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الجامعة للعلوم الطبية

مجلة علمية محكمة تصدر عن جامعة الإسراء

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بسم الله الرحمن الرحيم

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
دائرة البحث والتطوير

Republic of Iraq
Ministry of Higher Education & Scientific Research
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Date:

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 أعتماد المجلة المذكورة في الترقيات العلمية والنشاطات العلمية المختلفة الاخرى وتسجيل المجلة في موقع المجلات الاكاديمية العلمية العراقية ، وحسب ما جاء بأعمامنا المرقم ب ت ١٠٩٨٨/٤ في ٢٠١٩/١١/٢٤ **(تقرر اعطاء موافقة مؤقتة لمدة ٦ أشهر على ان يتم تزويدنا بالرقم المعياري الدولي المطبوع والالكتروني وانشاء موقع الكتروني للمجلة وبخلافة تلغى الموافقه وأعلامنا الاجراءات لاحقاً).**

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

السيد المدير العام المحترم

للتفضل بالتوقيع مع التقدير

د.هناء /المعاون

أ.د. غسان حميد عبدالمجيد
المدير العام لدائرة البحث والتطوير
٢٠٢١/٣/ ١

نسخة منه الى:

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

مهند ، أنس
٢٤/ شباط

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 يبين فيها 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 والايسر.
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 إنجازه أكثر من أربع سنوات.

1. يجب أن يكون عنوان البحث موجزاً قدر الإمكان ومعبر 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.Us) 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 وهادف 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 المرسلة 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 و**متبوعا بكلمات مفتاحية 4-6**.

دليل المقيّم Reviewer Guidelines

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

1. ملء استمارة التقويم المرسلة رفقة البحث المطلوب تقييمه بشكل دقيق وعدم ترك أي فقرة بدون إجابة.
2. على المقيّم التأكد 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 وحداثتها.
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 المرسلة له رفقة البحث المرسل له للتقييم.

المصادر

1. يشار إلى المصادر في متن البحث كما يلي:

اللقب او 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 والانتهاء 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 والانتهاء 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.

التوقيع :

التاريخ :

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Conclusion

According to the results of this study, some hematological and biochemical parameters were significantly different between the (HCC, HBV, and HCV) groups and the control group. One parameter can be used as a reference for each liver disease, including. MPV is the only element that is exclusive to "HCC" while MID is the only element that is exclusive to "HBV", and P-LCC is the only element that is unique to "HCV". Consequently, these parameters can be used as biomarkers for the diagnosis of these three liver diseases, allowing the differentiation of HCC, HBV, and HCV from the control group. In addition, the parameters GOT (AST) and indirect bilirubin have been found to be essential in diagnosing all three liver diseases, making them valuable indicators for the presence of a liver condition.

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and indicate liver function. In this study, the results are consistent with those in the previous studies. (19) In their study, they noted that these tests may aid in determining where damage is occurring in the liver and, depending on the pattern, may aid in establishing a differential diagnosis. Hepatocellular disease is indicated by elevations in ALT and AST that are disproportional to increases in alkaline phosphatase and bilirubin. A cholestatic pattern is characterized by elevated levels of alkaline phosphatase and bilirubin in comparison with ALT and AST. Liver function is determined by its ability to produce albumin and vitamin K-dependent clotting factors.

There was an increase in GOT (AST), total bilirubin, and indirect bilirubin levels in HCV patients. This study confirms the findings in the previous studies (20). In comparing the control group with the HCV +ve group, serum AST and ALT were statistically significantly higher in the HCV +ve group ($P<0.001$). In contrast, the prothrombin concentration was significantly lower in the HC +ve group ($P= 0.001$).

Comparing hematological and biochemical parameters between HCC, HBV, and HCV patients

From HCC, HBV, and HCV patients' blood samples, it is evident that the major hematological parameters are quite similar. In "HCC" one element is specifically included, in "HBV" one element is exclusively included, and in "HCV" one element is exclusively included. In contrast, blood samples from HCC, HBV, and HCV share significant biochemical parameters. "HCC", "HBV", and "HCV" share two common elements: GOT (AST), and indirect bilirubin. In addition, "HBV" and "HCV" share two elements: GPT (ALT), and Alkaline Phosphatase. Total bilirubin, however, is exclusive to "HCV."

observed among CHB subjects compared to healthy controls. Furthermore, there were no significant changes in the total WBC, granulocytes, RBCs, PCV, hemoglobin concentration, and platelet count. In the previous studies (17). found that WBC, RBC, PLT, and MIX% in hepatitis B patients were significantly higher than in controls ($p< 0.05$). A number of hematological parameters (HPs) were significant, such as HCT and WBC, HB and RBC with PLT, as well as RBC, HCT, and PLT with WBC.

In HCV patients, the majority of the hematology parameters examined changed significantly, including LYM%, LYM, RBC, HGB, HCT, MCV, MCHC, PLT, PCT, P-LCR, and P-LCC. The results of this study are in agreement with those of Rasheed and colleagues in 2022 (15). Among hepatitis C patients, they found that hepatitis C patients had statistically significant differences in hemoglobin, platelets, white blood cells, HCT, neutrophils, and neutrophil/lymphocyte ratios (NLR). A similar pattern was observed for RBCs, MCVs, MCHs, MCHCs, lymphocytes, monocytes, and eosinophils. The peripheral hematological parameters of patients with HCV may serve as a valuable biomarker for HCV diagnosis.

HCC, HBV, and HCV biochemistry parameters

HCC patients had abnormal levels of indirect blood bilirubin, creatinine, and GOT (AST). This study confirms the findings in the previous studies (18), which found that serum bilirubin levels (total, direct, and indirect) and the presence of amyloid particles are higher in men than in women with HCC.

A high level of Indirect Bilirubin, GPT (ALT), GOT (AST), and Alkaline Phosphatase is present in HBV patients. Liver function tests are blood tests that diagnose liver disorders, detect inflammation, and prevent liver damage,

mean age of 30-40 years within the group of 30-40-year-olds. This result is in line with previous findings in the previous studies (14). Most acute cases of hepatitis B occur among those 15-44 years old, while the majority of chronic cases occur among those 30-44 years old. From 2012 to 2018, chronic hepatitis B incidence rates increased in those over 45 but decreased differently in others. Men had a higher incidence rate than women.

The hematological parameters of HCC, HBV, and HCV patients

There were significant abnormalities in several parameters in patients with HCC, including Granulocytes (GRAN), Platelet Distribution Width (PDW), and Platelet Concentration (PCT). As compared with the control group, red blood cell counts (RBC), hemoglobin levels (HGB), hemoglobin saturation (HS), platelet counts (PLT), and mean platelet volume (MPV) were significantly altered. The results of this study are in agreement in the previous studies (15). Among hepatitis C patients, they found that hepatitis C patients had statistically significant differences in hemoglobin, platelets, white blood cells, HCT, neutrophils, and neutrophil/lymphocyte ratios (NLR). A similar pattern was observed for RBCs, MCVs, MCHs, MCHCs, lymphocytes, monocytes, and eosinophils. The peripheral hematological parameters of patients with HCV may serve as a valuable biomarker for HCV diagnosis.

Patients with HBV also had abnormal levels of lymphocytes (LYM), red blood cells (RBCs), hemoglobin (HGB), and platelet-large cell ratio (P-LCR) (p0.001). According to our study, some of the results are in agreement the previous studies (16). Based on their study, patients with CHB have significantly higher monocyte counts and lower lymphocyte counts than healthy controls. A significant decrease in CD4+ T cell counts was also

Discussion

The complete blood count (CBC) with differential leukocyte count is one of the most requested blood tests in medical laboratories. A CBC test can be used to detect anemia and leukemia, as well as to assess general health. The test measures three types of blood cells: red blood cells (RBC, HCT, HB, erythrocyte indices), white blood cells (numbers and differential counts), and platelets. The results of the CBC test provide vital data for early diagnosis of a number of diseases, as well as for monitoring the progress of treatment and assessing overall health. Therefore, we analyzed the blood and biochemical test results of patients with HCC, HBV, and HCV liver disease to identify the most significant and co-related parameters. According to the results, some hematological and biochemical parameters significantly changed from their respective levels in the control group as compared to the (HCC, HBV, and HCV) groups.

Distribution of ages

Age is associated with an increased risk of hepatocellular carcinoma. HCC cases were most prevalent among patients aged 51 to 60. In people over 44 years of age, hepatocellular carcinoma is more likely to occur. In addition, HCV incidence rates are strongly correlated with age and gender, particularly among younger males and females. Between 21 and 30 years of age, incidence rates increased for both males and females. People over 44 are more likely to develop hepatocellular carcinoma, according in the previous studies (12). Furthermore, in 2020, acute hepatitis C cases were high among those aged 20 to 39. In the United States, overdoses and injection drug use are most prevalent at this age (13). HBV patients aged 30-40 had a

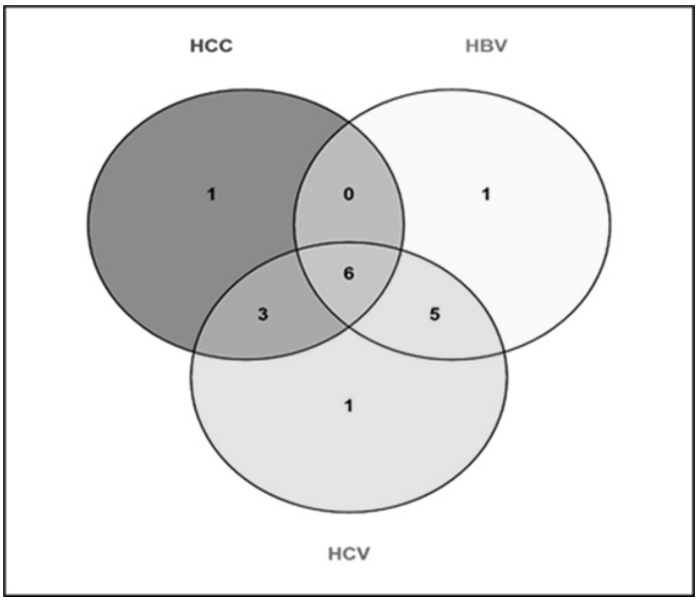

Figure 1. Venn diagram showing commonalities between the different significant hematological parameters between HCC, HBV, and HCV patient blood samples.

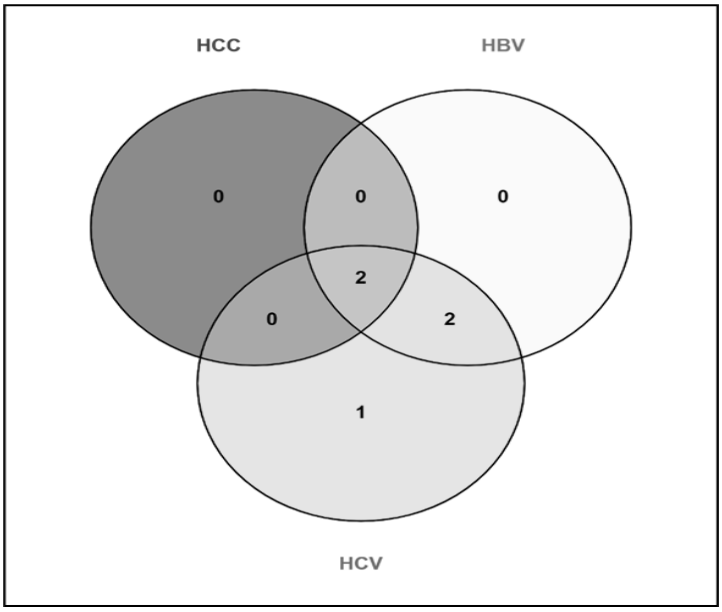

Figure 2. Venn diagram showing the significant biochemical parameters shared by blood samples from patients with HCC, HBV, and HCV.

Table 7. Indicates the correlation coefficient between biochemistry parameters related to HCV.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
Blood Sugar	10(10)	100%	0(10)	0.00%	10	0.993
Urea	9(10)	90.00%	1(10)	10.00%	10	0.945
Creatinine	5(10)	50.00%	5(10)	50.00%	10	0.0322*
GPT (ALT)	2(10)	20.00%	8(10)	80%	10	0.00714**
GOT(AST)	0(10)	0.00%	10(10)	100%	10	0.001***
Alkaline Phosphatase	1(10)	10.00%	9(10)	90.00%	10	0.002**
Total Bilirubin	0(10)	0.00%	10(10)	100%	10	0.001***
Indirect Bilirubin	0(10)	0.00%	10(10)	100%	10	0.001***
Chi Square	P = 2.805×10-9					

3.9 Comparison of common parameters from hematology and biochemistry tests among different samples of HCC, HBV, and HCV patients

Venny2.0, an interactive tool for comparing lists with Venn's diagram, was used to analyze the data generated from previous Tables. Based on the Venn diagram in Figure (1), it can be seen that the major hematological parameters are quite similar between blood samples from patients suffering from HCC, HBV, and HCV.

Alternatively, Figure (2) shows that blood samples from patients with HCC, HBV, and HCV share significant biochemical parameters.

Table 6. Correlation between biochemistry parameters and HBV infection.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
Blood Sugar	10(10)	100%	0(10)	0.00%	10	0.993
Urea	7(10)	70.00%	3(10)	30.00%	10	0.065
Creatinine	5(10)	50.00%	5(10)	50.00%	10	0.0322*
GPT (ALT)	0((10)	0.00%	10(10)	100%	10	0.001***
GOT(AST)	1(10)	10.00%	9(10)	90.00%	10	0.002**
Alkaline Phosphatase	0((10)	0.00%	10(10)	100%	10	0.001***
Total Bilirubin	9((10)	90.00%	1(10)	10%	10	0.945
Indirect Bilirubin	0((10)	0.00%	10(10)	100%	10	0.001***
Chi Square	P = 3.806×10-9					

3.8 Analysis of the biochemistry of patients with HCV

According to Table (7), HCV patients have elevated levels of, GOT (AST), Total Bilirubin, and Indirect Bilirubin. A normal ratio of (0.00%) and abnormal (100%) with a correlated significant P value (P=0.001). The normal percentage of GPT(ALT) is 20.00 percent and the abnormal percentage is 80.00 percent, and the normal levels of Alkaline Phosphatase are 10.00 percent and the abnormal percentage is 90.00 percent.

Table 5. Statistical correlation between biochemistry parameters of HCC.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
Blood Sugar	17(24)	70.83%	7(24)	29.17%	24	0.793
Urea	20(24)	83.33%	4(24)	16.66%	24	0.562
Creatinine	14(24)	58.33%	10(24)	41.67%	24	0.0422*
GPT (ALT)	22(24)	91.67%	2(24)	8.33%	24	0.792
GOT(AST)	14(24)	58.33%	10(24)	41.67%	24	0.0422*
Alkaline Phosphatase	19(24)	79.17%	5(24)	20.83%	24	0.913
Total Bilirubin	18(24)	90.00%	6(24)	24%	24	0.845
Indirect Bilirubin	2(24)	8.33%	22(24)	91.67%	24	0.001***
Chi Square	P = 1.597x10-9					

3.7 Biochemistry parameters in patients with HBV

As shown in Tables (6), HBV patients have elevated levels of Indirect Bilirubin, GPT (ALT), GOT (AST), and Alkaline Phosphatase as compared to normal individuals with highly significant p values ($p \leq 0.001$). GPT (ALT), Alkaline Phosphatase, and Indirect Bilirubin are normal (0.00%) and abnormal (100%), while GOT (AST) is normal (10.00%) and abnormal (90.00%). In contrast, there was no significant correlation between other variables. These tests are all indicators of liver function. Liver function tests are a group of blood tests that aid in diagnosing liver disorders, detecting inflammation, and preventing liver damage. Chi Square P = 3.806×10-9

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
MCV	0(10)	0.00%	10(10)	100%	10	0.001***
MCH	1(10)	10.00%	9(10)	90.00%	10	0.002**
MCHC	0(10)	0.00%	10(10)	100%	10	0.001***
RDW-CV	1(10)	10.00%	9(10)	90.00%	10	0.002**
RDW-SD	3(10)	30.00%	7(10)	70.00%	10	0.0214*
PLT	0(10)	0.00%	10(10)	100%	10	0.001***
MPV	8(10)	80.00%	2(10)	20.00%	10	0.203
PDW	3(10)	30.00%	7(10)	70.00%	10	0.0214*
PCT	0(10)	0.00%	10(10)	100%	10	0.001***
P-LCR	0(10)	0.00%	10(10)	100%	10	0.001***
P-LCC	0(10)	0.00%	10(10)	100%	10	0.001***
MID%	9(10)	90.00%	1(10)	10.00%	10	0.945
MID#	9(10)	90.00%	1(10)	10.00%	10	0.945
Chi Square	P = 3.858x10-15					

3.6 Biochemistry parameters in patients with HCC

Table (5) shows that the distributions of indirect bilirubin normal (8.33%) and abnormal (91.67%) in HCC patients were highly significant (P=0.001). Additionally, there was a significant correlation between Creatinine levels that were normal (58.33%) and abnormal (41.67%), as well as GOT (AST) levels that were normal (58.33%) and abnormal (41.67%). Other parameters, however, did not show a significant correlation.

3.5 Hematological parameters in patients with HCV

Most of the hematology parameters that were examined in this study changed significantly, including LYM%, LYM, RBC, HGB, HCT, MCV, MCHC, PLT, PCT, P-LCR, and P-LCC. When compared with respective normal ranges, the percentage of normal patients was (0.00%) and the percentage of abnormal patients was (100%) at (P=0.001). Additionally, a significant change was observed in MCH and RDW-CV, both of which were normal (10.00%) and abnormal (90.00%) in correlation (P=0.002). Furthermore, significant modifications were observed in the percentages of GRAN with normal ratios of (40.00%) and abnormal ratios of (60.00%), in GRAN# normal (60.00%) and abnormal (40.00%), in RDW-SD normal (30.00%) and abnormal (70.00%), and in PDW normal (30.00%) abnormal (70.00%) with a correlation of p value (p = 0.05). However, there was no significant correlation between other parameters. The following Table (4) illustrates the above-mentioned results (6).

Table 4. An analysis of the correlation coefficient between hematological parameters and HCV infection.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
W.B.C	7(10)	70.00%	3(10)	30.00%	10	0.065
LYM%	0(10)	0.00%	10(10)	100%	10	0.001***
GRAN%	4(10)	40.00%	6(10)	60.00%	10	0.0254*
LYM#	0((10)	0.00%	10(10)	100%	10	0.001***
GRAN#	6(10)	60.00%	4(10)	40.00%	10	0.0473*
RBC	0((10)	0.00%	10(10)	100%	10	0.001***
HGB	0((10)	0.00%	10(10)	100%	10	0.001***
HCT	0(10)	0.00%	10(10)	100%	10	0.001***

Table 3. Statistical correlation between hematological HBV parameters.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
W.B.C	7(10)	70.00%	3(10)	30.00%	10	0.065
LYM%	7(10)	70.00%	3(10)	30.00%	10	0.065
GRAN%	8(10)	80.00%	2(10)	20.00%	10	0.203
LYM#	0((10)	0.00%	10(10)	100%	10	0.001***
GRAN#	7(10)	70.00%	3(10)	30.00%	10	0.065
RBC	0((10)	0.00%	10(10)	100%	10	0.001***
HGB	0((10)	0.00%	10(10)	100%	10	0.001***
HCT	10(10)	100%	0(10)	0.00%	10	0.993
MCV	5(10)	50.00%	5(10)	50.00%	10	0.0322*
MCH	6(10)	60.00%	4(10)	40.00%	10	0.0473*
MCHC	5(10)	50.00%	5(10)	50.00%	10	0.0322*
RDW-CV	6(10)	60.00%	4(10)	40.00%	10	0.0473*
RDW-SD	5(10)	50.00%	5(10)	50.00%	10	0.0322*
PLT	6(10)	60.00%	4(10)	40.00%	10	0.0473*
MPV	8(10)	80.00%	2(10)	20.00%	10	0.203
PDW	10(10)	100%	0(10)	0.00%	10	0.993
PCT	4(10)	40.00%	6(10)	60.00%	10	0.0254*
P-LCR	1(10)	10.00%	9(10)	90.00%	10	0.002**
P-LCC	7(10)	70.00%	3(10)	30.00%	10	0.065
MID%	7(10)	70.00%	3(10)	30.00%	10	0.065
MID#	2(10)	20.00%	8(10)	80.00%	10	0.00714**
Chi Square	P = 3.806×10-9					

3.4 Hematological parameters among patients with HBV

It was found that several parameters, including lymphocytes (LYM), red blood cells (RBCs), hemoglobin (HGB), and Platelet-large cell ratio (P-LCR) were significantly abnormal compared with their respective control levels ($p \leq 0.001$). The percentages of normal and abnormal hematology distributions of HBV patients are also presented in Table (3). Participants with HBV had significantly abnormal lymphocyte ratios (100%) and normal (00.00%), abnormal RBC ratios (100%) and normal (0.00%), and abnormal HGB ratios (100%) and normal (0.00%), as well as P-LCRs that were normal (10.00%) and abnormal (90.00%) in significant correlation (0.002).

A significant correlation was found (p 0.05) between mean corpuscular volume (MCV) normal (50.00%) and abnormal (50.00%), and mean corpuscular hemoglobin (MCH) normal (60.00%) and abnormal (40.00%) in participants with HBV. Furthermore, the mean corpuscular hemoglobin concentration (MCHC) is normal and abnormal (50.00%), the Red Blood Cell Distribution Width CV (RDW-CV) is normal and abnormal (40.00%), the Red Cell Distribution Width (RDW-SD) is normal and abnormal (50.00%), the PLT is normal (60%) and abnormal (40.00%), the minimum inhibitory dilution (MID) is normal (20.00%) and abnormal (80.00%), and the procalcitonin level in the blood (PCT) is normal (40.00%) and abnormal (60.00%). However, no significant correlation was found with any other parameters.

