Republic of Iraq

Ministry of Higher Education & Scientific Research

University of Thi-Qar

College of Science

Study of Homocysteine Levels and Some Biochemical parameters in Pregnant Women in Thi-Qar Governorate

A Thesis

Submitted to the College of Science University of Thi-Qar in Partial Fulfillment of the Requirements for the Master Degree of Science in Biochemistry

By

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B.Sc. in Chemistry (Thi – Qar University) 2008

Supervisor

Asst. Prof. Dr.

Jamal Harbi Hussein Alsaadi

2021A.D **1442A.H**

بسم الله الرحمن الرحيم

{ قال رَبِّ اشْرَحْ لِي صَدْرِي
(25)
وَيَسِّرْ لِي أَمْرِي (26)
وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (27)
يَفْقَهُوا قَوْلِي (28) }

صدق الله العلي العظيم

سورة طه ٢٥-٢٨

The Ministry of Higher Education & Scientific Research
University of Thi-Qar
College of science
Dean's Office

وزارة التعليم العالي والبحث العلمي
جامعة ذي قار
كلية العلوم
مكتب العميد

Ref:
Date: / / 20

العدد: ٢٠٠٥
التاريخ: ٢٠٢١/٦/٢٤

الى / رئاسة قسم الكيمياء / كليتنا

م/ استلال الكتروني

تحية طيبة ...

إشارة الى طلبكم في تاريخ ٢٠٢١/٦/١٤ نحيل اليكم تقرير استلال المعد قبل اطلاع المقوم العلمي لرسالة طالبة الماجستير (رؤى حسن ناصر) والموسومة :

Study of Homocysteine Levels and Some Antioxidants in Pregnant Women in Thi-Qar Governorate

والذي بلغت نسبة استلاله (١١٪) فقط.

... مع خالص التقدير ...

المرفقات:

- تقرير ورقي
- قرص CD

23.6.2021

أ.م.د. هيثم عبدالأمير ميناس
العميد وكالة

نسخه منه إلى:

- مكتب السيد العميد المحترم/للتفضل بالاطلاع.....مع التقدير.
- ملفة الاستلال.
- الصـــــادرة.

استمارة تدقيق الاستلال للرسائل والاطاريح الجامعية

جامعة ذي قار – كلية العلوم

رقم كتاب لجنة الاستلال (في القسم) : تاريخه :

الرسالة/ الاطروحة الموسومة: Study of Homocysteine Levels and Some Antioxidants in Pregnant Women in Thi-Qar Governorate

الاختصاص العـام للرسالة / الاطروحة:

الاختصاص الدقيق للرسالة / الاطروحة:

المحتويات الخاضعة لتحديد نسبة الاستلال	نسبة الاستلال المعتمدة من قبل الوزارة	النسب الواردة في تقرير برنامج الاستلال
١- عنوان الرسالة / الاطروحة ٢- الخلاصة ٣- النتائج ٤- الاستنتاجات ٥- التوصيات	(صفر%)	٠٪ فقط
١- طرق البحث	لا تزيد عن (5%) لرسائل الماجستير واطاريح الدكتوراه	
١- الجانب العملي ٢- بناء المشكلة وتصميمها ٣- الفرضيات ٤- الخوارزميات	○ (صفر%) لاطاريح الدكتوراه ○ لا تزيد عن (5%) لرسائل الماجستر	٥٪ فقط
١- المقدمة ٢- الجانب النظري ٣- ادبيات البحث	لا تزيد عن (10 %)	٦٪ فقط

ملاحظات هامة :

١- التأكد من عدم اقتباس فقرات كاملة نصا الا مع وجود تبرير مثل التعاريف المقرة رسميا او دوليا والا يتوجب إعادة الصياغة.
٢- لا تسلم الرسالة / الاطروحة من قبل القسم العلمي لأغراض التقويم العلمي وتشكيل لجنة المناقشة ما لم تحقق النسب الواردة في أعلاه.
٣- مصادقة وتوقيع رئيس القسم وأعضاء اللجنة على استمارة التدقيق، حيث يكتب إضافة للقب العلمي امام كل عضو من أعضاء لجنة الاستلال بما فهم رئيس اللجنة الاتي :

