridge that appears knife-edge and a diminished maxillary alveolar ridge(23).

Likewise, Shahen *et al.* (27) observed that patients with poorly controlled insulin-dependent diabetes exhibited heightened alveolar bone resorption during socket healing, which was associated with diminished synthesis of the collagen matrix. Although patients with uncontrolled type 2 diabetes often experience poor socket healing, the application of hyaluronic acid to the socket after extraction may offer therapeutic benefits (28). Hyaluronic acid has been shown to play a beneficial role in the initial stages of post-extraction socket healing by enhancing fibroblast migration, proliferation, and survival, as well as promoting osteogenic differentiation. Additionally, it upregulates the expression of key wound healing-related genes, such as type III collagen and transforming growth factor- β 3(29-31). Furthermore, research has indicated that post-extraction application of mouthwashes containing chlorhexidine may contribute to a reduction in alveolar bone loss, despite the precise biological mechanisms responsible



(iatrogenic) factors, localized anatomical or pathological influences, and environmental determinants.

Among the local determinants influencing socket healing, the anatomical location of the extraction site plays a significant role. Mandibular molar regions have been associated with the most inconsistent and irregular healing patterns, whereas the maxillary incisor and canine areas tend to exhibit the most favorable and predictable healing responses(19). In a retrospective analysis, Pramstraller et al.(20) reported that extraction sites in the molar region experienced more substantial bone loss compared to premolar areas. They suggested that this discrepancy may be due to the increased complexity involved in extracting posterior teeth, which typically results in a broader post-extraction socket. Furthermore, the elevation of the entire muco-periosteal flap prior to tooth removal was identified as an additional local factor potentially affecting alveolar socket healing. This procedure has the potential to contribute to bone resorption, as it has been demonstrated to promote periodontal attachment loss and impair vascular supply to the regenerating socket, thereby negatively impacting the healing process(21). Moreover, studies have indicated that multiple adjacent tooth extractions tend to result in more extensive alveolar bone resorption compared to isolated single-tooth extraction sites(18).

The molecular and cellular mechanisms underlying post-extraction socket healing can be significantly influenced by systemic factors, with smoking being a notable contributor to impaired regenerative processes(22), uncontrolled systemic diseases such as post-menopausal osteoporosis(23), and diabetes mellitus(24). The specific mechanism by which smoking impacts socket healing remains unclear, but Kuśnierek *et al.* reported that the



extraction is thought to be associated with heightened osteoclastic activity during the early phases of alveolar bone modeling and remodeling(14). Schropp *et al.* carried out a forward-looking investigation assessing alterations in both osseous and soft tissues following tooth extraction, employing clinical assessments and radiographic analysis. Their findings revealed that nearly half of the buccopalatal ridge width was lost within the first three months of the healing process(9, 15). A systematic review encompassing 12 studies evaluated dimensional changes during the post-extraction healing phase and reported a mean horizontal reduction of approximately 3.87 mm in ridge width. Additionally, vertical bone loss was observed, ranging from 1.67 to 2.03 mm clinically, and approximately 1.53 mm as measured radiographically(16). A detailed analysis conducted by Tan *et al.* (17) demonstrated that within six months following tooth extraction, vertical bone loss ranged from 11% to 22%, while horizontal dimensional reduction in ridge width varied between 29% and 63%. Resorptive activity was found to be more substantial on the buccal aspect of the alveolar bone compared to the palatal side, with the mandible exhibiting more significant changes than the maxilla. These alterations were most prominent in the molar region relative to the premolar area. As a result, the remodeling process of the alveolar ridge leads to a ridge that is both reduced in height and width, predominantly due to buccal bone resorption, ultimately causing a palatal displacement of the residual alveolar structure(18).

4. Factors that affect socket healing

The variability in alveolar socket healing outcomes can be attributed to a multitude of factors, including systemic conditions, clinician-induced



2. Socket healing

Tooth removal initiates a complex cascade of localized biological events involving both the alveolar bone and adjacent soft tissues. These processes are essential for promoting socket closure and reestablishing tissue homeostasis, collectively described as the phenomenon of socket healing(8). Alveolar healing progresses through distinct phases, beginning immediately after tooth extraction and extending over several months, typically up to six. Extensive research has shown that both modeling and remodeling of the alveolar bone can continue beyond the initial healing period, often lasting over a year post-extraction (9). Socket healing is influenced by a diverse array of determinants, encompassing local tissue conditions, systemic health status, clinician-related (iatrogenic) interventions, and external environmental factors(10). "Socket healing is typically associated with notable morphological changes in the alveolar bone, including a reduction in its dimensional volume and alterations in its structural configuration(11).