Table 2. Statistical correlation between hematological parameters of HCC.

Analysis Name	Normal	Percentage%	Abnormal	Percentage%	Total	P
W.B.C	19(24)	79.17%	5(24)	20.833%	24	0.431
LYM%	18(24)	75.00%	6(24)	25.00%	24	0.362
GRAN%	18(24)	75.00%	6(24)	25.00%	24	0.362
LYM#	18(24)	75.00%	6(24)	25.00%	24	0.362
GRAN#	0(24)	00.00%	24(24)	100%	24	0.001***
RBC	11((24)	45.833%	13(24)	54.17%	24	0.0491 *
HGB	9(24)	37.5%	15(24)	62.5%	24	0.0463*
HCT	9(24)	37.5%	15(24)	62.5%	24	0.0423*
MCV	14(24)	58.33%	10(24)	41.66%	24	0.0831
MCH	16(24)	66.66%	8(24)	33.33%	24	0.6473
MCHC	8(24)	33.33%	16(24)	66.66%	24	0. 384
RDW-CV	5(24)	20.833%	19(24)	79.17%	24	0.0233*
RDW-SD	7(24)	29.83%	17(24)	70.17%	24	0.0322*
PLT	10(24)	41.33%	14(24)	58.66%	24	0.0373*
MPV	12(24)	50.00%	12(24)	50.00%	24	0.0482*
PDW	1(24)	4.17%	23(24)	95.83%	24	0.001***
PCT	0(24)	0.00%	24(24)	100%	24	0.001***
P-LCR	17(24)	70.83%	7(24)	29.17%	24	0.786
P-LCC	16(24)	66.66%	8(24)	33.33%	24	0.647
MID%	23(24)	95.83%	1(24)	4.16%	24	0.895
MID#	24(24)	100%	0(24)	80.00%	24	0.9998
Chi Square P = 7.006×10-25						

3.3 Hematological parameters among patients with HCC

Results indicate that several parameters, including Granulocytes (GRAN), Platelet Distribution Width (PDW), and Platelet Concentration (PCT), were significantly abnormal compared to the respective control levels ($p \leq 0.001$). The levels of Red Blood Cells (RBC), Hemoglobin (HGB), and Hematocrit (HCT) as well as the red blood cell distribution width (RDW- CV), (RDW- SD), Platelet Count (PLT), and Mean Platelet Volume (MPV) were significantly altered compared with the control group ($p<0.05$).

Table (2) illustrates the Hematological parameters and correlation coefficients and normal values. Furthermore, the percentage of normal and abnormal hematology distributions of HCC patients according to normal levels is also provided. It was noted that participants with HCC showed a significant correlation between the ratio of GRAN normal (00.00%) and abnormal (100%), PDW normal (4.17%) and abnormal (95.83%), and PCT normal (0.00%) and abnormal (100%). In addition, a significant correlation ($p<0.05$) has been found between RBC normal (45.333%) and abnormal (54.17%), HGB normal (37.5%) and abnormal (62.5%), HCT normal (37.5%) and abnormal (62.5%), RDW-CV normal (20.833%) abnormal (79.17%), RDW-SD normal (29.83%) and abnormal (70.17%), PLT normal (41.33%) and abnormal (58.66%) and MPV normal (50.00%) and abnormal (50.00%). Other parameters, however, did not show a significant correlation.

3. Results

3.1 Distribution of the gender

A total of twenty-four patients with HCC were studied, including four samples of patients who had not been treated with HCC. Six of the patients were males and 18 were females. Twenty subjects with recurrent HBV were also included, ten males and ten females. A total of 20 subjects with recurrent HCV were included in the study, 10 of whom were males and 10 of whom were females. In addition, twenty-four healthy subjects were included as apparently control subjects, including ten males and fourteen females.

3.2 Distribution of age

According to this study, hepatocellular carcinoma risk increases with age. Based on the results illustrated in Table (1), the mean age at diagnosis was 43 years, ranging from 20 to 70 years. It was found that patients between 51 and 60 had the highest number of HCC cases. Hepatocellular carcinoma is more likely to occur in people over 44 years of age. HCV incidence rates were strongly related to age and gender, with the highest rates observed among younger men and women. Both males and females experienced a gradual increase in incidence rates between 21 and 30.

Table 1. Distribution of HCC, HVC, HBV and apparently healthy control subjects by age group.

Group	HCC	HVC	HBC	Control
10-20	1	2	-	1
21-30	1	6	4	4
31-40	-	2	4	6
41-50	6	6	8	6
51-60	8	4	4	2
61-70	7	-	-	3
71-80	1	-	-	2

2.2 Specimen collection (Blood Samples Collection)

Each patient had five milliliters of blood taken by venipuncture using disposable syringes at Medical City Hospital (Oncology Teaching Hospital, Gastroenterology and Hepatology Teaching) Hospital and Al-Yarmook Teaching Hospital in Baghdad and apparently healthy control people obtained at various places.

A CBC test was performed by adding 2ml of blood to an EDTA tube K3 and mixing for 2 minutes to anticoagulant, then analyzing the samples using the CBC 3DIF device (Mindry).

The Spectrophotometer was used for the Biochemistry test to measure Blood sugar, Urea, Creatinine, Alkaline Phosphate, and Total Bilirubin. Biosystem devices, however, were used for GPT and GOT.

2.3 Statistical analysis

The statistical was performed software R package (10) and used Student's T-test evaluated the relation between normal and abnormal results for each analysis to get P value, and the Chi square test to get the percentage to normal, abnormal from the study sample to compare categorical data. Independent sample t-tests were used to compare two means, while one-way analysis of variance (ANOVA) was used to compare more than two means. The p-value was used ≤0.05.

In addition, Venny2.0, an interactive tool for comparing lists with Venn's diagrams, was used to analyze the data generated from previous tables (11).

Furthermore, a biochemical profile involves a series of blood tests that assess the function of several critical organs and systems. In addition to a complete blood count (CBC), tests can also be conducted on these organs and systems. Biochemical profiles can detect the presence of kidney disease, liver disease, diabetes, and other metabolic disorders. Additionally, they can be used to monitor the effectiveness of treatments and predict potential health risks (9).

Therefore, we analyzed the blood and biochemical test results of patients with HCC, HBV, and HCV liver disease to identify the most significant and co-related parameters.

2. Materials and methods

2.1 The collection of samples

64 blood samples were collected for this study, including 20 samples from individuals with HCC (under treatment), 4 samples from individuals before treatment, 10 samples from individuals with HBV, 10 samples from individuals with HCV, and 20 samples from healthy individuals. Patients were sampled after an oncologist diagnosed them with Hepatitis B and C infections and hepatocellular carcinoma.

Samples were collected from Baghdad hospitals provided samples, including the following:

1. Medical City Hospital (Oncology Teaching Hospital)
2. Medical City Hospital (Gastroenterology and Hepatology Teaching Hospital)
3. Al-Yarmook Teaching Hospital

Between November 2022 and April 2023.

Ethics committee approval was given by the Iraqi Ministry of Health.

1.Introduction

Various types of cancer can affect the liver. Hepatocellular carcinoma (HCC) is one of the most common types of liver cancer and a leading cause of cancer death in humans. HCC is highly aggressive and has a poor prognosis, making it a particularly dangerous form of cancer (1), (2). Cancer can form new tumor colonies as it migrates from other tumors to the liver, making it difficult to detect the disease throughout its course (3). HCC is caused by a variety of viral and non-viral diseases (4). HCC kills approximately two million people worldwide. As a result, the aggressive nature of HCC makes it particularly dangerous and hard to identify in its early stages (5).

The Hepatitis B Virus (HBV) is one of the most common and deadly diseases in the world and infects over 2 billion people. HBV accounts for up to 80% of all HCC cases, which are prevalent in Chinese and African populations (6). HBV infection persisting in liver tissue has been linked to cancer, inflammation of the liver, and chronic oxidative damage (7).

There is no doubt that the Hepatitis C Virus (HCV) is a major cause of HCC. HCV is associated with HCC, the fifth most common malignancy worldwide, accounting for 85 to 90% of all primary liver cancers. Despite the reduction in HCC risk with HCV treatment, patients with cirrhosis still face the risk of HCC. The prevention of HBV-associated HCC can also be achieved through immunization and antiviral therapy, but these methods are not widely available everywhere. To address HBV-related HCC effectively and affordably, further research is needed. (8).

As part of a routine health examination, a complete blood count, or CBC, is often performed. Complete blood counts detect a variety of disorders, such as infections, anemia, immune system diseases, and blood cancers.

المستخلص

يعد سرطان الخلايا الكبدية (HCC) ثاني أكثر أنواع السرطان شيوعًا بين البشر وخامس أكثر أنواع السرطان انتشارًا في جميع أنحاء العالم. على الرغم من أن معدل البقاء على قيد الحياة لسرطان الكبد قد زاد مع مرور الوقت، إلا أن تكرار الإصابة بعد العملية الجراحية يظل مرتفعًا بعد عقد من الزمن. يمكن أن يحدث سرطان الكبد بسبب أمراض فيروسية وغير فيروسية. هناك نوعان من عوامل الخطر العالمية الرئيسية لسرطان الكبد هما فيروس التهاب الكبد B (HBV) وفيروس التهاب الكبد C (HCV).

كجزء من الفحوصات الصحية الروتينية، يمكن لتعداد الدم الكامل اكتشاف مجموعة متنوعة من الاضطرابات، بما في ذلك العدوى وفقر الدم واضطرابات الجهاز المناعي وسرطانات الدم. يمكن استخدام تعداد الدم الكامل (CBC) لإجراء ملف كيميائي حيوي، والذي يقيم العديد من الأعضاء والأنظمة الحيوية. بالإضافة إلى ذلك، يمكن لفحص تعداد الدم الكامل اكتشاف اضطرابات خلايا الدم الحمراء والالتهابات وغيرها من الاضطرابات الأيضية. كان الهدف من هذه الدراسة هو تحديد أهم العوامل المرتبطة بأمراض الدم والكيمياء الحيوية بين المرضى الذين يعانون من سرطان الكبد، وفيروس التهاب الكبد الوبائي B، وفيروس التهاب الكبد الوبائي C.

أظهرت النتائج أن بعض المعايير الدموية والكيميائية الحيوية تغيرت بشكل ملحوظ في المجموعات الثلاث (HCC، HBV، وHCV) يمكن تقييم كل مرض كبدي باستخدام معامل تاثير واحد بما في ذلك (MPV لـ HCC)، و(MID لـ HBV)، و(P-LCC لـHCV) على الرغم من ذلك، هناك معاملان مشتركان في أمراض الكبد الثلاثة، وهما GOT (AST) والبيليروبين غير المباشر.

الكلمات المفتاحية: سرطان الخلايا الكبدية، فيروس التهاب الكبد B، فيروس التهاب الكبد C، أمراض الدم، الكيمياء الحيوية

Abstract

Hepatocellular carcinoma (HCC) is the second most common cancer in humans and the fifth most prevalent worldwide. Although the survival rate of HCC has increased over time, postoperative recurrences remain high after a decade. HCC can be caused by both viral and non-viral diseases. There are two major global risk factors for HCC these are: hepatitis B virus (HBV) and hepatitis C virus (HCV). As part of routine health exams, complete blood counts can detect a variety of disorders, including infections, anemia, immune system disorders, and blood cancers. A complete blood count (CBC) can be used to perform a biochemical profile, which evaluates several critical organs and systems. Additionally, the CBC can detect red blood cell disorders, inflammation, and other metabolic disorders. The aim of this study is to identify the most significant and co-related haematological and biochemical parameters among patients with HCC, HBV, and HCV. The results revealed that some haematological and biochemical parameters changed significantly in the three groups (HCC, HBV, and HCV). Each liver disease can be evaluated using a single parameter, including the MPV for HCC, the MID for HBV, and the P-LCC for HCV. Despite this, two parameters are common to all three liver diseases, namely GOT (AST) and indirect bilirubin.

Keyword: Hepatocellular carcinoma, HBV, HCV, Hematology, Biochemistry

Determination of the Hematological and Biochemical Parameters Associated with Liver Cancer, Hepatitis Types B, and C in Iraqi patients

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تحديد المعايير الدموية والبيوكيميائية المرتبطة بسرطان الكبد والتهاب الكبد النوعين C و B لدى المرضى العراقيين

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Jlassi agree with The current study, which showed correlation coefficients with CTLA4 expression at p value = < 0.01 [31]. Liu also showed patients a higher expression in CTLA4 and were positively correlated similar to the current study [32]. Egiz agree with present study which showed VEGF expression was correlated with broad metastasis in OC [33]. Ding showed similar results to current study in G1 and G2 of VEGF which found In OC case group, the high serum VEGF-A levels correlated significance at (p = 0.008) [34]. Raspollini agree with present study in G3 of VEGF which found VEGF were not correlated with responsiveness to chemotherapy [35].

5 - Conclusion

The study, showed the relationship between the immunological markers of the CTLA4 and VEGF and patient responses to Avastin and chemotherapy in (OC). Through analysis of studied groups and control group, we reached several significant conclusions. Firstly, The high significance difference of age, occupation, and family history as risk factors for ovarian cancer, Conversely, the lack of significant findings for residency and BMI suggests that these factors alone may not be sufficient indicators. Secondly, high significant difference in CTLA4 and VEGF between studied groups and control group. Thirdly, CTLA4 and VEGF high Sensitivity and Specificity in ROC and positive correlation between them in current study.

6 - Recommendations

We recommended a study of immunotherapy (anti-CTLA4) and effect on patients on different dose, study mutation causes (OC) and follow up for patients after and before dose.

infections, illnesses, and some types of cancer due to immune-senescence, a decrease in immunological function. A low immune system in elderly patients is one of the causes leading to ovarian cancer [19, 20]. Ilic agree with current finding when found not significance difference among the three BMI groups at (p = 0.3) in (OC) [21].while Beeghly-Fadiel disagree with the current study where found significant differences between BMI and ovarian cancer "each 5-unit increase in mean peri diagnosis" [22].Huang supported the present study in their studies they discovered a highly significant difference at (P value = 0.01) between CTLA4 and the usage of Avastin, which was highly significant difference [23]. Abodunrin and Silberstein agree with the present study which found in meta-analysis showed a significant difference at (p value = 0.02) indicating a significant benefit with Avastin [24].In the present study high sensitivity and specificity of CTLA4 in all groups with high significant difference at p value < 0.01 among OC patients, resemble to the study presented by James, which showed results similar to these findings [25]. Świderska agree with current study, that founded the serum CTLA4 concentration was high, the sensitivity was (70.3%) and the specificity was (90.7%) at (p = 0.000004) [26]. Maryam Agree with current finding, in G1 and G3 of VEGF, which found Sensitivity was 61.3% and specificity was 82.2% [27]. Faruq supported current result in G3 of VEGF the patients malignance (OC) with a best combination of specificity and sensitivity which gave with (93.5%) specificity, (90.1%) sensitivity as the value for identifying the malignance (OC) [28]. Trifanescu disagree with current finding which found VEGF to predict recurrence with 30% specificity [29]. Obermair disagree with present study, which found (ROC) curves shown the (VEGF) does not represent a beneficial tool for early diagnosis of (OC), sensitivity of (54%) and a specificity of (77%) [30].

4 - Discussion

The significant difference in the OC is more prevalent at an older age compared to the health control group. This could be due to various factors, including biological changes, risk factors that accumulate with age, or screening practices that identify the disease more often in older individuals. This study similar to other studies [11 and 12]. The no significant difference shown that residency (urban vs. rural) does not appear to be a distinguishing factor for ovarian cancer in this study. Both groups have a similar distribution of urban and rural residents, suggesting that environmental or lifestyle factors related to residency might not be a major effect on the incident of ovarian cancer in this sample This study agree to other studies [13 and 14]. This significant difference underscores the importance of family history as a risk factor for (OC). Genetic predisposition plays a critical role, and having a family history of OC increases the likelihood of developing the disease. This finding aligns with other research that highlights genetic factors in the etiology of OC This study similar to other studies [12 and 15]. The association between the study groups and control group was high significance ($P < 0.01$) for age groups where in patient group older age (≥ 60) more significant for ovarian cancer. In contrast younger age in control group is healthy. Matsas and Huang showed the high significant of age when found the risk of (OC) increases with age at (p-values ≤ 0.05) [16, 17] that agree with current study. Brezis supported current study when found the median overall survival (mOS) for elderly patients with epithelial ovarian cancer (EOC) was significantly lower compared to the control cohort, with values of 41.26 months for the elderly and 69.78 months for the control group at (p value < 0.0001) [18]. Overall, The efficiency of the body's defenses often declines with age in those who are older than 60. Older persons are more vulnerable to

The level of samples of CTLA4 for each groups showed high significant difference in G1,G2 and G3 at (p = 0.0001, 0.0001 and 0.000 respectively). The level of samples of VEGF for each groups showed high significant difference at (p = 0.0001, 0.0001 and 0.000 respectively) effectively distinguishing OC patients. with elevated of the sensitivity and specificity of CTLA4 (80 and 86.67 in G1, 76.6 and 86.6 in G2 and 80 and 86.67 in G3 respectively), Also higher sensitivity and specificity of VEGF (60 and 93.9 G1, 80 and 100 G2, and 73.3 and 100 G3 respectively).

3.4- Person's correlation coefficients between CTLA4 and VEGF in ovarian cancer patients.

The results represented in Table (3-4) showed that both CTLA4 and VEGF were had highly significant correlation between levels of CTLA4 with VEGF in G1 and G2 at (p value = 0.0001 and 0.0003 respectively), but not significant correlation observed in G3 at (P value = 0.088).

Table (3-4) Person's correlation coefficients of biomarkers in OC patients.

Groups		Variable(s)	CTLA4
G1	VEGF	"Pearson Correlation"	0. 562**
		"Sig. (2-tailed)"	0.0001
G2	VEGF	"Pearson Correlation"	0.516**
		"Sig. (2-tailed)"	0.0003
G3	VEGF	"Pearson Correlation"	0.257**
		"Sig. (2-tailed)"	0.088

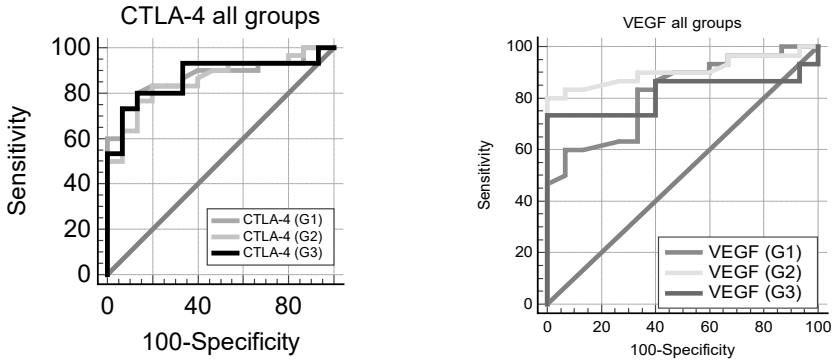
****high significant difference *significant difference**

3.3- Receiver operative characteristic Curve among studies groups.

The results presented in Table (3-3) for the patients Groups (G1, G2, and G3) illustrate that the concentrations markers CTLA4 were (0.873, 95 % CI 0.740-0.954 , 0.859, 95 % CI 0.723-0.945 and 0.871, 95 % CI 0.765-0.977) and for VEGF were (0.820, 95 % CI 0.677-0.918 , 0.906, 95 % CI 0.781-0.972 and 0.818, 95 % CI 0.674-0.917), respectively. Furthermore, applying ROC curve for analyzing data in the studied groups. Figure (3.1) represent ROC curve concerning the CTLA4 and VEGF markers for the studied groups.

Table (3-3) ROC of concerning study groups and biomarkers

Groups	Markers	Area under curve	Asymptotic Sig.	"Asymptotic 95% Confidence Interval"		The best Cut off	Sensitivity %	Specificity%
				"Lower Bound"	"Upper Bound"			
G1	CTLA4	.873	.0001	.740	.954	≤280	80	86.67
	VEGF	.820	.0001	.677	.918	≤234.2	60	93.3
G2	CTLA4	. 859	.0001	.723	.945	≤293.3	76.6	86.6
	VEGF	. 906	.0001	.781	.972	≤215.8	80	100
G3	CTLA4	.871	.000	.765	.977	≤290	80	86.67
	VEGF	.818	.000	. 674	.917	≤202.6	73.3	100



A: CTLA4 all groups. **B: VEGF all groups.**

Figure (1): ROC curve (A, B) of studied marker (CTLA4 and VEGF).

Table (3-1) Demographical characteristic of the studied groups.

Parameters		Patients	Control	Sign.
Age	Mean ±SD	54.888 ±14.1544	42.8000 ± 11.716	"T-test = 4.2 p-value = 0.001"** HS
Residency No. (%)	Urban	82 (91)	38 (95)	Chi-square= 0.59 p-value = 0.4 NS
	Rural	8 (9)	2 (5)	
Family History No. (%)	Yes	9 (10)	0 (0)	Chi-square= 4.29 p-value = 0.04* S
	No	81 (90)	40 (100)	
Occupation No. (%)	Employed	11 (12)	15 (37.5)	Chi-square= 11.01 p-value = 0.001** HS
	Unemployed	79 (88)	25 (62.5)	
BMI No. (%)	Normal Weight	24 (26.7)	2 (5.0)	T-test = -.941 p-value = 0.3 NS
	Over Weight	25 (27.8)	26 (65.0)	
	Obese	41 (45.5)	12 (30.0)	
	Mean ± SD	29.5389 ± 6.2284	31.1333±11.9948	

3.2- Distribution of CTLA4 and VEGF in study groups

The study of (OC) were detected high significance between the study groups and healthy control group in CTLA4 and VEGF at (p value = 0.0001) was shown in Table (3-2).