- الاسم :
- التخصص العام :
- التخصص الدقيق :

ختم وتوقيع رئيس القسم واعضاء اللجنة على استمارة تدقيق استلال البحوث والاطاريح الجامعية

نسبة الاستلال الكلي (١١٪) فقط

23-6-2024

أ.م.د. هيثم عبدالامير ميناس

العميد وكالة

استمارة تدقيق الاقتباس النصي للرسائل و الاطاريح الجامعية

عنوان (الرسالة / الأطروحة) Study of homocysteine levels and some antioxidants in pregnant women with Thi-Qar Governorate

الاختصاص العام للرسالة/الأطروحة :

الاختصاص الدقيق للرسالة /الأطروحة:

المحتويات الخاضعة لتحديد نسب الاقتباس النصي	نسبة الاقتباس النصي (%)
عنوان الرسالة / الأطروحة	% صفر
الخلاصة	
النتائج (*)	
الاستنتاجات	
التوصيات	
طرق البحث	
الجانب العملي (*)	% ٤
بناء المشكلة و تصميمها	
الخوارزميات(*)	
المقدمة	% ٥
الجانب النظري	
ادبيات البحث	
المجموع الكلي لنسبة الاقتباس يجب ان لا يزيد عن (١٥%)	

١- (*) باستثناء العبارات الشائعة الاستخدام في مجال الاختصاص عند فحص المحتويات

٢- استنادا الى كتاب دائرة البحث و التطوير المرقم (ب ت ٥٨٦٨/٥) في ٢٠١٥/٧/٢٧ الفقرة (٨) تعتمد نسبة ١٥% للاقتباس العلمي من اجمالي الرسائل / الأطاريح الجامعية على ان لا تتجاوز (٥%) من مصدر واحد.

٣- في حالة وجود نسبة اقتباس أكثر من (١٥%) تعاد الرسالة / الاطروحة الى الطالب لغرض تقليل نسبة الاقتباس.

٤- في حال وجود انتحال (سرقة علمية (تذكر بالتفصيل و المصادر التي تم السرقة منها) و يتحمل الطالب و المشرف مسؤوليتها و تعامل على اساسها كحالة غش .

ملاحظة :-
لا تستلم الرسالة / الأطروحة من قبل (القسم / الفرع) العلمي في الكلية لأغراض التقويم العلمي و تشكيل لجنة المناقشة مالم تحقق النسب الواردة في أعلاه.

ت	الصفة في اللجنة	الاسم واللقب العلمي	الاختصاص العام/الدقيق	محل العمل	التوقيع
١	رئيساً	م.د سعد [illegible]	كيمياء / فيزياوية	كلية العلوم	[illegible]
٢	عضواً	م.د. زينب مجيد كريم	احياء / كيمياء حياتية	كلية العلوم	[illegible]
٣	عضواً	م.د. [illegible]	[illegible]	كلية العلوم	[illegible]

جامعة ذي قار
كلية العلوم

وزارة التعليم العالي
والبحث العلمي

**استمارة تعهد الالتزام
بأخلاقيات البحث العلمي**

نتعهد بالتقيد التام بما جاء في مضمون كتاب وزارة التعليم العالي والبحث العلمي/جهاز الإشراف والتقويم المرقم ج ت/425 الصادر بتاريخ 2014/3/24 والمبلغ الينا بكتاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 والصادر بتاريخ 2014/3/31 عند أعداد الرسالة أو الأطروحة الموسومة

Study of Homocystene levels and some antioxidants in pregnant women in Thi Qar governorate

وبخلافه نتحمل نحن الموقعين أدناه المسؤولية القانونية حال وجود ما يخالف مضمون الكتاب أعلاه أو ما ينافي أخلاقيات البحث العلمي.