3. Morphological Alterations in Alveolar Bone Post-Socket Healing:

Following tooth extraction, remodeling affects all surfaces of the alveolar bone—including the buccal, palatal, mesial, and distal regions—resulting in dimensional alterations. The bundle bone, which is a tooth-associated component, has been identified as the initial site of resorption and plays a key role in the subsequent vertical reduction of the alveolar ridge(12). The most pronounced decrease in alveolar ridge dimensions occurs within the initial three months following tooth extraction, after which bone resorption persists at a more gradual rate over the individual's lifetime(13). The accelerated bone loss observed shortly after tooth



1. INTRODUCTION

Tooth extraction initiates a physiological remodeling process within the alveolar bone, characterized by a gradual loss in both crystal height and buccolingual width of the ridge. This dimensional reduction can significantly hinder the optimal placement, stability, and esthetic integration of prosthetic restorations, particularly endosseous dental implants (1). Socket preservation is an essential technique aimed at minimizing bone loss following tooth extraction. A range of materials, such as bone grafts, platelet-rich fibrin derivatives, and barrier membranes, are employed to enhance healing and preserve the natural shape of the alveolar ridge (2). The collagen plug, made from a biocompatible extracellular matrix, promotes osteoblast migration, helps stabilize the blood clot, improves soft tissue repair, and contributes to bone regeneration and the preservation of the alveolar ridge(3). Its porous architecture offers a scaffold that enables the migration and proliferation of cells such as fibroblasts and endothelial cells, which is essential for the development of new connective tissue and vascular networks(4). I-PRF is a novel autologous blood-derived biomaterial that remains in a liquid state while incorporating a fibrin meshwork resembling that of solid PRF. Unlike traditional PRP, it offers a unique combination of flowable consistency and regenerative potential, making it ideal for enhancing tissue healing(5). Injectable PRF retains its liquid form temporarily before coagulating, allowing for straightforward mixing with grafting materials or direct injection into the surgical site(6). This substance contains high concentrations of platelets, leukocytes, and essential growth factors such as PDGF, TGF- β , and VEGF, which play crucial roles in promoting angiogenesis, soft tissue regeneration, and osteogenesis(7).



المستخلص

تُحدث عملية خلع الأسنان سلسلة من التغيرات البيولوجية التي تؤدي إلى ارتشاف العظم السنخي، مما ينعكس سلباً على النتائج الجمالية والوظيفية لإعادة التأهيل السني المستقبلية، لا سيما في حالات زراعة الأسنان. وقد برزت استراتيجيات الحفاظ على تجويف الخلع كإجراءات مهمة تهدف إلى تقليل انهيار الحافة السنخية بعد الخلع. تستعرض هذه المراجعة التأثيرات التآزرية للفيبرين الغني بالصفائح الدموية القابل للحقن (I- PRF) وسدادات الكولاجين في تعزيز نتائج الحفاظ على تجويف السن. إذ تعمل سدادات الكولاجين كدعامات متوافقة حيويًا، تُسهّل عملية الإرقاء، وهجرة الخلايا، وتكوين الأوعية الدموية، كما تساهم في التئام الأنسجة الرخوة وتجديد العظم. وفي الوقت نفسه، يوفر الفيبرين الغني بالصفائح القابل للحقن —وهو مادة ذاتية المنشأ وغنية بعوامل النمو— مستودعًا ديناميكيًا يحتوي على الصفائح الدموية، والكريات البيضاء، وسيتوكينات رئيسية مثل عامل النمو المشتق من الصفائح الدموية (PDGF)، وعامل النمو المحوّل بيتا ($TGF-\beta$)، وعامل نمو بطانة الأوعية الدموية (VEGF)، التي تُسهم مجتمعةً في تسريع تجديد الأنسجة وتكوين الأوعية.

وتُتيح الصيغة القابلة للحقن تطبيقًا مرناً، سواء بشكل منفرد أو عند دمجها مع مواد التطعيم العظمي. تسلط هذه المراجعة الضوء على الأدلة الحالية المتعلقة بالآليات البيولوجية والفوائد السريرية لاستخدام I-PRF بالاقتران مع سدادات الكولاجين للحفاظ على تجويف الخلع، مما يُظهر إمكانيات واعدة في تحسين صيانة الحافة السنخية وتسريع الشفاء في مواقع الخلع.

الكلمات المفتاحية: الحفاظ على تجويف الخلع، أبعاد التجويف، فقدان العظم السنخي، سداة الكولاجين، مركز الصفائح الدموية القابل للحقن.