Table (3-2) Comparison between patients studied groups and control

Groups			CTLA4	VEGF
Patients	G1	Mean ±SD	231.440b±109.119	221.393b±57.837
		SE	19.92236	10.5596
	G2	Mean ±SD	254.5567b±98.752	192.990b±68.160
		SE	18.0296	12.444
	G3	Mean ±SD	255.7667b±110.108	228.060b±150.59
		SE	20.102	27.494
Control		Mean ±SD	399.5600a±105.2752	297.0133a±62.9140
SE		27.1819	16.2443	
P-VALUE			0.0001**	0.0001**

linked immunosorbent assay" (ELISA). catalogue number (SL0594Hu and SL1811Hu respectively) www.sunlongbiotech.com.

2.3- Ethical approval

The ethical committees of the Middle Technical University College of Health and Medical Techniques gave their approval for the study.

2.4- Statistical analyses

The Analysis of Statistical 26 program in SPSS was applied to detect the effect of different parameters. "One-way ANOVA and T-test" was applied to significance difference between means. "Chi-square" test was applied to significance difference in percentage 0.05 and 0.01 probability and computer program "MedCalc" in Windows for applications in laboratory medicine. Receiver operative characteristic Curve (ROC) and Person's correlation coefficients [9, 10].

3 - Results

3.1- Demographical characteristic of the study groups

The study of (OC) were detected high significant difference at (P value = 0.00) between the means of age and occupation in studied groups. while not significant difference at (P value = 0.4 and 0.3) between the means of residency and body mass index (BMI) respectively in studied groups. However, the significant difference at (P value = 0.04) between the mean of family history in studied groups. shown in Table (3-1).

treatments this strategy has the potential to address tumor-induced immune tolerance and enhance the overall effectiveness of chemotherapy [7].

Avastin is a monoclonal antibody that targets VEGF in an attempt to prevent it from binding to receptors through the inhibition of VEGF, this activity may limit the formation of blood vessels in tumors, hence impeding the progression and spread of the tumor. Research demonstrates that Avastin is effective in treating several malignancies by inhibiting VEGF, which reduces the blood supply to the tumor and slows tumor growth and metastasis [8].

2 - Materials and methods

2.1- Patients and samples

Adjuvant (OC) women were taken 5 ml in gel tube to get clear serum. The patients were attending the rapid treatment taking unit to receive treatment were at Al-Amal National Hospital for Oncology, Al-Nahrain Center for Cancer Diagnosis and Treatment Laboratory in Al-Harithiya, Al-Yarmouk Teaching Hospital and Fallujah Teaching Hospital. in Iraq during the period from January 2024 – May 2024, and 40 as healthy control group.90 adjuvant (OC) patients were divided into three groups 30 taking (1-6) dose of biotherapy Bevacizumab (Avastin) group 1 (G1), 30 taking > 6 dose of avastin group 2 (G2) and last 30 taking chemotherapy group 3 (G3) and 40 as control group. Quantitative measurement of human (CTLA4 and VEGF). This was achieved by “Enzyme-linked immunosorbent assay” (ELISA).

2.2- Evaluations of CTLA4 and VEGF serum level

The “cytotoxic T lymphocyte associated antigen 4” (CTLA4) and “Vascular Endothelial Growth Factor” (VEGF) “solid-phase sandwich enzyme-

1 - Introduction

The most frequent cause of death for females with gynecological cancer diagnoses is ovarian cancer (OC). Furthermore, generally speaking, it ranks as the 5th most frequent cause of death for females. The majority of cases had advanced diagnoses, which worsens the disease's prognosis [1]. In ovarian cancer (OC), “cytotoxic T lymphocyte associated antigen 4” (CTLA4) is a protein receptor an immunological checkpoint that is necessary to regulate immune responses and T-cell activation trans membrane protein that is crucial for immune system modulation because it inhibits T-cell activation [2].“Vascular endothelial growth factor” (VEGF) “is a signal molecule that stimulates blood vessel growth”. The new blood vessels production from preexisting vasculature, which is called (angiogenesis), and the formation of the embryonic circulatory system, which is called vasculogenesis, depend on this subfamily of growth factors. VEGF is essential for the production of new blood vessels during fetal growth, after damage, and after exercise [3].Tumor debulking surgery and chemotherapy are the standard therapy for advanced (OC). While many different kinds of chemotherapy regimens have been tried to treat advanced (OC), carboplatin plus paclitaxel is now the most effective and standard first-line therapy [4]. By preventing angiogenesis and boosting the effects of chemotherapy, bevacizumab, also known as asvastin, is a vital component of the therapy of (OC). Research indicates that when bevacizumab is used in conjunction with chemotherapy, like paclitaxel, patients with recurrent platinum-sensitive (OC) may have longer progression-free survival times and better responses to therapy. [5, 6]. Blockading CTLA4 enhances the infiltration of T cells and suppresses the regrowth of cells of cancer between chemotherapy

المستخلص

الخلفية: السبب الأكثر شيوعا للوفاة بين الإناث هو الاصابة بالسرطاب وخصوصا سرطان المبايض. علاوة على ذلك فأن هذا النوع من السرطان يحتل المرتبة الخامسة من بين السرطان المسبب للوفاة بالاناث. غالبية الحالات لديها تشخيصات متقدمة، مما يؤدي إلى تفاقم تشخيص المرض. الأهداف: تهدف الدراسة إلى تقدير المعلمات المناعية لـ "المستضد 4 المرتبط بالخلايا اللمفاوية التائية السامة للخلايا " (CTLA4) و"عامل نمو بطانة الأوعية الدموية" (VEGF) لدى النساء بعد الاستئصال الجراحي لسرطان المبايض. **المواد والطرائق العمل:** شملت الدراسة 90 امرأة بعد الاستئصال الجراحي (30 منهن تناولن (1-6) جرعة من العلاج الحيوي بيفاسيزوماب (أفاستين) المجموعة الاولى (30 ،(G1 منهن تناولن (< 6) جرعة من أفاستين المجموعة الثانية (G2)، و اخر 30 مثلوا المجموعة الثالة (G3)و أخذوا العلاج الكيمياوي و 40 عينة مثلت مجموعة السيطرة الاصحاء. أجري القياس الكمي بواسطة مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA) للإنسان (CTLA4 وVEGF) النتائج: لوحظ فرق ذو دلالة عالية عند (P = 0.001) بين متوسطي العمر والمهنة للمجموعات المدروسة بينما لا يوجد فرق معنوي عند (قيمة P = 0.4 و 0.3) بين متوسطي الإقامة ومؤشر كتلة الجسم (BMI) في المجموعات المدروسة، إلا أن الفرق المعنوي عند (قيمة P = 0.04) بين متوسط التاريخ العائلي في المجموعات المدروسة. فرق معنوي كبيرسجل ايضا بين المجموعات المدروسة والمجموعة السيطرة لـ CTLA4 وVEGF عند قيمة(P <0.001) ، كما و درس منحنى الخصائص التشغيلية المستقبلة (ROC) وان وجود CTLA4 و VEGF كان عالياً. سجل فرق معنوي بين جميع المجموعات المدروسة (G1,G2,G3). مع ارتفاع حساسية ونوعية ل (80 CTLA4)و86.67 في 76.6 ،G1 و86.6 في G2 و80 و86.67 في G3 على التوالي)، وكذلك ارتفاع حساسية ونوعية ل (60 VEGF) و93.9 في 80 ،G1 و100 في G2، و (73.3 و 100 في G3 على التوالي)، كما أن الارتباط بين مستويات CTLA4 مع VEGF كان ذا أهمية كبيرة في G1 وG2، ولكن لم يكن هناك ارتباطات هامة في G3 عند (0.0003 ، p= 0.0001 و 0.088 على التوالي). الخلاصة: قد تلعب العوامل المدروسة دورًا مهمًا في تقييم الاستجابة العلاجية لدى النساء المصابات بسرطان المبيض بعد الاستئصال الجراحي.

الكلمات المفتاحية: سرطان المبيض، VEGF ،CTLA4، العلاج الحيوي، أفاستين، العلاج الكيميائي.

Abstract

Background: The most frequent cause of death for females with gynecological cancer diagnoses is ovarian cancer (OC). Furthermore, generally speaking, it ranks as the 5th most frequent cause of death for females. The majority of cases had advanced diagnoses, which worsens the disease's prognosis. **Objectives**: The aims of the study is to Estimate the immunological markers "cytotoxic T lymphocyte associated antigen 4" (CTLA4) and "Vascular Endothelial Growth Factor" (VEGF) in Adjuvant women (OC). **Materials and methods**: 90 Adjuvant women OC were included in this study (30 of them were taking (1-6) dose of biotherapy Bevacizumab (Avastin) Group 1 (G1), 30 of them were taking (> 6) dose of avastin Group 2 (G2), and the last 30 taking chemotherapy Group 3 (G3) in addition to 40were healthy used as control group. The "Quantitative measurement by Enzyme-linked immunosorbent assay" (ELISA) of human were used to estimate the immunological marker (CTLA4 and VEGF). **Results**: High significance difference at (P = 0.001) between the means age and occupation of OC studied groups while no significant difference at (P value = 0.4 and 0.3) between the means of residency and body mass index (BMI) in studied groups. However, the significant difference at (P value = 0.04) between the mean of family history in studied groups. High significant difference between studied groups and the control group for CTLA4 and VEGF at (P value < 0.001) also in Receiver operative characteristic Curve (ROC) The presence of CTLA4 and VEGF were highly significant difference of all studied groups (G1, G2 and G3). with elevated of sensitivity and specificity of CTLA4 (80 and 86.67 in G1, 76.6 and 86.6 in G2 and 80 and 86.67 in G3 respectively), Also higher sensitivity and specificity of VEGF (60 and 93.9 in G1, 80 and 100 in G2, and 73.3 and 100 in G3 respectively), also the correlation between levels of CTLA4 with VEGF were highly significance in G1 and G2, but no significant correlations in G3 at (P = 0.0001, 0.0003 and 0.088 respectively). **Conclusion**: The studied parameters may play an important role for the evaluation of therapeutic response on adjuvant OC women patients.

Keywords: Ovarian cancer, CTLA4, VEGF, Biotherapy, Avastin, Chemotherapy.

Estimation of the Immunological Markers (CTLA4 and VEGF) of the Adjuvant Ovarian Cancer in Iraqi Women Patients.

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تقدير المعلمات المناعية (CTLA4 و VEGF) المرافقة لعملية أستئصال سرطان المبايض في مرضى النساء العراقيات

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investigation (Jim, *et al*., 2022) that reported increase of serum and urine nephrin in early stage of diabetic nephropathy .

Conclusion

Kidney injury molecule-1 as biomarker for kidney proximal tube specific damage and increase in diabetic nephropathy complications of diabetes. Nephrin protein is specific for podocyte cell damage in kidney and increase in DN .This study suggested that serum level kim-1 and nephrin associated with the patients with diabetic nephropathy inflammation .The measurement of kim-1 and nephrin level was value importance for screening out the DM patients with more risk of diabetic nephropathy and use to predictor disease in early stage .

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various pathophysiologic pathways, including activation of insulin-like growth factor1, transforming growth factor-b, endothelin-1, and the renin–angiotensin–aldosterone system, impaired insulin sensitivity and compensatory hyperinsulinemia have been suggested to contribute to the development of renal injury (Aljorani, *et al*., 2023).

Pro-inflammatory cytokines, adipokines, and oxidative stress are strongly linked to insulin resistance and may potentially exacerbate kidney damage. However, the converse scenario is also conceivable: persistent kidney damage could lead to increased inflammatory activity, which would reduce insulin sensitivity (Mansoor, *et al*.,2022) Although not all diabetes patients with nephrinuria go on to develop kidney disease, nephrin may offer an early warning sign of renal impairment. The current study was consistent with a previous study that indicated nephrin loss significantly and redistribution in the glomeruli of diabetic patients with microalbuminuria. It also revealed that patients with diabetes and nephropathy have structural changes to the glomerular filtration unit, such as increased width of podocyte foot processes and filtration slits (Veluri, *et al*., 2022) . As a result, one can infer that nephrin can be found in the systemic circulation or that nephrin secreted by podocytes while passing through the nephron can be reabsorbed in the renal tubular system and discovered in the serum. Nephrin, a protein exclusive to podocytes found in serum, indicates only podocyte injury, unrelated to the other two elements of the glomerular filtration barrier. Because podocyte damage is assumed to exist before to the onset of microalbuminuria and proteinuria, podocyte proteins, including nephrin, are regarded as more accurate and early indicators of diabetic kidney disease (DN) (Kondapi et el., 2021).In keeping with the findings of the present

Discussion

Results from this study indicate that diabetes mellitus (DM) not only raises blood levels of urea and creatinine but also causes weight loss. Additionally, the results demonstrated a rise in the mean KIM-1 in diabetic nephropathy. This is because DM causes oxidative stress in the kidney tissue, which boosts serum KIM-1 levels and renal expression. KIM-1 is a phosphatidylserine receptor that identifies apoptotic cells and guides them to lysosomes, where it changes kidney proximal epithelial cells into semi-professional phagocytes. (Al-Bataineh, *et al*., 2021) showed significantly increase in serum KIM-1 in chronic kidney disease patients .

due to its function in facilitating the removal of dead cells by the tubular cells that are still alive. Via mechanisms aided by apoptotic cell uptake, KIM-1 protects the kidney in the early stages following damage(De Silva, *et al*.,2021) .

Due to proximal cell inflammation in diabetic nephropathy, KIM-1 is expressed in chronic kidney disease (Ghasemi, *et al*., 2019). Hypoxia is a potent inducer of proximal tubular cells' increased KIM-1 expression, which may lead to the development of persistent interstitial inflammation. In the context of signaling connections between the cells of injured renal proximal tubules and macrophages serving as autocrine-paracrine factors in relation to the epithelial and stromal cells, both membrane-bound and free KIM-1 were thought to be implicated.(Siddiqui, *et al*., 2020).that increase KIM-1 in DN groups.

Corresponding to this finding (Dong, *et al*., 2022) was the finding that insulin resistance in elderly people was associated with a considerably higher KIM-1 level. Through the promotion of mutagenic and fibrotic processes via

KIM-1 and nephrin had a strong capacity to distinguish and predict DN patients from healthy individuals (AUC=0.93, 0.94, respectively). The P value in terms of prior probability was discovered to be 0.001. Nephrin exhibits high sensitivity and specificity values of 99.6 percent and 83.3 percent, respectively, while KIM-1 displays very high values of 99.3 percent and 93.3%, respectively. It suggests that this marker has an equal role in both confirming and ruling out illness. The optimal threshold for KIM-1 (0.66 ng/ml) and nephrin (9.44 ng/ml) was determined by analyzing the ROC curve (Figure 2). When comparing the patients to normal individuals, a value of KIM-1 > 0.66 ng/ml and nephrin > 9.4 ng/ml suggests that the patients most likely have DN.

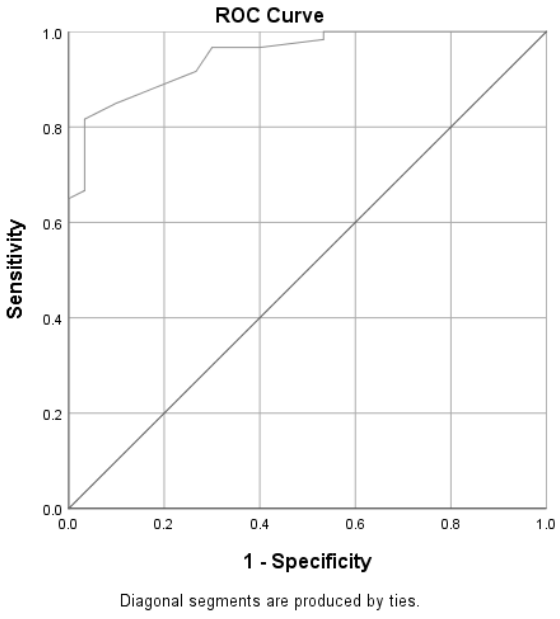

Figure (2) Receiver operatng characteristic curve for Nephrin showing sensitivity and specificity

correlation with FBS (r = 0.453, p < 0.05), KIM-1 showed positive significant correlation with nephrin (r = 0.867, p < 0.01) and showed in Figure (1)

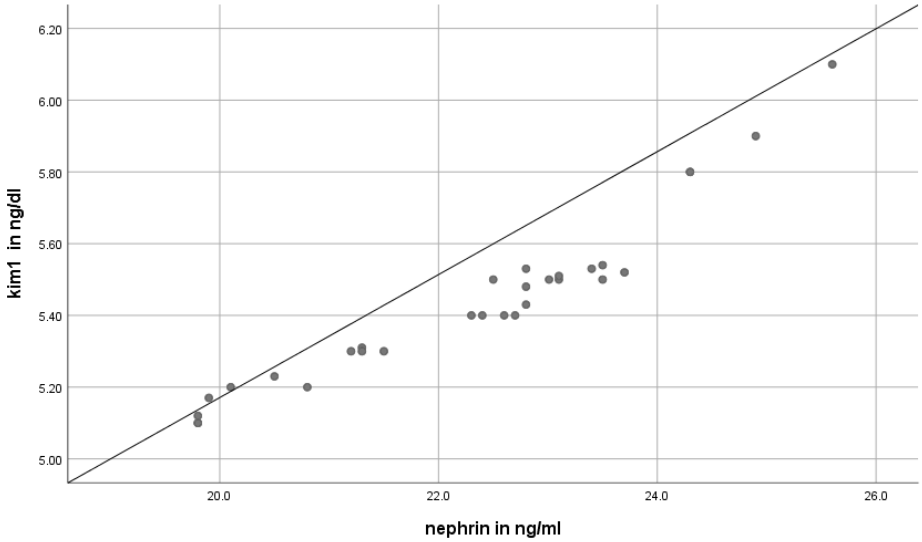

Figure (1) The correlation between serum levels of KIM-1 (ng/ml) with nephrin (ng/ml) in the Diabetic nephropathy group (P value =0.01) ,(R= 0.867)

When compared to the Control group, the ROC analysis data showed that KIM-1 had a superior ability to predict DN in the diabetic patients (Table 3), which included groups DM and DN. This conclusion was attained by investigations that looked at the test's sensitivity and specificity values, area under the curve (Figure 2).

Table (3) The ROC results of KIM-1 biomarkers

Test Variable positive actual critical state.	AUC	P value	Cut-off points ng/dl	Specificity	Sensitivity
KIM-1	0.939	0.001	0.66	99.3%	93.3%
Nephrin	0.94	0.001	9.4	83.3 %	99.6%

These results significant increase (P < 0.05) in mean serum levels of B. urea between control groups and DM group, also there were very high increase significantly (P < 0.001) in mean of B. urea between control group and diabetic nephropathy group.

There were no statistical differences (P > 0.05) in mean serum levels of creatinine between control group and DM group, in contrast very high significant increases (P < 0.001) in mean of S. creatinine in diabetic nephropathy group when compared with control group. The level of eGFR in DM and DN groups high significant decreases (P < 0.001) when compared with control group. These results clearly illustrated in Table (2).

Table (2) The mean ± SD levels of biomarkers (B. urea, S. creatinine, eGFR, and FBS)

Parameters	Control mean±SD n=30	DM mean±SD n=30	DFU&CKD mean± SD n=30
B. Urea (mg/dl)	28.7 ±5.6	34.4 ±2.2 a▲*	100.9 ±18.6 a▲*** b▲***
S. Creatinine (mg/dl)	0.76 ±0.13	0.84±0.10 a=ns	2.9±0.38 a▲*** b▲***
eGFR (ml/ min/1.73m2)	109.6± 10.2	95.7±10.4 a ▼**	21.4±4.2 a▼*** b▼***
FBS (mg/dl)	102.3 ±9.8	256.7±29.6 a▲***	363.7 ±28.3 a▲*** b▲***

N: Number, SD: Standard deviation, ▲; significant increase , ▼: significant decrease , * : (P <0.05),**: (P <0.01),***: (P <0.001) ns= non-significant, a= ANOVA test between control, DM, DFU and DFU &CKD groups ,b= ANOVA test between DM and DFU, DFU & CKD groups, c= ANOVA test between DFU and DFU & CKD groups.

The correlation of the KIM-1 values of DN group showed positive significant correlation with B. urea (r= 0.675, p < 0.05) and positive correlation with S. creatinine (r=0.626 p <0.05), as well showed positive significant

Results

The results of mean ± SD of serum KIM-1 in control group and patients groups (DM, DN) were (0.54 ±0.04), (0.71±0.11), (5.4±0.4) respectively. There were significant increase (P < 0.05) in mean of serum KIM-1 in DM group when compared with the control group . There were increased high significantly (P < 0.001) in mean of serum KIM-1 in (DN) group when compared with control group, also there were increased high significantly (P < 0.001) in mean serum KIM-1 found in(DN) groups when compared with DM group as shown in Table (1)

The results of the mean ± SD of nephrin for control group and patient's groups (DM, DN) were 5.03 ±0.66, 8.2±1.2, 25.7±2.4 respectively. There were increases significantly (P < 0.05) in mean serum nephrin in the patients group (DM) compared with the control group, also there were high significant increases (P < 0.001) in mean serum nephrin found in (N) group when compared with control group and this results clearly demonstrated in Table (1) .