	الاسم	القسم والكلية أو المعهد	التوقيع	التاريخ
1- المشرف الأول	أ.م.د. جمال حرزي حسين	الكيمياء / العلوم	[signature]	/ /
2- المشرف الثاني				/ /
3- طالب الدراسات	رؤى حسن ناصر	الكيمياء / العلوم	[signature]	/ /

ملاحظات هامة

1- تعتبر استمارة التعهد هذه جزء من متطلبات إعتماد مشاريع طلبة الدراسات العليا وتوزيع المشرفين على الطلبة.
2- تطبق استمارة التعهد هذه على السادة المشرفين على الرسالة أو الأطروحة وعلى طلبة الماجستير والدكتوراه.
3- السطو العلمي هو سرقة نتاج فكري وعند الاقتباس ضرورة الإشارة للمصدر وفي حالات الاقتباس ضرورة إعادة صياغة الفقرة المقتبسة دون الخروج من حدود فكرة الفقرة الأصلية المقتبسة والمشار لمصدرها في متن الرسالة أو الأطروحة.
4- تشمل استمارة التعهد هذه الرسائل والأطاريح التي لم يتم مناقشتها حتى تاريخ كتاب جهاز الإشراف والتقويم أعلاه.
5- أعدت استمارة التعهد هذه استنادا للفقرة-4 من كتاب وزارة التعليم العالي والبحث العلمي/جهاز الإشراف والتقويم المرقم ج ت/425 الصادر بتاريخ 2014/3/24 والمبلغ الينا عن طريق كتاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 بتاريخ 2014/3/31

جامعـــــة ذي قار
كليـــــة العلـــوم
القسم: ....

استمارة المقوم الاحصائي

Thi-Qar University

عنوان الرسالة/الاطروحة: Study of Homocystein levels and some Antioxidants in pregnant women in thiqar Governorate

السنة الدراسية: | **تاريخ التسليم:** 2021/7/ | **تاريخ الاستلام:** 2021/7/15 | **التقييم**

التقييم		السؤال
5%	متقدم ☐ متوسط ☑ بسيط ☐ لا يوجد ☐	1- مستوى التحليل الاحصائي المستخـــــدم؟
10%	تحليلية ☑ وصفية ☐	2- نوع تصميم الدراسة الاحصائيـــــــة؟
10%	نوعي ☐ كمي ☑	3- نوع المتغير المستخـــــــــم؟
10%	تجريبية ☑ توقعية ☐ تشخيصية ☐ تقييمية ☐	4- نوع الدراسة الاحصائيـــــــة؟
10%	ملائم جدا ☐ ملائم ☑ نوعا ما ☐ غير ملائم ☐	5- ملائمة نوع تصميم الدراسة للبيانات المستخدمة؟
10%	كلا ☑ نعم ☐	6- هل تم التحقق من توفر شروط الاختبارات الإحصائيـة قبل استخدامهـــــا (على سبيل المثـــــال اعتداليـة التوزيع والاستقلاليـــة)؟ اذا كانت الإجابة ب(نعم) تذكر الأساليب المستخدمة في التحقق: .1 .2
10%	كلا ☐ نعم ☑	7- هل ان حجم العينة المستخدمة في التحليل الاحصائي مناسب للتحليل الاحصائي؟
10%	كلا ☐ نعم ☑	8- هل تم استخـدام التحليـــل الإحصـــــاني بالشكل الصحيــــح؟
10%	كلا ☐ نعم ☑	9- التحليل يحقق الهدف الرئيسي من الدراســـــة؟
10%	نعم (اذكر اسمائها) SPSS كلا ☐	10- أستخدمت برامج احصائية ؟
5%	كلا ☐ نعم ☑	11- هل ان نتائج التحليل الاحصائي تدعم ما تم توصل اليه الباحث من استنتـــاجات؟
	كلا ☑ نعم ☐	12- هل تقترح أضافـــات للتحليـــل الاحصائي الموجود في الرسالـة او الأطروحة؟ اذا كانت الإجابة بنعم تذكر الفقرات الإضافية: .1 .2 .3

المجموع 100%

درجة التقييم كتابة: ــــ
درجة التقييم رقما:
اسم المقوم الاحصـــائي: د. رياض حسن كشكه
اللقب العلمي: أستاذ
الاختصـــاص الدقيق: احصاء رياضي
العنـــوان: كلية علوم الحاسوب والرياضيات
توقيع المقوم الاحصائي:

ختم وتوقيع الشعبـــة القانونية في كلية المقوم:
أسم م.الشعبة القانونية:

هل تقترح أضافة مناقش إحصائي الى لجنة المناقشة نعم ☐ كلا ☑

ملاحظة: لا تتجاوز فترة التقييم أسبوعين من تاريخ أستلامها وبخلافها يتم استبدال المقوّم

أسم وختم وتوقيع رئيس القسم

أسم وختم وتوقيع م.العميد للشؤون العلمية والدراســـــات العليـــا

أعدت الاستمارة: 1) أستنادا للامر الجامعي: ذي العدد 10553 بتاريخ: 2018/9/3، 2) وأشاره الى كتاب وزارة التعليم العالي والبحث العلمي ذي العدد: ج ع/ ق/ 2631 بتاريخ:16/ 2018/8

نموذج رقم-1-

وزارة التعليم العالي والبحث العلمي
جامعة ذي قار/ كلية العلوم
شعبة الشؤون العلمية والدراسات العليا
العدد: ٣/ ١٧٣ (ت ٥٠)
التاريخ: ٢٧ / ٧ /2021

أمر إداري

م/ تشكيل لجنة مناقشة

استناداً لمصادقة السيد رئيس الجامعة على محضر الجلسة الاعتيادية الصباحية التاسعة المفتوحة (الاجتماع الاول) لمجلس كليتنا المنعقدة بتاريخ (2020/8/19) بموجب كتاب رئاسة جامعة ذي قار /امانة مجلس الجامعة السري ذي العدد (4س /744) في 2020/9/7 والخاصة بالفقرة (ثالثا-3) المتضمنة تخويل السيد عميد الكلية صلاحية تشكيل لجان مناقشة طلبة الدراسات العليا (الماجستير والدكتوراه) و استنادا للصلاحيات المخولة لنا تقرر تشكيل لجنة مناقشة طالبة الدراسات العليا / الماجستير في قسم الكيمياء بكليتنا (رؤى ناصر حسين) عن رسالته الموسومة:-

(Study of Homocysteine levels and some antioxidants in pregnant women in Thi-Qar governorate)

من السادة الذوات المدرجة اسمائهم أدناه:-

ت	اللقب العلمي	الاســـــم	الشهادة	التخصص الدقيق	محل العمل	نوع التكليف في اللجنة
1	استاذ مساعد	لمياء ماجد حميد	دكتوراه	الكيمياء الحياتية	كلية الطب / جامعة ذي قار	رئيسا
2	استاذ مساعد	فاطمة صيوان صباح	دكتوراه	الكيمياء الحياتية	كلية العلوم / جامعة البصرة	عضوا
3	استاذ مساعد	هديل رشيد فرج	دكتوراه	الكيمياء الحياتية	كلية العلوم / جامعة ذي قار	عضوا
4	استاذ مساعد	جمال حربي حسين	دكتوراه	الكيمياء الحياتية	كلية العلوم / جامعة ذي قار	عضوا ومشرفا

علماً أن الرسالة قومت علميا من قبل أ.د. عدنان جاسم محمد – كلية العلوم / جامعة البصرة و أ.د. عباس دواس مطر / كلية التربية للعلوم الصرفة / جامعة البصرة ولغويا من قبل م.د. محمد عبد العلي عباس / كلية العلوم جامعة ذي قار واحصائيا من قبل أ.د. رياض رستم محسن / مليةعلوم الحاسوب والرياضيات / جامعة ذي قار وستجري المناقشة يوم الثلاثاء الموافق 17 / 8 / 2021 الساعة التاسعة صباحا وعلى قاعة (سمنار قسم الكيمياء) وتكون المناقشة الكترونية في حال الحظر الشامل شريطة توفر وسائل اتصال مناسبة فضلا عن تسجيل المناقشة بالكامل.