Abstract

Tooth extraction initiates a cascade of biological changes that result in alveolar bone resorption, compromising both the esthetic and functional outcomes of future dental rehabilitations, particularly implant placement. Socket preservation strategies have emerged as crucial interventions aimed at minimizing post-extraction ridge collapse. This review explores the synergistic effects of injectable platelet-rich fibrin (I-PRF) and collagen plugs in enhancing socket preservation outcomes. Collagen plugs serve as biocompatible scaffolds, promoting hemostasis, cellular migration, and angiogenesis, while supporting soft tissue healing and bone regeneration. Concurrently, I-PRF—an autologous, growth factor-rich biomaterial—provides a dynamic reservoir of platelets, leukocytes, and key cytokines such as Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-beta (TGF- β), and Vascular Endothelial Growth Factor (VEGF), which collectively accelerate tissue regeneration and vascularization. The injectable form allows for versatile application, either alone or in combination with grafting materials. This review highlights current evidence on the biological mechanisms and clinical benefits of combining I-PRF with collagen plugs for socket preservation, demonstrating promising potential for improved ridge maintenance and accelerated healing in post-extraction sites.

Keywords: socket preservation, socket dimensions, alveolar bone loss, collagen plug, injectable platelet concentrate.



The Impact of Utilizing Injectable Platelet-rich Fibrin and Collagen Plugs for Maintaining the Tooth Extraction Socket: A Review Study

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تأثير استخدام الفيبرين الغني بالصفائح الدموية القابل للحقن
وسدادات الكولاجين في الحفاظ على تجويف السن بعد الخلع:
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dentists for their daily work-life. There is a need to establish generally accepted digital standards of education—at least among the different dental universities within individual countries. Digitalization present the prospect to revolutionize the entire field of dental education which will arise that motivation and provide a stimulating, enjoyable, and meaningful educational experiment with proper outlet 24 h a day.



photos and files of any size. The other tool is concurrent/ asynchronous technique which is more advanced one; it requires the practitioners working on common tasks to remotely share computer display workspaces simultaneously, without leaving their workplaces^{32, 33}.

Future Sample Cooperation How will be?

Maxillofacial prosthesis cooperation effectiveness in many developing countries has been extended recently, sample of upcoming include pattern of effective network in perseverance sharing of data. This proposed model will simplify collaborative work on maxillofacial prosthetic different situations through real time high fineness digital information delivery and bring such data for sharing work table and enable navigation of its content³⁴.

The main goal of developing such future sample is to facilitate collaboration between different dental and maxillofacial clinic and centers to provide the required data concerning patients with maxillofacial defects by using various digital technologies; CT, digital photography, and 3D photogrammetry equipment to share information through effective network^{34, 35}. Prosthesis then can be designed and fabricated from 3D modeling and 3D RP technology, it would be possible to give some guidance and instructions during prosthetic insertion with many consultations³⁶.

Conclusion

Digital tools and applications are now widespread in routine dental care. Therefore, this trend towards digitization and outstanding developments must be considered in dental curricula generally and maxillofacial rehabilitation in specific way. In order to provide the new generation of



Rapid prototyping techniques have been used in digitized maxillofacial prosthetic production. The unique characteristic of RP is that, models and/or prostheses are produced by adding material from the main source layer by layer to form a 3D model in which all the layers are bound together.^{28, 29} physicians through this technique can get information about fine inner details of difficult structures and undercut areas so this will efficiently employ the production on facial prosthesis with practical steps²⁹.

Matrix using has takeoff the conventional process and makes prosthesis in shorter time and phases, this mold can be used for numerous prosthetic duplication. This long-term mold allow multiple pouring time³⁰.

Implication of Internet Multimedia

It's an application developed in relation to time importance, provide resolution to health-related problems using collective path. To achieve comprehensible communication there should be joining of high quality patient data shared over internet between many institutions at many places over world. In medical implication there is definite need for secure data and information partnership to explore different aspect of the case in real time manner, and that's sometimes inflexible due to different time zone^{7, 31}.

Real time high quality digital data submission is an important characteristic in maxillofacial prosthetic repair, and with all digital aided equipment still the voice communication is an essential achievement as source of references³¹.

Sharing patient's data need a secure database to preserve patient's details and information within working organized schedule; this include using of both video/audio internet based tool consist of sharing screens,



CAD CAM Technology for Prosthodontics

The rising of CAD CAM mechanisms has developed in prosthodontics and more chiefly in maxillofacial apparatus. Digital prosthetic reforming is becoming an important oncoming for maxillofacial prosthetic branch. Most recent maxillofacial studies converging on using the newly advanced technologies for making and designing these prosthetic appliances^{23, 24}. In digital maxillofacial prosthesis there are three important procedures to be considered; digitalization (visualization), designing and modeling, and making (processing). All these steps include using many forms of computer aids like intraoral scanner, optical scan system, computed tomography (CT), and magnetic resonance images (MRI)^{1, 25}.