Table (1) The mean ± SD of Ssrum levels of nephrin for both control group and patient groups (DM and DN)

	Control No=30	DM No=30	DN No=30
KIM-1(ng/ml)	0.54 ±0.04	0.71±0.11 a =ns	5.4± 0.4 a▲*** b▲***
Nephrin(ng/ml)	5.03 ±0.66	8.2±1.2 a▲*	25.7± 2.4a▲*** b▲***

N: Number, SD: Standard deviation, significant increase, ▼: significant decrease , * : (P <0.05),**: (P <0.01),***: (P <0.001) ns= non-significant, a= ANOVA test between control, DM and DN groups ,b= ANOVA test between DM and DN, groups

Inclusion criteria: Patients group whose age varied from 18 to 80 years, patient with and who have undergone a clinical examination by endocrinologist and nephrologist. The control group was selected as apparently healthy individuals.

Exclusion criteria: Cancer Patients, patients less than 18 years old, pregnancy, congestive heart failure, systemic lupus erythematous, acute kidney injury, and, incomplete data were excluded from the study.

Blood sampling

Venous blood was drawn from all participant using disposable syringes. The blood was discharged into plain tubes, where the blood was allowed to clot before being centrifuged to separate the serum into two parts. The first was used right away for a routine test that included serum FBS, B. urea, and S. creatinine. The second was kept in a deep freezer to be analyzed using the ELISA method for nephrin and KIM-1.,

Statistical analysis

Statistical program (SPSS version 22.0) was used. A one-way analysis of variance (ANOVA) test was used to analyze the differences in variable means between the control and patient groups. The results were expressed as mean ± standard deviation (SD). Using Pearson's correlation coefficient (r), correlations between all of the variables under study were assessed, and data analysis methods included linear regression analyses. Statistics were deemed significant when the P-value was less than 0.05. The study variables' cut-off values, sensitivity, specificity, and area under the curve were displayed using the receiver operating characteristic (ROC) curve.

Kidney diseases are frequently caused by persistently elevated blood sugar. Research suggests that a significant number of patients with diabetes mellitus may experience renal impairment over an extended period. For an extended period without any symptoms, diabetic nephropathy is thought to be a silent illness. The many types of kidney cells that are subsequently impacted by chronic hyperglycemia eventually develop progressive fibrosis, glomerular and tubular damage, and renal failure. (Chen *et al*., 2018). These indicators typically capture a single mechanism of the disease process, such as glomerular or tubular damage, inflammation or oxidative stress. These findings suggest that podocyte destruction may occur in DM patients prior to the development of microalbuminuria (Dumont et el., 2017) . Several studies showed that nephrinuria was associated with higher urine albumin concentrations and diabetes status, thus, given that hyperglycemia is likely to cause further damage to renal vasculature and glomerular filtration barrier over time (Jim *et al*., 2012).

Materials and methods

Study design

A case control study was carried at AL-Imam AL Sadiq general teaching Hospital in Hilla city during the period from December 2023 to March 2024 .An endocrinologist and nephrologist diagnosed all diabetic patient that included in this study based on clinical findings and measurements of FBS , B.urea , S.cr. All participants in this study divided into three group each group consist of 30 person, diabetic mellitus patients without any complication, diabetic nephropathy and 30 person as control group (apparently healthy). sample size was determined according to G power program for sample size determination.

2015). While KIM-1 is not expressed in healthy kidneys, it is expressed in many different types of kidney disorders in humans, mainly in the proximal tubular cells' apical membrane. A significant factor in the development of autosomal dominant polycystic kidney disease (ADPKD) is cyst growth, and the degree of compression that developing cysts impose on neighboring renal tubules may elevate KIM expression (Abid *et al*.,2019) .

The transmembrane proteins known as kidney injury molecule-1, which have Ig-like and mucin domains in their ectodomain, were only recently found. In addition to being expressed by injured kidney proximal tubules, TIM-1 regulates CD4+ T-cell responses. (Sadar *et al*., 2016).

Renal failure and progressive fibrosis are caused by persistent KIM-1 expression, and the cause of this is thought to be oxidized lipids. By means of the PI3K pathway, KIM-1 can initiate signalling. It was found that in cases of acute ischemia and toxic damage, KIM-1-mediated phagocytosis functions suppress innate immune responses and inflammation. According to theory, tubular interstitial damage is influenced by KIM-1. It is believed that urine concentrations of KIM-1 reflect the expression of tubular KIM-1, which is unique to continuous tubular cell injury and dedifferentiation. Additionally, in certain forms of renal illness, KIM-1 is linked to renal interstitial fibrosis and inflammation. (Satirapoj, ,2018).

Nephrin is a transmembrane protein of the immunoglobulin superfamily. It is a 180 KD and 1242- amino-acid , It is has been localized to the slit membrane between adjacent podocytes of the glomerulus (Kostovska *et al*., 2020) . comprises a fibronectin type III-like domain, eight extracellular immunoglobulin-like modules, and a cytosolic C-terminal tail. It is a transmembrane protein with a single pass that interacts with other trans and cis nephrin proteins both within and outside of cells. (Kondapi *et al*., 2018).

Introduction

Diabetic nephropathy (DN) refers to Diabetic patients may experience kidney illness due to microvascular difficulties stemming from their diabetes, concurrent kidney disease of another origin, or a mix of both. Patients with type 1 diabetes are more likely to experience microvascular disease. (Feng *et al*., 2022). Numerous studies have confirmed that kidney disease in type 2 diabetes may be a more complex entity than that found in type 1 diabetes, and that individuals with type 2 diabetes are frequently older at the time of diagnosis and are more likely to develop renal disease from causes other than diabetes. End-stage renal disease (ESRD) is the final stage of diabetic nephropathy, which progresses through multiple clinical stages including hyperfiltration, microalbuminuria, macroalbuminuria, and nephrotic proteinuria (Elsayed *et al*.,2022) .

KIM-1 is a cell surface receptor found on lymphoid/myeloid and epithelial cells. It functions as an entrance receptor for the hepatitis A and Ebola viruses as well as a scavenger receptor for oxidized LDL and phosphatidylserine (Siddiqui *et al*., 2020). In proximal tubules from AKI and CKD patients, Kim-1 expression is noticeably elevated. The Federal Drug Administration and the European Medicines Agency have qualified the urinary excretion of the KIM-1 ectodomain for preclinical assessment of nephrotoxicity and for case-by-case clinical evaluation. is a transmembrane protein that, following ischemia injury, is up-regulated in renal tubular cells (Ghasemi *et al*., 2019).

While KIM-1 is primarily utilized in relation to drug-induced acute kidney injury (AKI), newer studies indicate that KIM-1 might also be useful in forecasting the course of chronic kidney disease (CKD) (El-Ashmawy *et al*.,

المستخلص

اعتلال الكلى السكري يعد احد المضاعفات التي تحدث نتيجة استمرار زيادة السكر في الدم لفترات طويلة بسبب قله افراز هرمون الانسولين او زياده مقاومه الانسولين وحدوث مضاعفات منها مشاكل في الأوعية الدموية الكبيرة والدقيقة ويمكن ان يحدث في مرضى السكري النوع الاول والثاني

جزيء اصابة الكلى هو بروتين موجود على سطح الخلايا الانبوبية في الكلى وعند تأثر تلك الخلايا لدى مرضى السكري تزداد نسب هذ البروتين في الدم يمكن استخدامه كمؤشر حيوي في الكشف عن الحالات المبكرة للمضاعفات الكلوية في داء السكري.

النفرين : هو بروتين موجود في خلايا الكبيبة في الكلى الدقيقة ويعد احد البروتينات الأساسية التي تلعب دور في تشكيل خلايا الكلى. الهدف من دراسة : معرفه مستويات النفرين وجزيء اصابه الكلى في مرضى اعتلال الكلى السكري. النتائج: وجدت زياده معنويه في مستويات النفرين وجزء اصابه الكلى لدى مرضى الذين يعانون من اعتلال الكلى السكري.الاستنتاج : يمكن استخدام النفرين وجزء اصابه الكلى في الكشف المبكر عن اعتلال الكلى لدى مرضى السكري.

كلمات مفتاحية : اعتلال الكلى السكري – النفرين –جزيء اصابة الكلى – داء السكري

Abstract

Introduction: Diabetes, particularly when uncontrolled for an extended length of time, was already related with a higher risk of complications and progression of both macrovascular and microvascular problems. Diabetic nephropathy is one of the complications of diabetes can be a result from micro vascular effect of diabetes, kidney disease can occur in both type one or type two diabetes patients kidney disease usually affects young and middle-aged patients. Kidney injury molecule and Nephrin is a biomarkers used to diagnosis of early biomarkers in diabetic nephropathy **Objective**: The aim was effectiveness of KIM-1 and nephrin for diagnosis of diabetic kidney disease and compares with other markers as blood urea and s.cr and eGFR. **Materials and methods**: A case-control study included 90 participant that divided into three groups: 30 diabetic mellitus DM ,30 diabetic nephropathy (DN), and 30 healthy control groups, the study starter in November 2023 until March 2024. Laboratory tests were performed on all patients and controls. These included measurements of fasting blood sugar, blood urea, serum creatinine, and by full automated analyzer and KIM-1, and Nephrin by ELISA method. **Results**: The biomarkers KIM-1, Nephrin showed high significant increase in diabetic nephropathy group, and the correlation between KIM-1 and Nephrin was positive and ,the ROC analysis of KIM-1 show high sensitivity and specificity (93.3%, 99.3%).conclusion: kim-1 and nephrin used for diagnosis diabetic nephropathy disease and more sensitivity and specificity from other markers and used to detect kidney disease without clear clinical signs.

Evaluate Levels of Nephrin and Kidney Injury Molecule in Diabetic Nephropathy Patients

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تقييم مستويات النفرين وجزيء اصابة الكلى في مرضى اعتلال الكلى السكري

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that is aerobic, aerobic and strength mixed, or mind-body exercise is just as secure as it is beneficial for preventing diabetes (Paulsen, *et al*., 2023).

Finally, present outcomes showed significant increased of IL-15 in patients >30 years due to increase inflammation in pregnant women with age progression than young age. In contrast, current findings showed decrease WBCs in patients >30 due to impaired immune status with age progression than young age.

Conclusions

Our study showed increases in the levels of interleukins (IL-15 and IL-11) in aborted women due to increase inflammation during abortion process.. There are no significant differences between hematological indicators in aborted women in comparison with the controls. Occurrence diabetes in most women with abortion. Finally, increases in the levels of IL-15 and decreases of WBCs indicators with age progression.

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platelet destruction. Pregnancy causes a rise in the amount of blood, which causes the spleen to enlarge and may kill additional platelets during the procedure of filtering (Salman, *et al*., 2021). In comparison with controls, those suffering from earlier loss of fetal tissue had platelets in an increasingly developed phase of stimulation, as evidenced by decreased platelet heights and membranes the degree of roughness values, a significant shift in the plasma membrane's modulus of flexibility, raised platelet microparticle manufacturing, and a higher level of procoagulant surface markers. These alterations were demonstrated to be correlated with platelet impulsivity, which was linked to the gestation stage at which the abortion transpired and a greater incidence of harboring polymorphisms in thrombophilic factor genes in patients relative to controls (Langari, 2023).

Similar to the current study, Wang, *et al*. (2023) found that aborted mothers had higher glucose levels versus controls. The exact cause of gestational diabetes in some women and not in others is still unknown to researchers. Overweight before to conception frequently contributes. Normally, a number of hormones regulate blood sugar levels. However, the body finds it more difficult to handle blood sugar effectively during pregnancy because to changes in hormone levels (Sangtani *et al*., 2023). Pregnant women with an abortion background had a higher chance of developing diabetes; this finding suggests that abortion history may be a risk factor for diabetes prediction. According to a recent investigation, women who have previously experienced abortions that were induced or abortions are more likely to develop diabetes during their first pregnancy after giving birth. Understanding this link will assist with diabetes screening and prevention (Vaajala, *et al*., 2023). According to earlier research, training during pregnancy

the placenta among other tissues (Menkhorst, *et al*., 2023). Nevertheless, this research also shows that reducing IL-11 action is necessary to enhance pregnancy outcomes and that merely blocking placental inflammasome activation to avoid preeclampsia-associated hypertensive is insufficient protection against insufficient placental supply (Nkhorst, *et al*., 2023).

Our research showed no differences between WBCs, PLT, PCV, RBCs and Hb parameters with study groups, and these outcomes were matcher with findings by Hajiabadi, *et al*., (2022). Evidence from the past points to a possible link between inflammatory and spontaneous abortion. In particular, pathogens have been linked to 66% of late miscarriages and 15% of the earliest abortions (Bas, *et al*., 2018). Preterm birth, premature membrane split, spontaneous miscarriage, fetal infections, and congenital deformities are only a few of the unfavorable obstetrical consequences that can arise from pathogens during gestation (Wall and Yemane, 2022). Although inflammation plays a crucial and essential function in healthy pregnancies, a number of pregnancy diseases can result from aberrant and prolonged inflammation and the failure of anti-inflammatory cytokine-producing cells to resolve it (Soysal, *et al*., 2023).

The authors demonstrated that spontaneous abortion was at risk due to low hemoglobin levels. Additionally, they noted that in order to reduce the risk of anemia throughout pregnancy, women of reproductive age should take iron pills both before and during their pregnancies (Larasati, *et al*., 2023).

Because PLR has a high sensitivity and specificity in the pathological processes of abortion, a new study proposed utilizing it as a predictive indication to identify women who experience recurrent miscarriages (Hantoushzadeh, *et al*., 2024). Pregnancy may cause a greater incidence of

Al-Kinani, *et al*.'s (2022) findings, which correlated with our analysis, revealed that aborted women had higher mean levels of IL-11 than controls. One important cytokine linked to implantation and decidualization is IL-11. Recurrent pregnancy losses (RPL) have endometrial tissues with lower expression of IL-11, indicating that RPL significantly prevents abortion (Yang, *et al*., 2022). Therefore, addressing IL-11 signaling deficits in the endometrium may be a possible treatment for RPL. In IL-11R animals, there was impaired decidualization and a reduced number of uterine stromal cells. However, it is not possible for these in vivo intervention studies carried out in mice to accurately represent alterations in people (Pantos, *et al*., 2022). Because an unplanned abortion happens in 30% of all female pregnancies, most of which occur prior to a clinical pregnancy test, defective implantation and placentation are serious issues in human pregnancy. Additionally, the human endometrium expresses IL-11. Therefore, in order to determine if the alterations in IL-11 and its receptors are connected to RPL, a pertinent research must be carried out (Yang, *et al*., 2023).

The researchers show that in an animal model of preeclampsia, IL-11 triggered the maternal inflammasome, resulting in pyroptosis in a human maternal villus. According to Menkhorst, *et al*. (2023), this is the first proof that IL11 triggers the inflammasome in any kind of tissue. In women with increased blood IL11 in the early stages of pregnancy, therapeutic suppression of the NLRP3 inflammasome may be able to avoid IL-11-induced activation of the umbilical cord and eventual preeclampsia. Current research in numerous illnesses is focused on developing medications to block IL11-induced inflammation and fibrosis. One such medicinal properties. might target the inflammasome to stop IL11-induced fibrosis and inflammation in

2021). Patients who experience repeated 8miscarriages may have disrupted implantation and vascularization with subsequent placental and fetal rejection, which might explain the increased production of IL-15 in their decidua (Toth, *et al*., 2010).

Throughout pregnancy, the amount of IL-15 in the uterus is copious and closely controlled. According to multiple sources of evidence, maintaining IL-15 homeostasis is essential for the mother's and the fetus's health. Many biological substances have been created to imitate or suppress the action of IL-15 on target cells because to the extensive interest in IL-15 as a modulator of anti-tumor immunity in preclinical investigations (Waldmann, *et al*., 2020). When used alone, IL-15 agonists are effective in growing killer cells but not in rejecting human malignancies. These findings, which are believed to stem from IL-15's compensating activation of anti-inflammatory communication, might be very significant for procreation, as it requires healthy inflammation to preserve fetal tolerance (Gordon, 2021).

Furthermore, the amount of uNK cells is correlated with the production of interleukin-15 (IL-15) and leukaemia inhibitory factor in the endometrium of women who have repeated unsuccessful implantation following in vitro fertilization (IVF), indicating a possible function for uNK cells in recurrent miscarriages (Lin, *et al*., 2024).

Serum levels of interleukin (IL)-11 are higher in pregnancy that lead to the occurrence of early-onset preeclampsia. In pregnant mice, pharmaceutical rise of IL-11 leads to the expansion a number of symptoms similar to early-onset preeclampsia, such as proteinuria, hypertension, and fetal growth restriction (Menkhorst, *et al*., 2023).

as well as infectious elements like bacteria, viruses, fungi, and protozoan pathogens (Kortsmit, 2021). The etiology of abortion is heavily influenced by both infectious and non-infectious factors. Selection faults, dietary and chemical variables, poisons, poisonous plants, genetic factors, heat stress, hormonal changes, and fluctuations in blood metabolite concentration are examples of non-infectious causes (Sorhaindo and Lavelanet, 2022). Present investigation showed no differences between age of aborted women and controls due to samples size of participants. Moradinazar, *et al*., (2020) showed the age period 18-30 years scored highest percentage of abortion than >30 years. A prior study discovered a statistically significant correlation between age and ending a pregnancy, with women 45–49 years old having greater probabilities than younger women (Sesay, *et al*., 2023).This might be partially caused by the reality that elderly women are more likely to experience medical conditions and pregnancy-related issues, such as diabetes, chromosomal abnormalities, and heart disease, which could make the pregnancy more difficult and have a worse prognosis (Ryan, *et al*., 2020). In a similar vein, the size of their family might have reached its ideal level (Saikia and Pradhan, 2024).

Outcomes of conducted study mentioned significant raised levels of IL-15 in aborted women than controls, and these outcomes associated with outcomes of Saldanha, *et al*., (2023). The primary source of IL-15 was found to be trophoblast cells, and the authors revealed that IL-15 secretion is elevated in placental tissue of disrupted the initial trimester of pregnancy. Unrestrained production of IL-15 is linked to unexpected and repeated miscarriages, even though it is extensively produced in the uterus throughout pregnancy and in the utero-placental unit when not pregnant (Gordon,

5- Relation of immunological and hematological markers with age of patients

Present outcomes mentioned increased levels of IL-15 in patients ≥30 years (57.73 ±1.74) than patients <30 years (53.62 ±1.93), and decrease levels of WBCs in patients ≥30 years (8.00 ±0.68) than patients <30 years (9.45 ±0.53) with significant differences ($p<0.05$). In contrast, another markers (IL-11, Hb, RBS, Platelet, PCV, an8d RBC) don't showed significant differences ($p>0.05$) with age patients (Table 5).

Table 5: Effect of age groups in parameters studied of the aborted women

Parameters	Mean ± SE		P-value
	<30 yr.	≥30 yr.	
IL-15 (pg/ml)	53.62 ±1.93	57.73 ±1.74	0.0498 *
IL-11 (pg/ml)	18.06 ±1.54	19.11 ±2.47	0.569 NS
WBC (10^9/L)	9.45 ±0.53	8.00 ±0.68	0.0387 *
Hb (mg/dl)	8.96 ±0.32	7.92 ±0.59	0.085 NS
Glucose (mmol/L)	84.74 ±3.42	78.82 ±5.39	0.147 NS
Platelet (10^9/L)	195.96 ±15.31	192.40 ±26.84	0.896 NS
PCV (%)	33.12 ±0.81	32.24 ±1.79	0.472 NS
RBC (x106)	4.26 ±0.16	4.09 ±0.24	0.238 NS

Discussion

Abortion is a complex phenomenon that has a major impact on economic output and production since it can result in large financial losses, particularly during the latter stages of pregnancy. Numerous elements influence it, comprising non-infectious elements like medications, nutritive and chemical substances, plant poisons, hormones, and unidentified causes,

(p<0.05). In contrast, our outcomes revealed no differences (p>0.05) between study groups according to WBCs and Hb markers (Table 3).

Table 3: The levels of WBC, HB, and Glucose for aborted women and the healthy group

Group	MEAN ± SD		
	W B C (10^9/L)	Hb (mg/dl)	Glucose (mmol/L)
Patients	8.97 ±0.43	8.61 ±0.30	82.77 ±2.90
Control	7.92 ±0.33	9.49 ±0.51	73.17 ±3.84
T-test	1.022 NS	1.191 NS	8.679 *
P-value	0.141	0.145	0.049
* (P≤0.05).			

4- Mean levels of platelets, PCV and RBC within study groups

Current research presented no significant differences (p>0.05) between study groups for platelets, PCV and RBC parameters as presented in Table (4).

Table 4: Comparison between the aborted women and the healthy group in PLT, PCV and RBC

Group	MEAN ± SD		
	Platelet (10^9/L)	PCV (%)	RBC (x106)
Patients	194.76 ±13.30	32.82 ±0.79	4.21 ±0.13
Control	186.29 ±13.88	31.98 ±0.69	5.34 ±0.29
T-test	38.521 NS	2.098 NS	0.645 NS
P-value	0.661	0.418	0.201

NS: Non Significant.

Table 1: The average ages of the aborted women and the healthy group

Group	Mean ± SE of Age (year)
Patients	27.80 ±1.07
Control	26.25 ±1.08
T-test	3.046 NS
P-value	0.315
NS: Non-Significant.	