أ.م.د. هيثم عبد الامير ميناس
العميد وكالة

نسخه منه إلى:

- وزارة التعليم العالي والبحث العلمي / جهاز الإشراف والتقويم العلمي / للتفضل بالاطلاع ..... مع التقدير.
- رئاسة جامعة ذي قار /قسم شؤون الدراسات العليا للتفضل بالاطلاع ..... مع التقدير.
- مكتب السيد العميد / للتفضل بالاطلاع .....مع التقدير.
- مكتب السيد معاون العميد للشؤون العلمية / للتفضل بالاطلاع .....مع التقدير.
- شعبة الاعلام / للتفضل بالاطلاع .....مع التقدير.
- قسم الكيمياء مذكرتكم بالخصوص بتاريخ 25 / 7 / 2021 للتفضل بالاطلاع ..... مع التقدير.
- موقع الكلية الالكتروني لنشر اعلان حول تفاصيل المناقشة ..... مع التقدير.
- السادة الذوات رئيس وأعضاء اللجنة المحترمون / للتفضل بالاطلاع .....مع التقدير.
- المالية / التدقيق / لاتخاذ ما يلزم وصرف مستحقات لجنة المناقشة والبالغة (75000) لكل واحد منهم و(15000) وخمسة عشر الف دينار لكل من المقومين العلمي واللغوي .
- الادارية لملفا لا صدار امر اداري بالصرف.....مع التقدير.
- شعبة ضمان الجودة والاعتماد للتفضل بالاطلاع .....مع التقدير.
- شعبة الدراسات العليا.
- ملفة الطـــــــالب
- الصـــــادرة.

البريد الالكتروني: sci@utq.edu.iq
العنوان: العراق/ ذي قار/ الناصرية / قرب متنزه الناصرية

وزارة التعليم العالي والبحث العلمي
جامعة ذي قار/ كلية العلوم
شعبة الشؤون العلمية والدراسات العليا

العدد: ١٧٦٣ / ١٧ / ٣
التاريخ: ٢٩ / ٧ / 2021

أمر إداري

م/ تصويب

استناداً لمصادقة السيد رئيس الجامعة على محضر الجلسة الاعتيادية الصباحية التاسعة المفتوحة (الاجتماع الاول) لمجلس كليتنا المنعقدة بتاريخ (2020/8/19) بموجب كتاب رئاسة جامعة ذي قار /امانة مجلس الجامعة السري ذي العدد (4س /744) في 2020/9/7 والخاصة بالفقرة (ثالثا-3) المتضمنة تخويل السيد عميد الكلية صلاحية تشكيل لجان مناقشة طلبة الدراسات العليا (الماجستير والدكتوراه) و استنادا للصلاحيات المخولة لنا تقرر تشكيل لجنة مناقشة طالبة الدراسات العليا / الماجستير في قسم الكيمياء بكليتنا (رؤى حسن ناصر) عن رسالته الموسومة:-

(Study of Homocysteine levels and some antioxidants in pregnant women in Thi-Qar governorate)

من السادة الاتية المدرجة اسمائهم أدناه:-

ت	اللقب العلمي	الاســـــم	الشهادة	التخصص الدقيق	محل العمل	نوع التكليف في اللجنة
1	استاذ مساعد	لميس ماجد حميد	دكتوراه	الكيمياء الحياتية	كلية الطب / جامعة ذي قار	رئيسا
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3	استاذ مساعد	هديل رشيد فرج	دكتوراه	الكيمياء الحياتية	كلية العلوم / جامعة ذي قار	عضوا
4	استاذ مساعد	جمال حربي حسين	دكتوراه	الكيمياء الحياتية	كلية العلوم / جامعة ذي قار	عضوا ومشرفا

علماً أن الرسالة قومت علمياً من قبل أ.د. عدنان جاسم محمد – كلية العلوم / جامعة البصرة و أ.د. عباس دواس مطر / كلية التربية للعلوم الصرفة / جامعة البصرة ولغوياً من قبل م.د. محمد عبد العلي عباس / كلية العلوم جامعة ذي قار واحصائياً من قبل أ.د. رياض رستم محسن / ملية علوم الحاسوب والرياضيات / جامعة ذي قار وستجري المناقشة يوم الثلاثاء الموافق 17 / 8 / 2021 الساعة التاسعة صباحا وعلى قاعة (سمنار قسم الكيمياء) وتكون المناقشة الكترونية في حال الحظر الشامل شريطة توفر وسائل اتصال مناسبة فضلا عن تسجيل المناقشة بالكامل.