The advantage of using such digitalizing systems is to get a clear photograph so the distortion that may occurs with the conventional impression techniques can be eliminated that data can be collected without need to touch the dental and facial tissues and consequently having successful maxillofacial models with tissue adaptation and good contour.²⁶ according to the type of data cons-questing, CT or MRI photos, and any other digital imaging modeling process will develop, and these data group will be an easily information exchange tool between employers. Standard transformation language (STL) data can be saved according to the American Standard Code for Information Interchange (ASCII) and binary.¹ designing process can use many special software programs for various maxillofacial prosthetic restorations which contain an inclusive tool to erode prosthesis anatomical details and distortions of virtual clay model into any form desired^{26, 27}. The final make-up is constantly available for user update, and can be documented for evaluation and recheck defect model and fitness before manufacturing.



eyelashes, some treatment options use eye glasses for fixation.^{18,19} nasal prosthesis are used to treat partial or total nasal defects and sometimes may even include rehabilitation of corresponding cheek and upper lip soft tissue disorders and this reflect the fact that nose is important as it locate in the center of face^{20,21}.

Auricular defect can be repaired by ocular appliance to give excellent aesthetic results^{10, 21}.

All these types of treatment and repair of maxillofacial prosthetics have been developed all over time, they registered an advance approach in such therapy procedures concerning many newly progressive techniques with less time consuming, highly specialized laboratory and clinical skills, less no. of patient clinical attending, and more experience for individual training²².

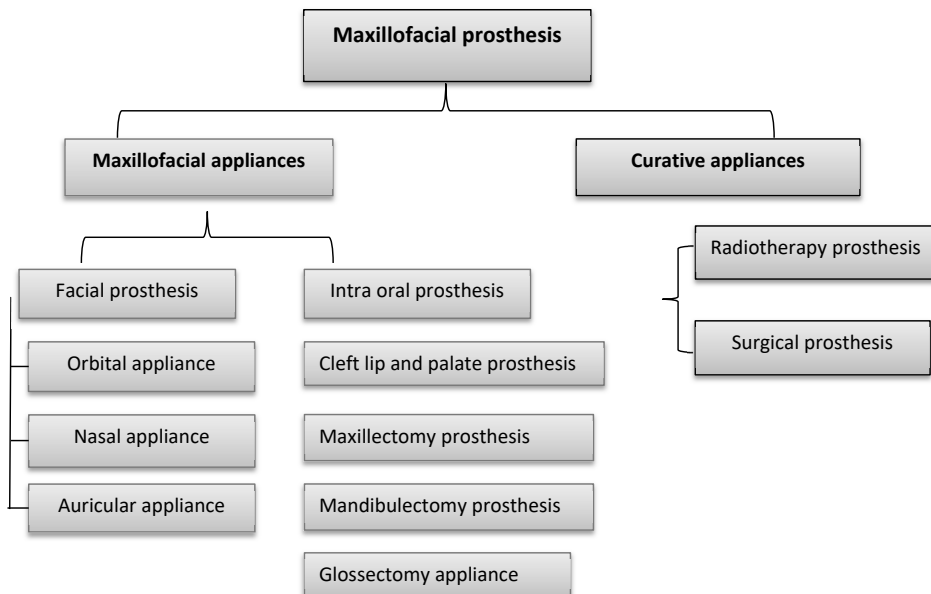


Fig.1 Descriptive chart representing maxillofacial prosthesis range.



Maxillofacial Prosthetics

Maxillofacial prosthesis is that department of prosthetics that attentive about replacement and rebuilding of craniofacial body with prosthetic appliance that considered as one of the confronts in the dental area which should deal with many deficiencies in head and neck parts whether congenital or acquired¹². The etiology of these defects is numerous according to its location and how it is behave in such area that may fellow a cancer, trauma, infection or burn causes and treatment will be according to treatment stage either repairing or curative devices such as surgical devices and radiological devices^{1, 13}. These treatment appliances can be divided into two main divisions; facial prosthesis which include any extra oral deficiency in the head and neck regions that contain orbital, nasal, ocular, and facial skin defects. The other group contains appliances that rehabilitate all intra oral defects including maxilla, mandible, and related soft tissue structures like tongue, soft palate and floor of mouth disorderes^{14, 15} Fig (1).