2- Mean levels of IL-15 and IL-11 within study groups

Our study revealed a significant ($p<0.05$) increases in the levels of IL-15 and IL-11 in patients (54.99 ±1.59 and 18.41 ±1.29) than controls (42.11 ±1.70 and 14.03 ±1.12) (Table 2).

Table 2: Comparison between the aborted women and the healthy group in IL-15 and IL-11

Group	Mean ± SE	
	IL15	IL11
Patients	54.99 ±1.59	18.41 ±1.29
Control	42.11 ±1.708	14.03 ±1.12
T-test	4.667 **	3.426 **
P-value	0.0001	0.0013
** (P≤0.01).		

3- Mean levels of WBCs, Hb and Glucose within study groups

Present investigation showed a high means of Glucose in patients (82.77 ±2.90) versus controls (73.17 ±3.84) with significant differences

Materials and Methods

Blood collection

This investigation was conducted in the Medical City Hospital's gynecology consultant for the period of March – May 2023. Thirty blood samples were collected from aborted women and thirty blood samples were collected too from healthy women that represented them as a control group. Study ages were ranged from 17 - 40 years.

Methods

The collected blood in gel tube was separated by centrifuge at (4000 rpm for 6 minutes) to has serum for detecting levels of IL-11 and IL-15 by enzyme immunoassay (ELISA) (Immunotech, France), and blood sugar by Biorex kit (UK). EDTA tube used to quantify levels of hematological indicators by complete blood count (CBC) machine (Sysmex, Japan company).

Statistical analysis

Statistical Analysis System (SAS) (2018) software was used, the impact of the patient and control groups' differences in the research variables was determined. The t-test was employed to contrast means statistically. In the present investigation, the chi-square test was utilized to compare percentages (0.05 and 0.01 likelihood) statistically significant.

Results

1- Average of age within study groups

Present research showed that no differences between patients than controls according to age as illustrated in Table (1).

preeclampsia, the exact mechanism by which it triggers placental injury and preeclampsia remains unclear (Singh and Seed, 2023).

At the maternal–fetal interface, a variety of cytokines and chemokines control the distinct makeup and functionality of immune cells. Killer cell formation, upkeep, and activity have historically been supported by the pleotropic cytokine interleukin-15 (IL-15) (Gordon, 2021). In mice lacking IL-15, frequencies of remember CD8+ T cells, natural killer (NK) cells, and NKT cells are significantly reduced. In the uterus, NK cells are seen in startlingly high quantities compared to both lymphoid and non-lymphoid organs before pregnancy and during the early stages of gestation. It is now evident that NK cells are important for both healthy and difficult gestations, even if their exact roles in pregnancy are unknown (Zhang, *et al*., 2024). A thorough investigation of IL-15 is necessary due to the significance and multiplicity of NK cells during gestation.

Among pregnant women, gestational diabetes mellitus (GDM) is a prevalent metabolic illness. Abortion history was linked to a higher incidence of GDM in expectant mothers, suggesting that it might be a risk factor for GDM prediction (Wang, *et al*., 2023).

According to Biyik, *et al*. (2020), the complete blood count (CBC) is a frequently performed, affordable, and easily accessible test that is advised during the early stages of pregnancy to rule out pathological issues such anemia, thrombocytopenia, bleeding disorders, thrombosis, and thrombophilia.

The objective of current investigation is acknowledgment roles of IL-15 and IL-11 with some physiological indicators in pathophysiology of aborted women.

Introduction

About 1-3 percent of women who want their own kids produce recurrent miscarriage (RM), an early pregnancy failure (Von Woon *et al*., 2022). Early pregnancy is a frequent and complex condition that can have etiological causes that are genetic, anatomical, hormonal, or infectious, or they might have no known cause. A maternal immune component that interferes with the maternofetal tolerance might be among the root causes of unexpected recurrent abortions (RA) (Shmeleva and Colucci, 2021).

An essential part of being pregnant, through the implantation of embryos to baby birth, is the control of pro- and anti-inflammatory cytokines. Immunological homeostasis needs to be preserved at the mother-fetal interface, just like it is in a myriad of other situations, to protect both the mother and the developing fetus. Unwanted pregnancy results are linked to both improper immune activity and quiescence (Andreescu, *et al*., 2023).

There is a pleiotropic cytokine called interleukin (IL)-11. Although IL-11 causes fibrosis in a variety of tissues, the exact process by which IL-11 causes fibrosis is unknown. Mother serum, deciduous tissue, and placenta from pregnancies with early-onset preeclampsia had higher levels of IL11 (Menkhorst, *et al*., 2023). We have demonstrated that IL-11 suppresses human 1st trimester primary trophoblast cell expansion and an invading indicating a causative role for IL-11 in the development of early-onset preeclampsia (Ozmen, *et al*., 2022). Furthermore, the mid-gestational treatment of exogenous IL11 to pregnant mice mimics the characteristics of preeclampsia, including proteinuria, high blood pressure, fetal growth limitation, and preterm delivery (Winship and Dimitriadis, 2017). Though IL-11 stimulates a number of pathways recognized to be changed in

المستخلص

الهدف من البحث الحالي: هو التعرف على أدوار IL-15 و IL-11 مع بعض المؤشرات الفسيولوجية في الفسيولوجيا المرضية للنساء المجهضات.

الطريقة والعمل: أجريت الدراسة في الاستشارية النسائية في مستشفى مدينة الطب للفترة من آذار – أيار 2023. حيث أخذت ثلاثين عينة دم من النساء المجهضات و ثلاثين عينة دم من نساء اصحاء كمجموعة مراقبة. وتم الكشف عن مستويات IL-11 وIL-15 بواسطة المقايسة المناعية الإنزيمية (ELISA) (France, Immunotech)، فضلا عن قياس سكر الدم بواسطة جهاز Biorex machine (UK) ومستويات المؤشرات الدموية الاخرى و التي قيست بواسطة جهاز تعداد الدم الكامل المصنع من شركة (Sysmex, Japan company) ، و استخدم برنامج نظام التحليل الإحصائي (System) Statistical Analysis لتحليل البيانات الحالية.

النتائج: أظهرت النتائج التي توصلنا إليها عدم وجود فروق بين المرضى مقارنة بالأصحاء حسب العمر.لكن اظهرت الدراسة زيادة معنوية ($P<0.05$) في مستويات IL-15 و IL-11 وكذلك في Glucose في المرضى مقارنة بالأصحاء. في المقابل، كشفت نتائجنا عدم وجود فروق ($P> 0.05$) بين مجموعتي الدراسة للقياسات الدموية. أخيرًا، أشارت النتائج الحالية إلى زيادة مستويات IL-15 في المرضى الذين تزيد أعمارهم عن 30 عامًا مقارنة بالمرضى الذين تقل أعمارهم عن 30 عامًا (53.62)، وانخفاض مستويات كرات الدم البيضاء في المرضى الذين تزيد أعمارهم عن 30 عامًا مقارنة بالمرضى الذين تقل أعمارهم عن 30 عامًا مع وجود فروق ذات دلالة إحصائية ($P <0.05$)، بالمقابل لم تظهر العلامات الأخرى (IL-11، Hb، Glucose، Platelet، PCV، و RBC) أي فروق معنوية ($P >0.05$) مع المرضى في العمر.

الاستنتاجات. أظهرت دراستنا زيادة في مستويات الإنترلوكينات (IL-15 و IL-11) في النساء المجهضات بسبب زيادة الالتهاب أثناء عملية الإجهاض. لا توجد فروق ذات دلالة إحصائية بين المؤشرات الدموية في النساء المجهضات مقارنة بالأصحاء . وحدوث مرض السكري عند معظم النساء اللواتي تعرضن للاجهاض . وأخيرا، زيادة مستويات IL-15 وانخفاض مؤشرات كرات الدم البيضاء مع تقدم العمر.

الكلمات المفتاحية: النساء المجهضات ، IL-11، IL-15، المؤشرات الدموية.

Abstract

Objective; The objective of current investigation is TO KNOW THE roles of IL-15 and IL-11 with some physiological indicators in pathophysiology of aborted women.

Methods; This investigation was conducted in the Medical City Hospital's gynecology consultant for period of March – May 2023. Thirty blood samples were taken from aborted women who visit hospitals and clinics otolaryngology. In addition to thirty blood samples were taken from healthy women who represented as a control group. The levels of IL-11 and IL-15 were detected by enzyme immunoassay (ELISA) (Immunotech, France), and random blood sugar observed by Biorex machine (UK). Levels of hematological indicators observed by complete blood count (CBC) machine (Sysmex, Japan company). Statistical Analysis System (SAS) program was used to analyze the current data.

Results; Our findings showed no differences between patients than controls according to age. Significantly increases ($p<0.05$) in the levels of IL-15 and IL-11 as well as in the RBS in patients in comparison with the healthy women. In contrast, our outcomes revealed no differences ($p>0.05$) between study groups according to hematological markers. Finally, Present outcomes mentioned increases in the levels of IL-15 in patients ≥30 years than patients <30 years (53.62 , and decreases in the levels of WBCs in patients ≥30 years than patients <30 years with significant differences ($p<0.05$). In contrast, another markers (IL-11, Hb, Glucose, Platelet, PCV, and RBC) didn't showed significant differences ($p>0.05$) with patients age.

Conclusions; Our study showed increases in the levels of interleukins (IL-15 and IL-11) in aborted women due to inflammation during abortion process. There are no significant differences between hematological indicators in aborted women than healthy women. Furthermore, diabetes observed in most women with abortion. Finally, increases the levels of IL-15 and decreases of WBCs indicators with age progression.

Keywords; Abortion women, IL-15, IL-11, Hematological indicators

Study of IL-15, IL-11, and Some Physiological Parameters in Aborted Women

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دراسة مستويات IL-15 و IL-11 وبعض المعايير الفسيولوجية في النساء المجهضات

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Conclusions

- Traces elements play very important role in human daily activity
- Traces elements are different between the three groups; Iron is in highest level in IVF group, while cobalt and magnesium appears in highest level in control group.
- The variation in the level of the traces between the three groups (pregnant, IVF and control) could be related to hormones, medication, genetic, and medications

Authors Contributions

The concept and study design was by AM, all the lab work and statistical analysis were carried out by LA, the critical revision were done by AM. Acquisition of data analysis, and the drafting of the manuscript was done by LA and AM.

Authors Declaration

No conflicts of interest

level is more expected to be reduced in pregnant especially those with IVF than control.

The results of the Mg among the groups were evaluated. The IVF group exhibits the lowest magnesium value whereas the control group records the highest level. Notably, there exists a noteworthy statistical significance between the groups ((P-Value≤ 0.000), Table 4. This could be justified that during pregnancy, a woman's body undergoes significant hormonal changes, including increased levels of hormones like estrogen which can impact the body's mineral balance, including magnesium. Magnesium metabolism is accurately controlled by estrogen through enhancing magnesium utilization and uptake by soft tissue and bone while level of plasma magnesium is decreased. So that it can be postulated that salivary magnesium reflects low serum value. This explains the increased demand of Mg during pregnancy that may lead to changes in magnesium levels in various bodily fluids, including saliva (Farias, *et al*., 2020).

The magnesium value showed a lower concentration in the pregnant women saliva because of the association between magnesium and alkaline phosphates that the latter increases in the saliva of pregnant women(Rio, *et al*., 2015). Interestingly, the salivary magnesium level was lower in IVF compared to pregnant since IVF procedures can be emotionally and physically taxing, potentially leading to stress and anxiety that affect magnesium levels in the body (Proskurnina, *et al*., 2023).

formation and effect on the divisions and growth (Abu-Mejdad 2013).The average levels iron (Fe) levels during pregnancy showed comprehensive statistical analysis that confirmed a highly significant variation across all groups with highest results obtained for IVF compared to pregnant (Table 4).

Studies observed that ferritin is presented in saliva with significantly higher levels compared to serum; moreover, it was noted that salivary ferritin serves as an indicator of iron deficiency anemia (Gawaly, *et al*., 2020). Consequently, it was suggested that iron plays a substantial role in pregnancy and in vitro fertilization (IVF). Notably, in the IVF group, ferritin levels were found to be elevated, possibly due to saliva conserves iron in the form of ferritin owing to its iron-dependent enzymatic function. Another potential explanation could be the endocytosis and excretion of ferritin by the salivary ducts (Rathnavelu, *et al*., 2021). Moreover, the rise in salivary ferritin levels may be attributed to the existence of high-molecular-weight iron-binding proteins and the uptake of ferritin from the intercalated ducts of the parotid gland. In addition, the increases in salivary ferritin levels could be linked to higher oxidative stress levels in the IVF group, which might have an impact on trace element levels (Gawaly, *et al*., 2020, Rathnavelu, *et al*., 2021).

Cobalt is an essential trace element that accumulates more in women than men at similar exposure levels which may be related to higher metabolic iron loss especially during pregnancy (Fort, *et al*., 2015). Lower serum vitamin B12 levels are associated with a lower clinical pregnancy rate in IVFis well known that the core component of cobalamin in vitamin B12 is the corrin ring, which houses a central cobalt ion (Osman, *et al*., 2021) so that depletion of vitamin B12 is concomitant with Cobalt reduction during pregnancy (with higher significance in IVF). Accordingly, it can be concluded that the cobalt

C. *albicans* is the most frequent species in the oral cavity(Talabani, *et al*., 2013, Rashak, *et al*., 2019). The recorded data verified non-significant positive correlation between salivary Fe and *Candida* in pregnant, IVF and control groups. The previous studies showed that salivary Fe was negative correlation with serum Fe. These explain the increased growth of *candida* in T2 due to the presence of excess amount of Fe in saliva which is essential for *candida* proliferation (Abbas, *et al*., 2009, Rajkumaar and Mathew 2020).

The results conveyed non-significance negative correlation between cobalt and *Candida* in pregnant, IVF and control groups. Cobalt is a necessary component of vitamin B12. As such, cobalt has no known nutritional function, except as a component of vitamin B12, so when we refer to the Co status, we are really referring to the vitamin B12 status(González-Montaña, Escalera-Valente *et al*. 2020). It was found that patients with heavy *Candida* colonization had low levels of cobalt. In this category of susceptible patients, it seems that lower Vitamin B12 and consequently cobalt values may facilitate epithelial invasion by hyphae of Candida and contribute to heavy colonization (Taha and Al Haidar 2019, González-Montaña, *et al*,. 2020).

The data showed no significance negative correlation between Mg and *Candida* in pregnant group while IVF group shows significance negative correlation. This mineral is needed for *Candida* to survive as do all living organism. this manifestation could be elaborated due to Mg impact on immune system recognition toward *Candida* (through reducing hyphal damage, enhanced β-glucan exposure and altered vacuole homeostasis) that increase their level accordingly (Mohammed and Fatalla 2020, Hans, *et al*., 2022). Mg considered as a decreasing factor in growth rates of *Candidaalbicans* because of the effect on the germination tube configuration to prevent

Concerning *Candida* spp., results presented the data of Candida viable count between the three groups with higher counts in IVF groups than Pregnant, while the control shows the lower value. There could be several reasons why *Candida* spp. counts higher in the IVF group compared to pregnant group. Regarding hormonal changes, IVF involves hormonal stimulation to induce ovulation and prepare the uterus for implantation. These changes can affect the oral environment, making it more favorable for the growth of organism and this may differ between IVF and natural pregnancies (Saadaoui, *et al*., 2021).

IVF treatment may affect the immune system, leading to changes in immune response and susceptibility to oral infections that contribute to higher Candida spp counts in the IVF group. IVF treatment can be associated with higher levels of stress and emotional strain which linked to changes in oral health and an increased risk of oral infections. Additionally, lifestyle factors such as diet and oral hygiene practices may differ between the IVF and pregnant groups, potentially impacting their colonization (Rivera-Yanez, *et al*,. 2021). Moreover, pregnancy is associated with immune system adaptations to accommodate the developing fetus. These adaptations can result in a slight suppression of the immune response, which can make pregnant individuals more susceptible to infections, including C. *albicans* (Rashak, *et al*., 2019).

Iron presence in saliva is one of a variety of nutritional factors has been associated in the pathogenesis of oral candidiasis, it is the most common fungal infection, caused by an overgrowth of opportunistic fungus Candida spp. in immunodeficiency hosts. Incidence of Iron deficiency anemia associated with pregnant women is more susceptible to oral candidiasis and

Table 4: The descriptive and statistical test of trace elements among the three groups (Pregnant, IVF, and Control)

		Mean (µg/dL)	±SD	±SE	Minimum	Maximum	F	P-value
Fe	**Pregnant**	93.200	16.171	3.234	69.000	121.000	62.048	0.000
	IVF	114.767	16.546	3.021	87.000	140.000		
	Control	70.000	13.965	2.550	47.000	110.000		
Co	**Pregnant**	0.138	0.017	0.003	0.110	0.170	2.830	0.066
	IVF	0.093	0.025	0.005	0.012	0.140		
	Control	0.142	0.159	0.032	0.070	0.900		
Mg	**Pregnant**	1.518	0.170	0.034	1.280	1.930	82.813	0.000
	IVF	1.285	0.127	0.023	1.060	1.520		
	Control	1.831	0.192	0.035	1.480	2.200		

Discussion

C. *albican* has lower frequency than C. *tropicalis* in IVF. The mixed biofilms formed with C. *tropicalis* indicated that C. *tropicalis* was able to limit the growth of C. *albicans*. Furthermore, the number of viable C. *albicans* cells in monotypic biofilms was significantly different from those in mixed biofilms, where C. *albicans* was reduced in the presence of C. *tropicalis* due to exhibited a significant decrease in metabolic activity in interaction group. Interestingly, decrease in the growth of C. *albicans* in presence of C. *tropicalis* could be attributed to an antagonistic relation between these two species. Therefore, C. *tropicalis* by reducing C. *albicans* virulence profile may limit the ability of this pathogenic fungus to cause infection. This agreed with previous study that evaluated the biofilm life cycle between C. *albicans* and C. *tropicalis* which proved the higher biofilm production of C. *tropicalis* (Atiencia-Carrera, Cabezas-Mera *et al*., 2022).

Table 3: Multiple comparisons of Candida using Games-Howell

Groups		Mean Difference	P value
Pregnant	IVF	4.933	0.002
	Control	6.247	0.000
IVF	Control	1.313	0.256

Description of traces elements (Fe, Cobalt and Mg) among the three groups (Pregnant, IVF and Control)

Three traces elements were measured and the mean values are listed in table 3.10, the findings were; 114.767µg/dL for iron (Fe) in IVF group, and for pregnant it was 93.200µg/dL but for control group it is 70.000µg/dL, all the results were compared statistically with a significant difference (P-Value 0.000), as it is shown in Table (4). As for the Cobalt (Co), the highest mean value is found in the control group with a value (0.142µg/dL), while the value in pregnant group is lower than control group (0.138µmol/dL), and the value of cobalt is the lowest in IVF group with a value of (0.093µg/dL) with no significant differences between the groups (P-Value 0.066), Table (5), Regarding magnesium (Mg), the statistical analysis of MCP test shows that the control group holds the highest value (1.83µg/dL), followed by pregnant group with a value of (1.518µg/dL), while the lowest value of magnesium is found in IVF group with a value (1.28µg/dL) with a significant statistical value between the groups ((P-Value 0.000), as depicted in Table (4).

Table 1: Frequency of *Candida* spp. among the three groups (Pregnant, IVF and Control)

Frequency of Candida spp. (%)		
Groups	***Candida Albicans* %**	***Candida Tropicals* %**
Pregnant	24(84%)	4(14%)
IVF	3(10%)	27 (90%)
Control	1(4%)	-

Viable count of *Candida spp*. among the three groups (Pregnant, IVF and Control)

Results in Table (2) shows that the data of viable count of *Candida* between the three groups and its statistics such as Mean, Standard Deviation (SD) and its Standard Error (SE), *Candida* counts are higher IVF groups (9.767*103*CFU/ml*) followed by pregnant (4.833*103*CFU/ml*) while the control shows the lower value (3.520*103*CFU/ml*) with significant difference (P-Value 0.000).

Table 2: Descriptive and statistical test of Candida among groups X 103

Groups	Mean	SD	SE	Minimum	Maximum	F	P value
Pregnant	4.833	3.896	0.711	1.000	19.000	14.579	0.000
IVF	9.767	6.420	1.172	2.000	25.000		
Control	3.520	2.044	0.409	1.000	7.000		

Table (3) shows that the mean differences between the three groups, Multiple Comparisons of *Candida* was carried out using Games-Howell, however, the multiple pair wise comparison indicates the significant difference between each group when compared with other with (P-Value 0.000) and P-Value 0.000) respectively except between IVF and control which does not shows any significance (P-Value = 0.256).

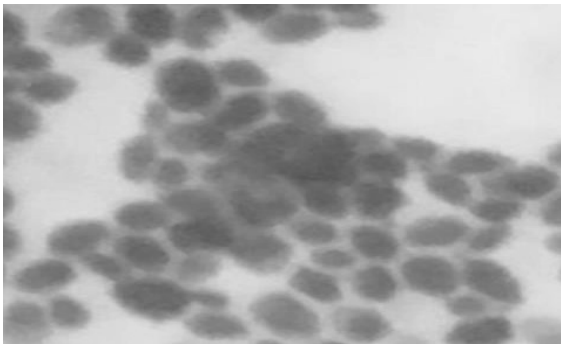

Figure 2: Gram's stain for Candida spp. cells (1000X magnification).

Germ tube formation

Under light microscope, the formation of germ tubes was observed which it is one of *Candida* Albicans characteristics, figure 3.

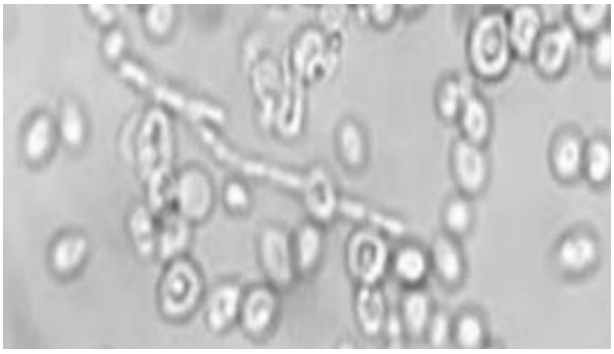

Figure 3: Germ tubes of Candida spp. (40X magnification)

Frequency of *Candida* spp. among groups (Pregnant, IVF and Control)

The results shown in Table (1) demonstrate that the Frequency of *Candida spp* in each pregnant group had a high proportion of *Candida Albicans* (84%) while in IVF group found a high proportion of *Candida Tropicalis* (90%). The control group reported *Candida Albicans* 4%.

Measurement of mineral elements

Perkin-Elmer (USA) Atomic Absorption Spectrophotometer model 305B fitted with Nitrous oxide acetylene burner head. Hollow cathode lamps were used as radiation emission source for (Ferrous Iron (Fe) / Magnesium (Mg)/ Cobalt (Co)). Absorption was measured in a Fuel-rich flame to obtain maximum sensitivity.

Results

Identification of *Candida spp*

Colony morphology

On SDA plates, colony of Candida spp. appeared smooth, creamy in color with distinguished yeast smell. They were about 3-4 mm in diameter and 2 days later they were developed into high convex, off-white large colonies as shown in figure 1.

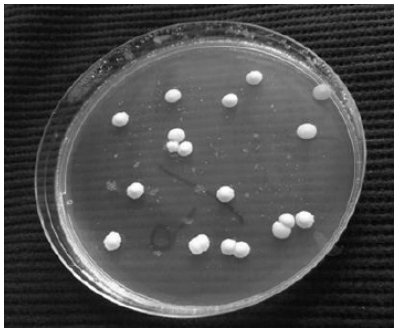

Figure 1: Colony of Candida spp. on sabouraud dextrose agar

Microscopic examination

Under light microscope, Candida spp. appear as rounded or oval yeast cells and were Gram positive when Gram staining was performed, as shown in figure 2.

Vitek system 2

Vitik-2 system was used in this study in order to diagnose the Candida spp. isolates which included several steps as follows:

1. Preparation of fungus suspension A sterile swab was used to transfer a sufficient number of C. albicans, colonies of a pure culture and separately suspended in 3 ml of sterile saline in clear plastic test tube. The turbidity was adjusted up to 2.0 O.D.
2. Inoculation of identification card Identification card was inoculated with Candida spp. Isolates suspension using an integrated vacuum apparatus. A test tube containing the Candida suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests or up to 15 tests.
3. Card sealing and incubation Inoculated card was passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are incubated on-line at 35. 1.0°C. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15 minutes intervals during the entire incubation period.

Sabouraud Dextrose Agar (SDA)

This medium is selective for the isolation and cultivation of fungus and was prepared, sterilized and stored according to the manufacturer's instructions; 65g were suspended in 1000ml of D.W. Sterilization was done by autoclaving at 121°C at 15psi for 15 minutes, left to cool till 45-50°C then poured into Petri dishes and left at room temperature to cool then store in refrigerator till use. (Add chloramphenicol 0.05g/L)

Sterilization methods

The media sterilized in an autoclave for fifteen min at temperature of 12°C and 15 pound/inch2 pressure. All cleaned glass tools were sterilized in a hot air oven for 1 hour at 180°C.The laboratory's benches and floor were cleaned with a bleaching antiseptic agent.

Culturing method

Saliva was placed on a vortex machine to be homogenized for 120 second. Then serial dilution was done Tenfold steps, 0.1 ml was withdrawn by micropipette from (10-3 to 10-5). A microbiological spreader was used to inoculate the 0.1mL on (SDA) and agar sabouraud from each dilution. An anaerobic jar was used to incubate the MSBA agar for 48 hours, at 37°C, second incubation done at 37°C, for 24 hours without the jar, followed by aerobic incubation for 24hrs; at 37°C. Sabouraud chloramphenicol agar was incubated aerobically for 45 hrs, then samples were placed in the centrifuge at 3000rpm for 15 minute then the superannuant collected and stored in the freezer.

Ethical approval

All the subjects received detailed information concerning the nature of the study and the procedures involved, and their informed consent was obtained on a form approved by ethical committee of College of Dentistry in University of Baghdad.

Saliva samples collection

All participants were instructed not to eat or drink (except water) at least 1 hour prior to donation of saliva; the subject should sit in a relaxed position. Saliva was collected between 8-9 am. Whole un-stimulated saliva was collected into cups. After collecting the salivary sample from each patient, the tubes were placed in a cool box with ice to transfer them to the laboratory to be cultured within less than an hour, then 0.1 ml would be taken from the salivary sample by micropipette for the serial dilution tubes using PBS.

Phosphate Buffer Solution (PBS) preparation

Preparation of solutions and culture media

This isotonic solution used serial dilution was prepared by adding 8g (NaCl), 1.21g (K2HPO4) and 0.24g (K2HPO4) to 1000 ml deionized water, mixed well using magnetic stirrer for dissolving the powder (AL-Qaralusi and Al-Mizraqchi 2023)(AL-Qaralusi and Al-Mizraqchi 2023), then sterilized by autoclaving at 15 pound/inch2 pressure (121℃) for 15 minutes. After cooling, PBS solution used by 9.9mL in each disposable tube and then add 0.1mL from saliva.

Materials and Methods

The human sample

This study had been started after gaining the protocol approval, the ethical and the scientific committee approval at College of Dentistry/University of Baghdad. A total (85) subjects were enrolled in this study. They were admitted to (Elwea Maternity, Al-Jadiriah Private, Kamal Al-Samarrai Specialist, Albinouk private Hospitals, College of dentistry university of Baghdad under follow up and control participant choose randomly), the study was carried out from 15 November to 25 of February, 2022. The subjects were divided in to three groups: first group consist of (30) normal pregnant women with age range (25 - 40) years, and second group consist of (30) Invitro fertilization pregnant women their age range between (25 - 40), and third group consist of (25) non-pregnant women their age range between (25 - 40) was chosen to be the control group who were matching the study group.

Inclusion criteria

- Pregnant women with age range between (25-40) years old.
- Second trimester of pregnancy.
- First pregnancy.
- The pregnant should seize any oral supplement prior sampling only keep folic acid.

Exclusion criteria

- Pregnant women with signs and symptoms of any systemic disease.
- Have history of chronic disease like diabetes mellitus, hypertension or heart diseases.
- History of smoking or alcohol drinking.

Candida, especially Candida albicans). Candida species are present in the oral cavity of up to 75% of the population. In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with Candida species are termed "oral candidiasis"(Pappas, Kauffman *et al*. 2009).

Minerals and trace elements (MTEs) are inorganic micronutrients found in a variety of plant and animal foods. The functions of mineral elements include being a structural component of a vitamin (Zinc (Zn) and magnesium (Mg) and Iron (Fe), catalytic components of numerous enzymes, are also structural components of other important proteins. Deficiencies in iodine, iron, and zinc, have the largest negative impact on the public health (Harika, *et al*., 2017). Minerals are key components of complex enzyme systems responsible for antioxidant protection of the organism. This feature seems to be particularly important during pregnancy, which is associated with a higher frequency of oxidative reactions. There was a relationship between reduced levels of mineral elements essential for antioxidant activity in the body of pregnant women and an increased risk of developing preeclampsia (Bakacak, *et al*., 2015).

This research aims to assess variations in salivary mineral element levels and investigate how these fluctuations may impact the overall count of Candida spp.

Introduction

Pregnancy is characterized by significant physiological, social and emotional changes which can impact on maternal and fetal health and well-being across multiple domains (Littleton, *et al*., 2010). There is comprehensive evidence that anxiety, depression, and stress in pregnancy are risk factors for adverse maternal and fetal outcomes ranging from preterm birth and low birth weight to adverse neuro developmental outcomes in infants and children (Baibazarova, *et al*., 2013). Pregnancy is associated with several structural and functional changes that influence the processes of drug absorption, distribution, metabolism, and excretion, besides these pregnancy-related changes attributable to alterations in maternal blood flow, increased fluid retention, and the effect of hormones (such as vasopressin, progesterone, relaxin, estrogen, and angiotensin II), transmembrane transporter function, expression, and regulation also significantly influencing the pharmacokinetics of many drugs during pregnancy (Sheffield, *et al*., 2014, Al-zahraa and Aldhaher 2017).

Much of the success of IVF relies on women undergoing multiple embryo transfers and oocyte retrievals. However, multiple embryo transfers and oocyte retrievals can be cost prohibitive and emotionally and physically burdensome resulting in reported treatment attrition rates of up to 35–50%, While some factors associated with lower success of IVF treatment, such as advanced female age, are not modifiable, there is growing interest in the impact of modifiable factors, such as diet, on treatment outcomes (Olivius, *et al*., 2004).

A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of

المستخلص

الخلفية: كان هناك ترابط بين انخفاض مستويات العناصر المعدنية الحيوية الضرورية للنشاط المضاد للأكسدة في أجسام النساء الحوامل والتعرض المرتفع لارتفاع ضغط الدم أثناء الحمل. **الهدف:** تقييم تأثير العناصر المعدنية في اللعاب على العدد الكلي لمجموعات كانديدا في اللعاب للنساء الحوامل، والنساء الحوامل بعمليات التلقيح في المختبر، والسيدات الغير حوامل. **المواد وطرائق العمل:** تم تضمين مجموعة مكونة من 85 مشاركًا في هذه الدراسة، حيث تتكون المجموعة الأولى من (30) امرأة حامل طبيعية، والمجموعة الثانية من (30) نساء حوامل بعمليات التلقيح في المختبر، والمجموعة الثالثة من (25) سيدة غير حامل، تم جمع العينات من مراكز مختلفة موجودة في بغداد، العراق. تم استخدام نظام Vitik-2 في هذه الدراسة لتشخيص

النتائج:

عزلات كانديدا spp.، وتم استخدام جهاز Perkin-Elmer (الولايات المتحدة) نموذج B 305 لقياس العناصر النقطية. كانت تردد مجموعات كانديدا spp. في كل مجموعة من النساء الحوامل عاليًا بنسبة كبيرة من Candida Albicans (84%) بينما وُجد في مجموعة الحوامل بالتلقيح في المختبر نسبة عالية من Candida Tropicalis (90%). كان عدد كانديدا بين الثلاث مجموعات أعلى في مجموعة التلقيح في المختبر (9.767×103 CFU/ml) تليها مجموعة الحوامل (4.833×103 CFU/ml) بينما تُظهر المجموعة الضابطة القيمة الأقل (3.520×103 CFU/ml) مع فارق معنوي (قيمة $P \leq 0.000$). تم قياس العناصر النقطية الثلاث 114.767 ميكروغرام/ديسيلتر للحديد (Fe) في مجموعة التلقيح في المختبر، وللحوامل كانت 93.200 ميكروغرام/ديسيلتر لكن للمجموعة الضابطة هي 70.000 ميكروغرام/ديسيلتر، وكانت جميع النتائج مُقارنة بشكل إحصائي بفارق معنوي قيمة($P \leq 0.000$). أما بالنسبة لـ (Co)، فإن أعلى قيمة متوسطة توجد في المجموعة الضابطة، بينما في مجموعة الحوامل أقل من المجموعة الضابطة (0.138 ميكرومول/ديسيلتر)، وهي الأدنى في مجموعة التلقيح في المختبر بقيمة (0.093 ميكرومول/ديسيلتر) دون وجود فروقات معنوية بين المجموعات (قيمة $P \leq 0.066$). أما بالنسبة لـ (Mg)، فإن المجموعة الضابطة تحتل أعلى قيمة، تليها مجموعة الحوامل بينما توجد أقل قيمة للمغنيسيوم في مجموعة التلقيح في المختبر بقيمة إحصائية معنوية بين المجموعات قيمة ($P \leq 0.000$)

الاستنتاجات: العناصر النقطية مختلفة بين الثلاث مجموعات؛ الحديد في مستوى أعلى في مجموعة التلقيح في المختبر، بينما يظهر الكوبالت والمغنيسيوم بمستوى أعلى في المجموعة الضابطة.

الكلمات المفتاحية: العناصر النادرة، التلقيح في المختبر، كانديدا سب، العراق.

Abstract

Background: A correlation existed between diminished levels of vital mineral elements crucial for antioxidant activity in the bodies of pregnant women and an elevated susceptibility to preeclampsia. **Objective**: To assess the effect of salivary mineral elements on the salivary total count Candida spp in pregnant, in vitro fertilization pregnant women and control. **Materials and Methods**: A total of 85 subjects were included in this study, first group consist of (30) normal pregnant women, second group consist of (30) In vitro fertilization (IVF), and third group consist of (25) non-pregnant women, the samples were collected from different clinics located in Baghdad, Iraq. Vitik-2 system was used in this study in order to diagnose the Candida spp. isolates, and Perkin-Elmer (USA) Atomic Absorption Spectrophotometer model 305B was used to measure the traces elements. Results:the Frequency of Candida spp in each pregnant group had a high proportion of *Candida Albicans* (84%) while in IVF group found a high proportion of *Candida Tropicalis* (90%). the count of *Candida* between the three groups were higher IVF groups (9.767*103*CFU/ml*) followed by pregnant (4.833*103*CFU/ml*) while the control shows the lower value (3.520*103*CFU/ml*) with significant difference (P-Value 0.000). theThree traces elements were measured 114.767µg/dL for iron (Fe) in IVF group, and for pregnant it was 93.200µg/dL but for control group it is 70.000µg/dL, all the results were compared statistically with a significant difference (P-Value 0.000),, for t(Co), the highest mean value is found in the control group, while in pregnant group is lower than control group (0.138µmol/dL), it is the lowest in IVF group with a value of (0.093µg/dL) with no significant differences between the groups (P-Value 0.066), as for (Mg), the control group holds the highest value, followed by pregnant group while the lowest value of magnesium is found in IVF group significant statistical value between the groups ((P-Value 0.000).**Conclusions**:Traces elements are different between the three groups; Iron is in highest level in IVF group, while cobalt and magnesium appears in highest level in control group.

Keywords: Traces elements, IVF, Candida Spp, Iraq

Effect of Ferritin, Magnesium and Cobalt on Total Count of Candida Spp. in Normal Pregnant and In-Vitro Fertilizations Women

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تأثير الفيريتين والمغنيسيوم والكوبالت على العدد الكلي لمجموعات كانديدا في النساء الحوامل الطبيعيات واللواتي أجريت لهن عمليات التلقيح في المختبر

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compared with the prevalence of IBS among females in the West which is higher than in the East (Lau *et al*., 2002; Lee *et al*., 2009). However, Tang *et al*., (2012) indicated that women with IBS in middle age are more common in type IBS-d.

Many risk factors contribute to the onset of IBS; which is a prevalent and chronic complicated GIT disease. According to the present study, found all factors in the study did not act as risk factors for the IBS subgroups. In contrast, Zamani *et al*., (2019) found that IBS-c had the greatest prevalence of both anxiety and stress in a study of IBS patients. The present study found no relationship between types of IBS with married/single it does not constitute a risk factor among the species. Furthermore, in occupational status, Yilmaz *et al*. (2005) also did not find significant differences between occupational status and disease development. In addition, results indicated that other GIDs among IBS types did not appear as a risk factor and therefore, there was no previous study on IBS with a family history of serious illnesses. It is reasonable that IBS/GIT symptoms are linked to a family history of reflux and other functional GIDs conditions that overlap with IBS (Lacy *et al*., 2016).

According to the findings reported in this study, it can be concluded that about two third of IBS patients getting the disease in second third decade of their age (average: 20.3 ± 5 ages), and about two thirds of them are females. Also, the most common type of IBS is constipation (IBS-c) followed by diarrhea (IBS-d) and mixed (IBS-m). However, family history, Anxiety, Marital status, occupational status, and other GIDs not acts as risk factor for getting specific type of IBS.

(2020) reported that the prevalence of IBS is usually more common in the third to fourth decades. In Norway, it was also said that the infection occurs at a young age and declines with age and that the causes for this are unknown. It has been proposed, however, that stress and unclear living conditions may be factors in increasing risk in younger age groups (Johansen and Ness-Jensen, 2022). In contrast, other studies disagree the present result, one study found no obvious link between age and onset of disease (Saito *et al*., 2002), while other studies reported that the prevalence of IBS increases with age and the cause of its increase in the elderly may be caused by concomitant medications or organic diseases (Gwee *et al*., 2004; Lee *et al*., 2009).

On the other hand result in figures, 3-2 show that IBS-c is the most common at young ages. This is in agreement with most studies instance Ford *et al*. (2013) refer to the IBS-c as more common in younger patients. Additionally, Bellini *et al*. (2017) also discovered that IBS-c is more common in younger persons, who had more severe symptoms, firmer stools, and a lower quality of life (QOL). As it is much known from previous studies, the IBS-c was the most frequent kind of IBS particularly in females; various studies found that IBS-c is more prevalent, especially among women (Arasteh *et al*., 2018; Üçüncü *et al*., 2020; Johansen and Ness-Jensen, 2022). It has been noticed that women have higher levels of inflammatory cytokines than males which may be due to having greater tiredness, depressed mood, less positive well-being and self-control, and higher anxiety (Blanchard *et al*., 2001; Choghakhori *et al*., 2017). In contrast, some researchers didn't found substantial difference in the development of IBS between men and women, particularly in Singapore, Korea, or Hong Kong, indicating probable cross-national variances in the distribution of IBS between the two genders when

patients of these subtype (Table 3-1) which disagree with those found by other studies that found the constipation is the most common bowel behavior along with abdominal bloating/distension and discomfort (Lewis and Heaton, 1997; Longstreth *et al*., 2006). Recently, Shah *et al*. (2020) also noticed that patients of IBS-c have more abdominal discomfort than other subtypes. This form of IBS-c might be described as painful constipation because difficulty passing stools or a low frequency of bowel movements which is frequently accompanied by straining during defecation or a sense of incomplete evacuation (Mearin *et al*., 2016). There are several factors that cause constipation such as; (damaged or reduced 5-hydroxytryptamine (5-HT) release, psychological issues, poor diet, enteric inflammation, genetic changes, high polyunsaturated fatty acid levels, low bile acid levels, and incorrect GIT motility all the way to methanogenesis inhibition) (Lee *et al*., 2017; Niewinna *et al*., 2020). 5HT is a member of the intestinal serotonin system, which aids in GIT motility and it is also present in the central nervous system as a neurotransmitter that aids in mental health (Manchia *et al*., 2017).

Many studies agreed with their results for instance; Tan *et al*,. (2003) refer to a high prevalence of IBS patients among young people. Also Yilmaz *et al*., (2005) According to the findings the prevalence was highest in the middle-aged group with the highest rates. This is also in line with findings from research conducted in Syria, which indicated that IBS was more prevalent among young adults (Al Saadi *et al*., 2016). Furthermore, Arasteh *et al*. (2018) recorded a distinct age limit at which the prevalence of IBS fluctuates, the age between 40 and 45 years is a changeable point at which the prevalence of IBS reaches its peak, and drops. However, Üçüncü *et al*.

diseases (GIDs) rather than IBS. Table-4 show non-significant difference in the occurrence of all these factors among patients of three groups, and not act as risk factors in differentiation between the three types of IBS.

Table (4) Risk factors for getting different types of IBS

Factor		Disease type			P value
		IBS-c	IBS-d	IBS-m	
Family history	Present	11 (32.4%)	1 (12.5%)	3 (37.5%)	0.479
	absent	23 (67.6%)	7 (87.5%)	5 (62.5%)	
Anxiety	Mild	4 (11.8%)	2 (25%)	0 (0%)	0.872
	Moderate	8 (23.5%)	1 (12.5%)	3 (37.5%)	
	Severe	22 (64.7%)	5 (62.5%)	5 (62.5%)	
Marital status	married	16 (47.1%)	5 (62.5%)	4 (50%)	0.734
	Not married	18 (52.9%)	3 (37.5%)	4 (50%)	
occupational status	Employee	15 (44.1%)	4 (50%)	2 (25%)	0.158
	Student	15 (44.1%)	2 (25%)	2 (25%)	
	No job	4 (11.8%)	2 (25%)	4 (50%)	
Other GIDs	Present	7 (20.6%)	1 (12.5%)	4 (50%)	0.152
	Absent	27 (79.4%)	7 (87.5%)	4 (50%)	

Discussion

These results are comparable with those reported by other studies (Siah *et al*., 2016; Zweig *et al*., 2018; Tack *et al*., 2019) which found that the main IBS subtype is constipation. Moreover, Mohammad *et al*. (2019) reported that IBS-c is more frequent in Iraqi patients, than IBS-d and IBS-m. In contrast, other studies found that IBS-m and IBS-d are the most common subtypes than IBS-c (Singh *et al*., 2015; Windgassen *et al*., 2019; Johansen and Ness-Jensen, 2022). Concerning with sustained pain after defecation the result of the present study reported non-significant differences among

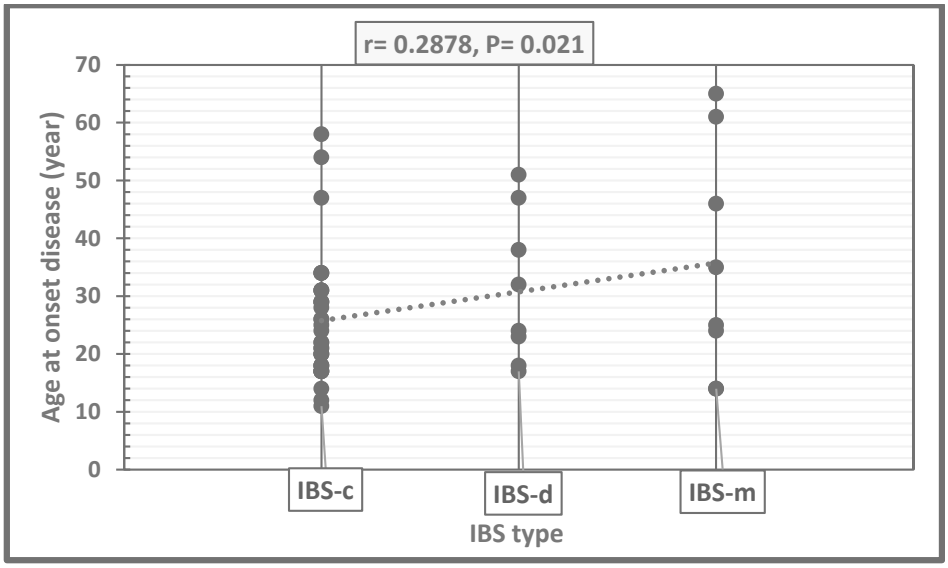

Figure (2) Correlation of IBS types with patient's age at onset of disease.