Mandibular defects can be reestablishing functionally and aesthetically by removable and/or fixed prosthetic appliances to fix the deviation in opening and closing, and related alteration in occlusion seen in the affected side. Sometimes such defects may involve tongue defects like glossectomy which apparently affect speech and swallowing^{1, 16}. Maxillary disorder can also be treated with such obturator prosthetic devices for either small and large defects and may include even palatal lift prosthesis in cases of soft palate defects^{16, 17}. For rehabilitation of facial defects include using of silicon material to restore them and even may include use of magnates or facial implants for more retention means; this covers all orbital defects with



Introduction

Repairing and reforming of maxillofacial prosthesis is aim to rehabilitate and regain aesthetic, speech and function to people having maxillofacial defects either by fixed or removable appliances. Though there is many variations in the range of maxillofacial prosthetic according to the defect size and location, it's still categorized as maxillofacial (maxillary and mandibular) prosthesis, while for facial prosthetic rehabilitation that includes (auricular, nasal, facial, ocular, and orbital prostheses as well as craniofacial implant production)¹⁻³. The traditional process for repairing maxillofacial prosthesis including taking an impression for the defect site, then proceeding to stone model and wax pattern to have the permanent final shape made of either acrylic or metal, with all these conventional work steps there is a considerable participation by human and material consumption and this showed a chance for errors, inexactness prosthesis, beside of time consuming, and additional cost⁴⁻⁶. The revolutionary effect of digital technologies in general and for maxillofacial prosthesis in specific has encouraged us to study and evaluate this fundamental path concerning facial prosthesis court^{4, 7}. Now a days in many developing countries the need for specialized centers which should be with unlimited expertise is essential to overcome the complications of maxillofacial prosthesis making and ensure that patient should have a proper prosthesis rehabilitation^{8, 9}. Accordingly involved human and submitting to the digitalized fabricating techniques which will facilitate and develop productivity of facial prosthesis^{8, 10, 11}. This article will display the role of digital system with participation of maxillofacial prosthesis to produce more satisfied future model by employing variable digital technologies.



المستخلص

تلعب التقنيات الرقمية دورًا هامًا في مجال طب الأسنان التعويضي، بشكل عام، وفي قسم طب أسنان الوجه والفكين بشكل خاص. وقد أصبح هذا النهج الثوري وسيلة مهمة لإعادة تأهيل مختلف الحالات في مجال طب الأسنان التعويضي. سنلخص في هذه المقالة العديد من محتويات أبحاث طب الأسنان، والبحوث على الإنترنت، وقواعد البيانات الإلكترونية، ومناقشات الخبراء والأطباء مع أحدث المنشورات والمنتجات الجديدة، ونقيّمها باستخدام تقنيات تصميم وتصنيع أطقم الأسنان للوجه والفكين (CAD/CAM) ومع تطور طب الأسنان الرقمي، الذي ساهم في التغلب على صعوبات بناء أطقم الأسنان ومحدودية الخبرة في المراكز المتخصصة، حيث أن مرضى عيوب الوجه والفكين يحتاجون إلى إعادة تأهيل أطقم الأسنان بشكل مناسب. لقد اظهرت نماذج أطقم الأسنان الوجه والفكين المصممة رقميًا العديد من المزايا المتعلقة بالجمال، والثبات، وسهولة الاستخدام، كما أنها أكثر قبولًا لدى المرضى.

الكلمات المفتاحية: طب اسنان الوجه والفكين، طب الأسنان الرقمي، أطقم الأسنان التعويضية، تقنية الحاسوب (CAD/CAM)



Abstract

Digital technologies play a great role in prosthodontics generally and specifically in maxillofacial prosthetics, this revolutionary approach has become an important way for rehabilitation of different modalities in maxillofacial prosthetic field. In this article we will summarize many contents of dental researches, web researches, on-line data bases, discussions of experts and clinicians with the most recent publications and new products releases and evaluate them. Describing the techniques in designing and fabrication for facial prosthesis applying computer aided method and manufacturing (CAD/CAM). As the development of digital dentistry took apart to overcome complexity of prosthesis construction and the limited expertise in specialized centers, maxillofacial defect patients seem to be deprived of appropriate prosthetic rehabilitation. Digitally designed facial prosthesis models show many advantages related to aesthetic, retention, and ease of use and more acceptable by patients.

Keywords: Maxillofacial Dentistry, Digital Dentistry, Facial Prosthesis, CAD CAM



Uses of Digital Technologies for Maxillofacial Prosthesis Manufacturing Review article

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