Table-3 shows that 33 (66%) of all IBS patients are females which is significantly (P= 0.001) higher than 17 (34%) of male patients. This table also reveals that 27 (73.5%) of IBS-c cases are females which is significantly (P= 0.0001) higher than 9 (26.5%) of male cases. However, the distribution of male and female cases in the IBS-d and IBS-m types reveals non-significant difference (50% for each gender) in both types of disease.

Table (3) Frequency of disease type based on patient's gender

Gender	Total patients (N=50)	Disease type		
		IBS-c (n=34)	IBS-d (n=8)	IBS-m (n=8)
Female	33 (66%)	25 (73.5%)	4 (50%)	4 (50%)
Male	17 (34%)	9 (26.5%)	4 (50%)	4 (50%)
P value	0.001	0.0001	1.0	1.0

From patients profile, five factors were studied which include family history, anxiety, marital status, occupational status, and other gastrointestinal

Table (1) Clinical manifestation in three types of IBS

Symptom		Disease type			P value
		IBS-c (n=34)	IBS-d (n=8)	IBS-m (n=8)	
Defecation times / week		3.6 ± 2	15.2 ± 10.8	8.7 ± 5.9	< 0.0001
Sustained pain after defecation	No	23 (67.6%)	7 (87.5%)	6 (75%)	0.519
	Yes	11 (32.4%)	1 (12.5%)	2 (25%)	

The age of patients at onset of disease was calculated by subtraction their disease duration from their present age. Table-2 shows that 33 (66%) of patients have experience of IBS at the second and third decade of their age (20.3 ± 5 year), which is significantly (P= 0.001) higher than 17 (34%) of them who have IBS at the fourth decade or more of their age (39.1 ± 14.2 year).

Table (2) Age of IBS patients at onset of disease

Parameter		Age of patient at onset of disease		
		2^{nd} – 3^{rd} decade	≥ 4^{th} decade	P value
Range (year)		11 - 29	≥ 30	0.001
Patient's	Number	33	17	
	frequency	66%	34%	
M ± SD (year)		20.3 ± 5	39.1 ± 14.2	< 0.0001

Moreover, the age at onset of disease is significantly correlated with the type of IBS (r= 0.2878, P= 0.021), in which IBS-c is mostly occur at young age in comparison with the incidence of IBS-d and IBS-m as shown in figure-2.

Results

Among 50 patients enrolled in this study, 34 (68%) of them are presented with constipation type of irritable bowel syndrome (abbreviated as IBS-c) which is significantly (P < 0.0001) more frequent than 8 (16%) of patients with the diarrheal type (IBS-d), and 8 (16%) of patients with mixed type of irritable bowel syndrome (IBS-m) as shown in Figure-1.

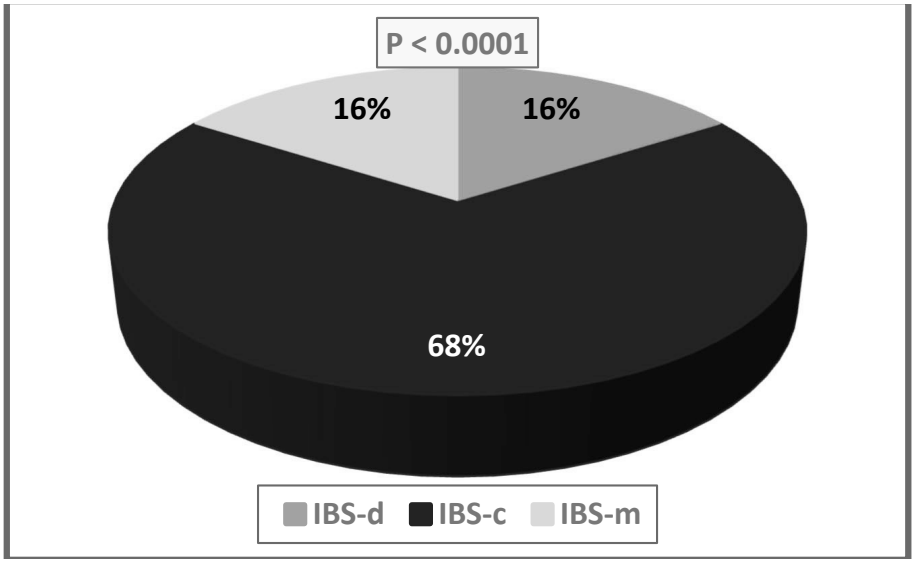

Figure (1) Frequency of three types of irritable bowel syndromes.

In these three types of IBS, defecation times per week and the sustained abdominal pain & discomfort after defecation were determined. Table-1 shows a significant difference (P < 0.0001) in the number of defecations per week among patients of IBS-c (3.6 ± 2 times/week), IBS-d (15.2 ± 10.8 times/week), and IBS-m (8.7 ± 5.9 times/week). However, pain after defecation is sustained in 32.4% of IBS-c patients which is higher than 12.5% and 25% in patients with IBS-d and IBS-m respectively, but difference is not significant.

Introduction

Irritable bowel syndrome (IBS) is a prevalent GIT disease that occurs because of a disruption in neuronal function along the brain-gut axis at different levels of enteric, autonomic, and/or central neurological systems (Öhman and Simrén, 2007; Öhman and Simrén, 2010). Patients with IBS are presented with abdominal pain and/or discomfort, bloating, and irregular bowel function (diarrhea and/or constipation) (Longstreth *et al*., 2006). IBS can be classified based on their primary stool pattern into three main types; constipation type (IBS-c), diarrheal type (IBS-d), and mixed type (IBS-m) which characterized with both diarrhea and constipation (Holtmann *et al*., 2016). The last criteria that used to diagnose and classify IBS into three types are based on Rome IV (Basnayake, 2018). In Iraq, previous studies reported that the prevalence of IBS affects 30% of the general population in Tikrit province (Hazaa and Lami, 2018), and about 29.7% of students in Baghdad governorate suffer from IBS (Rostami *et al*., 2017). Therefore, the present study is conducted on Patients with IBS to determine their subtypes as well as to investigate various risk factors that contribute in this disease.

Materials and Methods

All patients (males and females) with IBS were collected from private clinics specialized in GIDs. Other characteristic were obtained from patients profile such as gender, age at disease onset, disease duration, family history, marital status, occupation status and other GIDs disease rather than IBS. To classify patients in IBS subtypes based on Rome IV criteria (Hyams *et al*., 2016). Also, the anxiety score for each patient is calculated according to Generalized Anxiety Disorder Assessment (GAD-7) (Kroenke *et al*., 2007).

المستخلص

متلازمة القولون العصبي (IBS) هي مرض وظيفي في الجهاز الهضمي (GIT) يؤثر على 10-20٪ من السكان في جميع أنحاء العالم ويتميز بألم / عدم الراحة في البطن وتغيرات في عادات الأمعاء المصحوبة بالإسهال أو الإمساك أو كليهما. أجريت دراسة مقطعية على 50 مريضا عراقيا يعانون من القولون العصبي والذين تم تقديمهم إلى العيادات الخاصة لأمراض الجهاز الهضمي (GIDs). استمرت الدراسة من نوفمبر 2021 إلى مايو 2022، وتهدف إلى تحديد مدى تكرار أنواع فرعية من القولون العصبي بين المرضى العراقيين، بالإضافة إلى تأثير عدة عوامل على بدء المرض ونوعه وشدته مثل العمر والجنس والقلق. توصلت النتائج إلى أن أكثر أنواع المرض شيوعاً هو نوع الإمساك (IBS-c) مقارنة بالإسهال (IBS-d) والنوع المختلط (IBS-m). من بين جميع المرضى، يعتبر القولون العصبي مرضًا مرتبطًا بالجنس والعمر، حيث يكون المرضى من الإناث وفي سن مبكرة (العقد الثاني إلى الثالث) هم الأكثر تأثراً. ومع ذلك، فإن تاريخ العائلة، والحالة الاجتماعية، والحالة المهنية، والقلق، وغيرها من GIDs لم تكن بمثابة عوامل خطر للإصابة بنوع معين من القولون العصبي.

الكلمات المفتاحية: متلازمة القولون العصبي، الانواع الفرعية للقولون العصبي،المرضى العراقيين.

Abstract

Irritable Bowel Syndrome (IBS) is a functional disease of gastrointestinal tract (GIT) that affects 10-20% of the population worldwide and is distinguished by abdominal pain/discomfort and changes in bowel habits that is accompanied by diarrhea, constipation, or both. Cross-sectional study was conducted on 50 Iraqi patients with IBS who were presented at private clinics for gastrointestinal diseases (GIDs). The study lasted from November 2021 to May 2022 and aimed to determine the frequency of IBS subtypes among Iraqi patients, as well as the influence of several factors on disease's initiation, type and severity such as age, gender, and anxiety. The results found that the most common frequent type of disease is constipation type (IBS-c) in comparison with diarrheal (IBS-d) and mixed type (IBS-m). Among all patients, IBS is gender-related and age-related disease, in which patients who are females and at young age (2nd to 3rd decade) are the most affected. However, family history, marital status, occupational status, anxiety, and other GIDs didn't act as risk factors for getting certain type of IBS.

Key words: IBS, anxiety, Rome □, GAD-7.

Characterization of Irritable Bowel Syndrome Subtype in Iraqi Patients

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وصف النوع الفرعي لمتلازمة القولون العصبي لدى المرضى العراقيين

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Many reasons behind the spread of this dangerous zoonotic disease in Iraq through last years related, to increase population of the vector specially *Hylomma spp* ticks due to favorable climate and environment for its presence in addition to lack using the insecticides in the proper time from one side, beside the free animal trade and poor public health education that plays a critical role on other side. This fatal disease requires immediate attention for its timely prevention and control to limit its further expansion and that doesn't achieved without cooperation of the Ministry of Agriculture and Ministry of Higher Education and Scientific Research.

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The hemorrhage in skin and normal orifices (mouth, nose and ear) was observed as a prominent symptom in nearly all of patients which is consistently related to this disease. Sağmak, *et al*., (2019) also pointed to the same symptom on the infected patients in their study. The high fever and hemorrhage together consider a distinguish symptoms of this disease and good indicator for early diagnosis which is critical for patient survival and for preventing the spread of infection through well-documented human-to-human transmission. The most important issue should brought to the attention that, these symptoms and the high fatale cases occur for the people who are in direct contact with infected animals, and tick bites in addition to health worker by nosocomial infection which is the most dangerous factor. Many researchers reported these symptoms as Al-Shaliet, *et al*., (2022) and Sah, *et al*., (2022) who found, the health workers were the most affected with high fever, Beside they observed the mortality reached 30% which was higher than that in this study in 2022 ,which reached 27%, but lower than that in 2021,which recorded 47%. The high mortality in infected patients gave alarm to the seriousness of this disease and should be implementing various preventive measurement specially that related with control of *Hylomma spp* ticks the vector of the virus causing disease and reducing its population, and collaborating with veterinary staff to ensure the health of animals and standardizing slaughterhouse practices.

In Iraq the infection was increase with the alliance with social activities particulaly in Eid Al-adha, were large number of animals slaughter, especially in street without taking any health measure, in which the contaminated blood spread easily through the environment, this matter also mentioned in the study of Sisman (2013) and Ibrahim, *et al*.,(2014).

pointed to the presence of CCHF in middle and south of Iraq more than the north. and the high spread also, noticed in Iran by found high Chinikar, et al., (2010)infection in south and middle ,were higher than ,the north, nearly, same observation ,noticed in some of Arab countries (WHO,2019)

Since 2021, Iraq has reported a notable increase in cases and, in 2022, WHO issued an outbreak alert based on the country reporting 212 cases and 27 deaths in the first 5 months of the year, much higher than that reported in 2021(WHO, 2022)

It was noticed that, the disease affect both sexes in different ages with high incidence reached to 380 infected patients in 2022 which is about 20 times more than that in 2021 the ongoing spike in cases has been attributed to many reasons, the low slaughter house standardization with trade of contaminated meat in Iraq, the increased density of ticks and beside the decrease the campaign for eradication of insect mainly tick, this was hardening by the struck of Covid-19 which halt all the activities of ticks eradication, in the beginning of twenties.

The most affected age groups were, 15-24 year and 25-44 and low infection in low age and elderly people, this could be belong to the activity of these groups which may increase its contact with animal and vectors. The incidence in males and females were nearly the same; it appeared that, they are subjected to the similar factors. These had been reported also by Sabra (2012) and Al-Salehi (2018). According to profession, the most affected was the house holder in both years, this was unexpected, as they are mostly indoor, but it seemed they are dealing with fresh meat without taking preventive measure followed by the butchers' shop workers and Butures who were directly in contact with different types of animals and fresh meat.

The final assessment of the infection in 2022, revealed recovery of 62.8% (306 case) and death of 37.2%(74 case) as shown in Table (12)

Table (12): The Final assessment of the infection in 2022

Final situation		Total
Death	Recovery	
74	306	380

Discussion

This disease firstly appeared in Russia and Democratic Republic Congo, and then transmitted to other parts of the world and become endemic in Africa, Asia, Eastern Europe, and the Middle East (Bente, et a.l., 2013; WHO,2018; Mirembe, *et al*.,2021). A study by Tantwi, *et al*., (1980) and Al-Tikrity, *et al*., (1981) revealed that CCHF has been reported in Iraq as early as 1979 when the disease was initially diagnosed in ten patients. After those six cases were reported between 1989 and 2009; 11 cases 2010; three fatal cases were reported in 2018 (WHO, 2018).

The current study shed light on the spreading of CCHF infection in Iraq especially in the 2021 and 2022, It is clearly observed the incidence of disease was very high in 2022 in comparison with 2021. The outcome revealed that not all Iraqi governorates were affected with CCHF whether in 2021(4/18 governorates) or 2022 (16/18). It was found that, the disease in south governorates more prevailed than that of north and the highest number of cases were diagnosed in Thi Qar which considered the most infected governorate, include 15/19 (78.9%) of cases in 2021 and 164/380 (43.1%) of cases in 2022 Previously Al-Sulhi, *et al*.. (Al-Salihi, *et al*.,2012,2018,2022)

Table (8): Disruption of patients according to age groups of in 2022

Age groups by years						
1-4	5-14	15-24	25-44	45-64	65 >	Total
2	15	99	146	95	23	380

Table (9): Number of infected females and males in 2022

Sex		Total
Female	Male	
148	232	380

According to profession it was found that, the most affected criteria were in house holder (123 case), followed by butchers (62 case) and worker in butchers' shop (61) the lowest was in the children (7 cases) Table (10).

Table (10): Distribution of infection among different profession in 2022

Profession								Total
Butchers	butchers' shop workers	Livestock breeders	Governorate employee	House holder	Student	retired	children	
62	61	35	37	123	32	23	7	380

The prominent symptoms noticed on patients was fever (359 patients), followed by bleeding due to injection by syringe (109 patients) and bleeding under skin (104 Patients), whereas only 34 patients was suffered from nose bleeding (Table 11)

Table (11): Prominent symptoms of infected patients in 2022

Symptoms					
Bleeding under skin	Bleeding from ear	Bleeding from the mouth	Bleeding from the nose	Bleeding from injection by syringe	Fever
104	72	77	34	109	359

Table (7): Distribution of CCFH cases according to governorates and months in 2022

Governorate	Jan	Feb.	Mar	April	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Total
ThiQar	0	0	1	16	36	39	27	17	10	6	9	3	164
Mesan	0	0	0	0	18	9	4	1	4	0	0	1	37
Waist	0	0	0	0	13	10	0	0	1	0	1	0	25
Babylon	0	0	0	3	10	5	6	0	0	0	0	0	24
Muthna	0	0	0	2	7	4	5	1	0	0	1	0	20
Basra	0	0	0	0	3	3	5	2	1	0	1	4	19
Diywanai	0	0	0	3	3	4	0	2	0	4	2	0	18
Kerbela	0	0	0	1	3	4	0	1	1	0	4	3	17
Bag.Karch	0	0	0	0	5	4	1	0	0	1	2	1	14
Bagd-Rusafa	0	0	0	1	1	3	2	2	0	1	3	0	13
Ninawa	0	0	0	0	2	2	0	2	1	2	0	0	9
Diyala	0	0	0	0	1	1	1	1	0	0	0	0	4
Nagif	0	0	0	0	1	1	1	0	1	0	0	0	4
Erbil	0	0	0	0	1	2	0	0	0	0	1	0	4
Kirkuk	0	0	0	0	1	1	1	0	0	0	0	0	4
Dehouk	0	0	0	0	1	1	0	1	0	0	0	0	3
Anbar	0	0	0	0	1	0	0	0	0	0	0	0	1

The highest infection rate was (38.4%) of 164 case appeared in age group (25-44 year), followed by (26%) of 99 case in age group (15-24 year) whereas the lowest rate (0.5%) was detected in the group (1-4 year) with2 cases (Table 8). The males were more affected 232(61.05%) than females 148 (39%) as described in Table (9).

Regarding to a profession, the most affected criteria were house holder followed individuals work in butchers' shop (Table 4).

Table (4): Infection of individuals according to profession

Profession						Total
Butchers	butchers' shop workers	Governorate employee	Livestock breeders	House holder	Students	
2	3	2	1	8	3	19

The most important symptoms appeared on patients were fever (19), bleeding from injection by syringe (7) and bleeding from the skin (6). It was observed that, the number of recovered patients 10(52.6%) was slightly more than death 9 (47.4%) as demonstrated in Tables (5 and 6).

Table (5): Distribution of patients according to symptoms

Symptom					
Bleeding from ear	Bleeding under skin	Bleeding from mouth	Bleeding from nose	Bleeding from injection by syringe	Fever
5	6	3	5	7	19

Table (6): Final assessment of infection

Final situation		Total
Death	Recover	
9	10	19

The total of infected patents in the year 2022 was (380) cases. Mostly appeared in Thi Qar Governorate (164) discovered mainly in May and June reached to 36 and 39 respectively, followed by patients residence in Mesan governorate with 33 cases, appeared mostly in May and June (18 and 9) the lowest detected patients was in Anbar with one case only (Table 7).

Results

The infected individuals in 2021 were much lower (19) than that in 2022(380) and appeared in 4 governorates, most affected governorate was Thi Qar have been showed 15 case, and most of them (7) occurred in September and (5) in November as shown in Table (1).

Table (1): Distribution of CCFH cases according to governorates and months in 2021

Speared of infection according to governorates and months in 2021													
Governorate	Jan	Feb	March	April	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Total
Thi Qar	0	0	0	0	0	0	2	0	5	2	4	2	15
Nineveh	0	0	0	0	0	0	0	0	2	0	0	0	2
Diyala	0	0	0	0	0	1	0	0	0	0	0	0	1
Al-Muthna	0	0	0	0	0	0	0	0	0	0	1	0	1
Total	0	0	0	0	0	1	2	0	7	2	5	2	19

Most cases recorded in the age group (15-24 year) followed by (25-44) with 9 and 8 respectively, in same time the infection appeared in females (10) was slightly more than males (9) as shown in Tables (2 and 3).

Table (2): Distribution of patients according to age groups

Age group by Years						
1-4	5-14	15-24	25-44	45-64	<65	Total
0	0	9	8	2	0	19

Table 3: Infection in females and males

Sex		Total
Females	Males	
10	9	19

A review in the Arab world by Perveen and Khan (2022) included epidemiology, transmission, distribution, mortality, and clinical features of CCHF in 22 Arab countries, comprising the Arab world were conducted. Based on the analysis of 57 studies published from 1978 to 2021, found 20 tick species that could be associated with CCHFV transmission. During the 43-year period, 321 cases of CCHF were reported from 9/22 Arab countries, Iraq, Kuwait, UAE, Saudi Arabia, Oman, Sudan, Egypt, Tunisia, and Mauritania. The mean case fatality rate was 29% during various outbreaks. Individuals working in abattoirs/ slaughter houses, livestock farms, and healthcare were most at risk. Contact with blood or body secretions from infected animals and patients was the most common. Also CCHFV serological evidence has been recorded from 11 Arab countries including Iraq, Kuwait, UAE, Saudi Arabia, Oman, Sudan, Egypt, Tunisia, Algeria, Mauritania, and Morocco. However, deaths were reported in only seven counties, Mauritania, Oman, UAE, Saudi Arabia, Egypt, Iraq, and Sudan. Fatality rate ranged from 24 to 61% (mean: 29%) during the different outbreaks.

The aim of current research is highlighted the incidence of CCHF in Iraqi governorates through last few years.

Materials and Methods

The study depend on the data received from CDC - Ministry of Health during the period extended from the beginning of 2021 till the end of 2022, concerning the infection in all Iraqi governorates, in which the hemorrhagic fever prevail, taking into consideration, monthly spread in the governorates, Age, sex (male and female), professional, risk factors, symptoms, morbidity and mortality as mentioned in CDC protocol, depending on examination of serum samples of patients using Enzyme linked Immunosorbent Assay) ELISA)

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an important tick-borne viral infection with a fatality rate of up to 50% during outbreaks caused by Nirovirus belongs to the family Bunyaviridae (Belobo *et al*., 2021). This disease is considered as dangerous zoonotic disease transmitted from different field animals to human by ticks, *Hyalomma* spp ticks are the major source of human infection (Tylor,196). Crimean-Congo hemorrhagic fever virus (CCHFV) is sustained in the ecosystem in benign form through vertical and horizontal transmission cycles involving tick vectors, wildlife and livestock as reported by Ibrahim *et al*.(2014). The CCHF occurs most often among butchers, slaughterhouse workers, and farmworkers through infected tick bites or/and contact with blood and tissues of infected livestock. The nosocomial transmission can occur in auxiliary nurses and physicians through contact with the infected patients (Tsergouli *et al*.,2019). The widespread distribution of CCHFV most probably occurred by ticks on migratory birds, or through international travel and trade of livestock and wildlife (Tylor, 1967).

In 1979, a 24 year-old lady was diagnosed with CCHF at Al-Yarmouk hospital, Baghdad, making her the first confirmed case of CCHF in the country, later two close contacts, one physician and one health worker also contracted the infection and subsequently died (Tintawi *et al*.,1980,Tikrete *et al* .,1981). Thereafter, CCHF cases were reported in Iraq in different periods from 1980 to 2014 (Alsalehi *et al*. ,2022) and most of the cases had a history of contact with animals and others were physicians/health workers. Tantawi *et al*. (1981) carried out a study to determine the prevalence of CCHFV in animals and most of the animals tested positive with high prevalence for antibodies to the virus.

المستخلص

حمى القرم – الكونغو النزفية هي احد اهم الامراض المعدية في العالم و يعد هذا المرض من اخطر الامراض المشتركة اذ ينتقل من االحيوانات الى الانسان عن طريق القراد نوع الهايلوما. سجلت اول حالة في العراق في 1979 والتي ادت الى وفاة المريضة وطبيبها وبعض الطاقم الطبي في بغداد- مستشفى اليرموك. اجريت الدراسة الحالية ل 399 مريض يعانون من اعراض الحمى النزفية بناءا على المعلومات المستلمة من مركز الامراض الانتقالية خلال سنتين(2021-2022) المعتمدة على الفحص المصلي . بينت النتائج أن عدد المرضى الذين اصيبوا فى سنة 2022 اعلى من سنة 2021 و اكثر المحافظات اصابة كانت ذي قار ,واسط , نينوى خاصة خلال شهرى ايار وحزيران. وقد وجد ان المرض يصيب كلا الجنسين و لجميع الاعمار واكثر المجاميع العمرية اصابة هي (25-44) سنة, تليها المجموعة العمرية 19-24 سنة كذلك ,وان الاصابات عند الذكور (232) اعلى مقارنة مما عليه عند الاناث 148, وكانت اهم الاعراض هي الحمى و النزف من الفتحات الطبيعية . أكدت النتائج ان الفئات الاكثر خطورة للاصابة بالمرض هم, ربات البيوت ,والاشخاص الذين بتماس مباشر مع الحيوانات او منتجاتها مثل القصابين والمربين. وأخيرا ان اهم الاجراءات الوقائية من هذا المرض الخطيرلمنع الاصابة او التقليل من انتشارها تتضمن, التشخيص المبكر للمرض عند الانسان, الثقافة الصحية المجتمعية واستخدام المبيدات الحشرية للسيطرة على القراد.

الكلمات المفتاحية: حدوث حمى القرم - الكنغو النزفية, والامراض المشتركة, نيرو فايرس, قراد هايلوما, القصابين و ربات البيوت

Abstract

Crimean-Congo hemorrhagic Fever (CCHF) is one of the most important infectious diseases in the world. This disease is considered as a dangerous zoonotic viral disease transmitted from animals to human by *Hyalomma* ticks. The first case was registered in Iraq in 1979, which led to the death of the patient, her doctor and some of the medical staff in Baghdad/Al-Yarmouq hospital. The current study was conducting on the data of (399) patients suffering from symptoms of hemorrhagic septicemia for two years (2021and 2022) which received from Central Disease Control (CDC) in Baghdad depending on serological test. The results revealed that the number of patients who were registered in (2022) was higher than in (2021), the most affected governorates were Thi Qar, Waist and Nineveh in particular at June and May. It was found that, the disease affect both sexes in different ages, and the most affected age group (25-44) year followed by (19-24) also the highest infections were in males (232) compared to females (148). The fever and bleeding from natural opening were the prominent symptoms appeared on patients. The result confirmed that, the categories at high risk of infection were householders, individuals who had contact with the animals as butchers and breeders. Finally the important preventive measures for this dangerous disease should be taken to prevent infection or reduce its spread which include, early diagnosis the disease in human, public medical health education, and using insecticide for tick control.

Keywords: CCHF, Zoonotic disease, Nirovirus, Hyalomma ticks, Butchers and House holder

Incidence of Crimean-Congo Hemorrhagic Fever (CCHF) in Different Governorates of Iraq

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حدوث حمى القرم ـ الكنغو النزفية في محافظات مختلفة من العراق

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Conclusion

This article presents a literature review on five medications Glimepiride, Acarbose, Repaglinide, Glipizide and Metformin. It is particularly relevant in HPLC, as demonstrated in Tables 1-5. The study highlights the importance of determining diabetes drugs such as glimepiride, acarbose, repaglinide, glipizide, and metformin using high-performance liquid chromatography (HPLC). The study demonstrated the great effectiveness of this technology in analyzing these medications accurately and reliably, which contributes to improving the management of drug treatment for diabetes patients. The results also show the importance of determining the limits of detection (LOD) for these drugs, as this contributes to ensuring the delivery of accurate and safe doses, which enhances the effectiveness of treatment and reduces the potential risks of over- or under-doses.

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Table 5: HPLC methods for assay of repaglinide

No.	Stationary phase	Mobile Phase	Detection	FR (mL/ min)	RT (min)	Detection limit	Ref.
1.	3mmX 15cm column containing 5µm packing L20	Isopropyl alcohol: Hexane: glacial acetic acid (10:90:0.1)	UV detection at 228nm	0.5			(22)
2.	Shim-pack, RP-C18 column	methanol: 0.1% v/v triethylamine (pH adjusted to 7 with orthophosphoric acid)	UV detection at 235nm	NA	3.40	NA	(37)
3.	STAR C-18 analytical column (4.8mm×150 mm; 5 _m particle size).	The mobile phase composed of acetonitrile–ammonium formate (pH 2.7; 0.01 M) (60:40, v/v)		1.0	6.2	3.90 to 6.67%.	(38)
4.	µ-bondapack C18 column	acetonitrile-methanol-potassium dihydrogen phosphate	244	1.5	4.0	NA	(39)

Table 4: HPLC methods for assay of acarbose

No.	Stationary phase	Mobile Phase	Detection	FR (mL/ min)	RT (min)	Detection limit	Ref.
1.	3mmX 15cm column containing 5µm packing L20	Isopropyl alcohol: Hexane: glacial acetic acid (10:90:0.1)	UV detection at 228nm	0.5			[22]
2.	Column Lichrospher® –100–NH2 , 5 µm, 250 × 4.6 mm	acetonitrile–phosphate buffer	210	2			(34)
3.	An Alltima C18 column (4.6 mm×250 mm, 5 µm)	acetonitrile-10 mmol·L-1 ammonium dihydrogen phosphate (containing 0.04% sodium 1-octanesulfonate, adjusted pH to 3.3 with H3PO4) (15:85	200nm	0.8			(35)
4.	through thermo BDS (250mm 4.6mm, 5µ).	Buffer and Acetonitrile in the ratio of 35:65A	215nm	1.0	4.1	1.3ppm	(36)

Table 3: HPLC methods for assay of glipizide

No.	Stationary phase	Mobile Phase	Detection	FR (mL/ min)	RT (min)	Detection limit	Ref.
1.	3mmX 15cm column containing 5µm packing L20	Isopropyl alcohol: Hexane: glacial acetic acid (10:90:0.1)	UV detection at 228nm	0.5			(22)
2.	Grace Smart Altima C-8 column	acetonitrile : phosphate buffer (60: 40 (v/v), pH 3.0)	235 nm	1.0	2.57 min, 7.06 min, and 9.39 min		(31)
3.	C18 (250 mmL×4.6 mm I.D × 5µ)	acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15v/v/v)	UV detection at 239 nm	1.0	3.55 and 5.82	0.0040 ppm and 0.025 ppm	(32)
4.	y C18 column (75*4.6 mm, 3.5 m	ning Brij-35 (12.05 mM) and SDS (76.25 mM) at pH 3	225 nm 210nm and	1.0	2.7 min	0.4 µg/Ml	(33)

Table 2: HPLC methods for assay of metformin

No.	Stationary phase	Mobile Phase	Detection	FR (mL/ min)	RT (min)	Detection limit	Ref.
1.	3mmX 15cm column containing 5µm packing L20	Isopropyl alcohol: Hexane: glacial acetic acid (10:90:0.1)	UV detection at 228nm	0.5			(22)
2.	Column C18 250 x 4.6 mm, 5 µm	TEA buffer (50:50 v/v)	272 nm	1.0	3.6 min		(27)
3.	CN 250 mm × 4.6 mm × 5µ	ammonium formate buffer (pH 3.5) and acetonitrile at a ratio of (45:55, v/v)	227 nm	1.0			(28)
4.	C18 (250 mm×4.6 mm, 5 µ)	acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15v/v/v)	UV detection at 239 nm	1.0	3.55 and 5.82	0.0040 ppm and 0.025 ppm	(29)
5.	C18 column (150mm× 4.6 mm, 5 µm)	0.1% ortho-phosphoric acid buffer (pH 2.7): acetonitrile (65:35% v/v)	224 nm	1.0	2.170	0.025 ppm	(30)

Table 1: HPLC methods for assay of glimepiride

No.	Stationary phase	Mobile Phase	Detection	FR (mL/ min)	RT (min)	Detection limit	Ref.
1.	3mmX 15cm column containing 5µm packing L20	Isopropyl alcohol: Hexane: glacial acetic acid (10:90:0.1)	UV detection at 228nm	0.5	NA	NA	(22)
2.	C18 (250 mm×4.6 mm, 5 µ)	,, Sodium hexanesulfonate buffer adjusted to pH 2.5 with ortho-phosphoric acid and acetonitrile (45:55 v/v),,	UV detection At 229 nm	1.0	3.55 and 5.82	150-750 and 0.75-4.5 ppm	(23)
3.	C18 Hybrid) UPLC (100mm x 2.1mm ,1.8µm)	,,0.1% ortho phosphoric acid buffer (the pH was adjusted to 3.4 with 0.1 N NaOH) and methanol in the ratio 40:60% v/v,,	UV detection at 254 nm	0.25ml/ min			(24)
4.	C8	Methanol , KH 2PO4 adjusted to pH 3.2 utilizing ortho - phosphoric acid (70: 30, v/v)	UV detection at 235 nm	1.0	3.06, 4.33 and 6.00	0.05, 1.21 and 0.11 ppm	(25)
5.	C18 column (4.6 mm × 100 mm; 2.5µm)	methanol: phosphate buffer (pH corrected to 3.2 with 80% ortho phosphoric acid) in the ratio of 60:40 v/v	UV detection at 254 nm	1.0			(26)

inhibitors alpha: This class of drugs includes acarbose, miglitol, and voglibose. They work by inhibiting carbohydrate digestion in the small intestine by inhibiting enzymes that break down polysaccharides. Thiazolidinediones, Pioglitazone and rosiglitazone are part of this class(13-14). Through the activation of PPAR-γ in fat and muscle, they lessen insulin resistance. SGLT2 inhibitors: These medications cause increased glucose excretion in the urine by blocking the kidneys' sodium-glucose transport protein 2. (15—21).

In summary, diabetes medications are classified based on their mechanisms of action and chemical structures into different classes like sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and SGLT2 inhibitors. The aim of the review have compiled a list of published methods for the measurement of glimepiride in pharmaceutical formulations, discussed the importance of estimation, and listed important new methods in this regard name methods such as high-performance chromatography (HPLC) , and spectrophotometry, which are the most common methods. Glimepirides synthesized with various polymers have also been described.

Assay Methods for HPLC

Technique High-performance Liquid Chromatography for estimating some drugs for diabetes mellitus by using HPLC(21) that documented in literature, which is for determining drug DM. The literature review's illustrated in Tables 1-5 display an efficient, simple, and sensitive HPLC approach for the validation and development of a singles pharmaceutical, in addition to information on the stationary phase as well as the mobile phase, utilized, as well as their retention duration and flow rate with UV detection

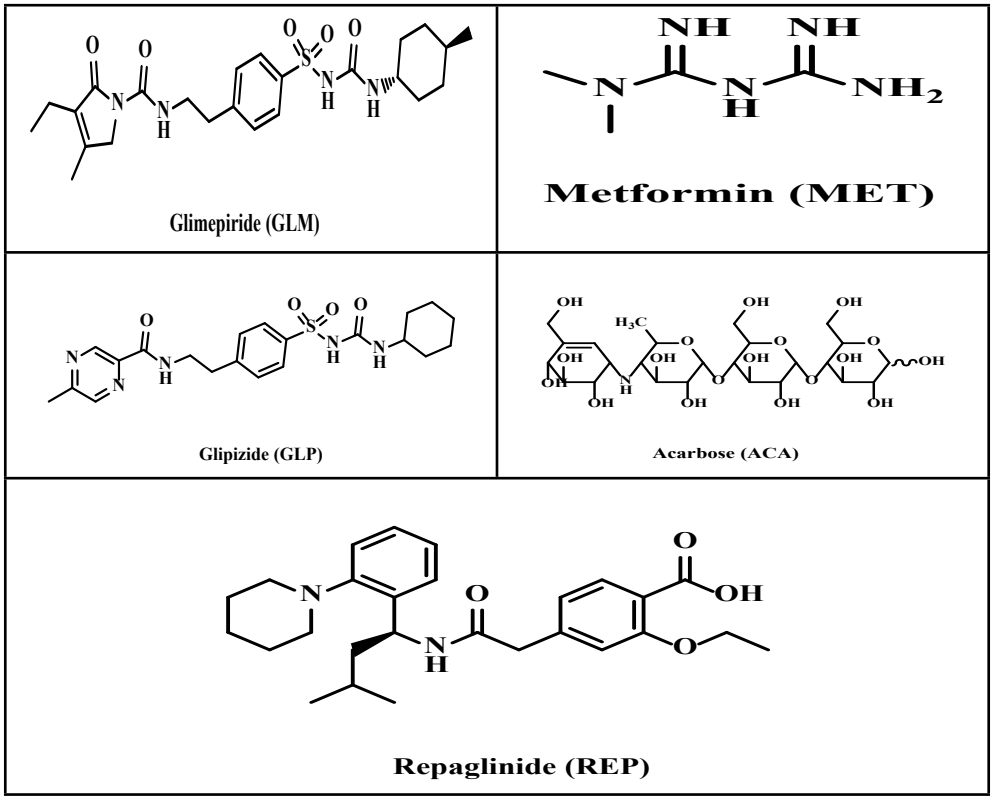

Figure (1): Structural representation of Glimepiride (GLM). Metformin (MET), Glipizide (GLP), acarbose (ACA), Glibenclamide (GLB), and, Repaglinide (REP).

Classification of Diabetes medication according their chemical structure

Different pharmacological classes are included in the classification of diabetes medications based on their chemical structures. sulfonylureas, Medication such as glimepiride, glipizide, and glyburide is included in this class. Sulfonylureas function by inhibiting the K ATP channel, which causes pancreatic beta cells to produce more insulin. (11-12). Metformin is categorized under the biguanides. Metformin increases AMPK signalling in the liver, which lowers gluconeogenesis and insulin resistance. Glucosidase

Introduction

Diabetes mellitus is a collection of diseases distinguished by alterations in glucose, protein, and lipid metabolism(1). Although additional variables might be implicated, the primary disruption in diabetes mellitus is an imbalance in insulin synthesis either action or both. This mostly causes increased Postprandial blood glucose levels and fasting. Diabetes mellitus has become a frequent condition that has had a major impact on human health in recent years. Diabetes affects an estimated 150 million people globally, and this estimate is expected to more than multiply by 2030(2). Diabetes mellitus is one of the major causes of mortality worldwide, according to "World Health Organization (WHO)" estimations, with those most at risk living in South East Asia and the Western Pacific. As a consequence, the world looks to be under the control of a diabetic epidemic (3). Approximately 90% of diabetic individuals who have diabetes mellitus (non-insulin-dependent) have type II diabetes (4). It is particularly important to assure the quality of anti-diabetic medications for type II diabetes, which has a less severe insulin deficit. Anti-diabetic drugs. Sulfonylurea medications (Glipizide (GLP), Glibenclamide (GLB), Gliclazide (GLC), and Glimepiride (GLM)) work by increasing the synthesis of insulin by pancreatic beta cells (5). This generation of hypoglycemic pharmaceuticals is far more powerful and hence effective at considerably lower doses (6). Repaglinide (REP) similarly stimulates cell insulin secretion, but it binds to locations other than the binding sites for sulfonylureas (7). Nateglinide (NGL) enhances the sensitivity of pancreatic cells to ambient glucose without increasing basal insulin production (8-9).

المستخلص

ان مرض الايضي المعروف باسم داء السكري (DM)يحدث بواسطة انخفاض إفراز الجسم و/أو نشاط الأنسولين الهرموني. مع تفاقم الحالة ، تظهراعتلال مرضي في الجسم في الجسم ، مثل اعتلال الكلية ، اعتلال الشبكية ، ومشاكل القلب والأوعية الدموية ، أمرًا لا مفر منه. النوع الأول والثاني DM هما النوعان الفرعيان الأساسيان من DM. تستخدم ادوية السكر عن طريق الفم لعلاج مرض السكري من النوع الثاني ، في حين يتم استخدام علاج الأنسولين عادة لعلاج مرض السكري من النوع الأول. يتلقى المرضى عادة علاجات دوائية مزدوجة, على الرغم من استخدام أدوية نقص السكر في الدم عن طريق الفم كعلاج أحادي ، لم يتمكنوا من تحقيق أهدافهم العلاجية مع العلاج من الخط الأول. في الدراسة المرجعية الحالية ، جمعت الطرق التحليلية المنشورة كمراجعة لتقدير الادوية الخاصة بمرض السكري في المستحضرات الصيدلانية. تم البحث عن معظم التقنيات المستخدمة على نطاق واسع مثل (HPLC) كما في الجدول1-5. تُظهر هذه الجداول ملخص طرق HPLC المستخدمة في تقدير ادوية مرض السكري و الأدوية مجتمعة على التوالي تشير إلى ظروف HPLC . هذه المعلومات المجدولة مفيدة لجميع الباحثين الذين يعملون حاليًا على دواء مرض السكري. لذلك يجب أن تكون هذه الورقة مفيدة للغاية لتطوير طريقة صياغة جديدة لدواء مرض السكري للدواء وتقديره.

الكلمات المفتاحية: مرض داء السكري، كرموتوغرافيا السائل عالية الاداء، ادوية مرض السكر (كلمبرايد، متفورمين، كلبازايد، اكاربوس، كلبني كلمايد والريباكلينايد)

Abstract

A metabolic illness known as diabetes mellitus (DM) is brought on by the body's decreased secretion and/or activity of the hormone insulin. The condition worsens, pathological alterations in the body, such as nephropathy, retinopathy, and cardiovascular problems, become inevitable. Type I and II DM are the two primary subtypes of DM. Oral hypoglycaemics are used to treat type II diabetes, while insulin replacement therapy is typically used to treat type I diabetes. Patients typically receive dual drug treatments. Despite using oral hypoglycemic medications as monotherapy, have not been able to accomplish their therapeutic goals with first-line therapy.

In the current research, compiled the analytical methods reported for the estimation of some drugs diabetes in formulations for medicines. Most extensively used Technique like High-performance liquid chromatography (HPLC) was reported in Table1, 2,3,4 and 5. These Tables show an overview of the reported HPLC methods for drugs of diabetes in combination respectively indicating the HPLC conditions.

Keywords: Diabetes Mellitus (DM), High-Performance Liquid Chromatography (HPLC), Glimepiride (GLM), Metformin (MET), Glipizide (GLP), Acarbose (ACA), Glibenclamide (GLB), and Repaglinide (REP).

High-performance Liquid Chromatography Analytical Techniques for Determining the Various Diabetic Type 2 Medications: A Review Article

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تقنيات كروماتوغرافيا السائل عالية الأداء لتقدير أدوية مرض السكري المختلفة \ دراسة مرجعية.

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