29-7-2021

أ.م.د. هيثم عبد الامير ميناس
العميد وكالة

جامعة ذي قار
الدراسات العليا
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نسخه منه الى:

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CERTIFICATE

We certify that this thesis **(Study of Homocysteine Levels and Some Biochemical parameters in Pregnant Women in Thi-Qar Governorate)** was prepared by Roaa Hassen Naser under our supervision at the Department of Chemistry, College of Science, University of Thi-Qar Iraq) in partial fulfillment of the requirements for the Master degree science chemistry

Asst.Prof. Dr. Jamal Harbi Hussein Alsaadi

Department of Chemistry

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In view of the available recommendations, we forward this thesis for debate by examination committee.

Asst. Prof .Dr. Mohammed A. Al-Tahan

Head of Dep. Of Chemistry

College of Science University of Thi-Qar

DEDICATION

To …

The shelter that has escape .... and the safety if I narrow my paths Who will always guide me to the best ... my Lord.

To…

The reason I am ... Dad.

To …

who the embodiment of the meaning of altruism in my view ... He wished and sought to be in the best possible position .... my husband

To…Who was my protector ... my mom.

To… Who was the blessing of help and support ... my sister Nour, and she was, as her name, a light for me

To… Everyone who wished me well and supported me even with a kind word.

ROAA

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In the beginning, thanks be to God Almighty, who helped me to complete my work ...

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ROAA

Contents index

Figures index

Tables index

List of abbreviations

Alb	Albumin
BMI	Body Mass Index
COPD	chronic obstructive pulmonary disease
PUFA	polyunsaturated fatty acid
MBPs	Metal-binding proteins
HSP	Human serum ceruloplasmin
HSA	Human serum albumin
Cp	Ceruloplasmin
CVD	cardiovascular disease
D	Dilution Factor
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immune sorbent assay
Hcy	Homocysteine
HDL	High-Density Lipoprotein
HHCY	Hyperhomocysteinemia
IUGR	Intrauterine Growth Restriction
LBW	Low birth weight
LDL	Low-Density Lipoprotein
LOO•	Lipid peroxyl radical
LOOH	Lipid hydro peroxide
MDA	Malondialdehyde
MTHFR	Methylene tetrahydrofolate reductase
THF	tetrahydrofolate
NTD	Neural Tube Defects
Se	Selenium
Cu	Copper
nmol	Nanomol
µmol	micromole

BCG	Bromcresol green
Mg	Magnesium
Di-Br-PAESA	(Di-bromo-2-pyridylazo)-N-ethyl-N-(3-sulphopropyl)aniline
LPL	Lipoprotein lipase
PE	Preeclampsia
CE	Cholesterol ester
SD	standard deviation
LSD	least significant difference
P	probability
AAP	Alanyl amino peptidase
CHOD	Cholesterol oxidase
GK	Glycerol kinase
GPO	Glycerol-3-phosphate oxidase
pmol	picomole
ng	Nanograms
µg	Microgram
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TBA	Thio Barbituric Acid
Tc	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglycerides
ε	Molar Extinction Coefficient

SUMMARY

Homocysteine is a sulfhydryl-containing amino acid that is formed when methionine is demethylated and is needed for intravascular metabolism. Homocysteine is thought to be a possible risk factor for the progression of atherosclerotic vascular disease processes that lead to cardiovascular disease (CVD) and death a stroke.

Aims of study Investigation of the relationship between elevation of homocysteine levels and levels of trace elements (selenium, copper, magnesium and ferritin) and antioxidants (Albumin, Ceruloplasmin) and Vitamins (B12 , C) and Lipid profile in serum of pregnant women with preeclampsia and risk of preterm labor . The study included (130) subjects , Average age (18-41)years, It devided to four groups (30) women a healthy pregnancy, (30) non-pregnant women, (35) women suffer from the onset of pre-eclampsia and (35) women at risk of premature labor .

The results shows a significant increase in the level of homocysteine in the preeclampsia and preterm labor groups compared to the healthy groups .The results show a presence of a significant decrease in Mg , Cu and Se in all groups of patients in preeclampsia and premature birth while The results show a presence of a significant increase in Ferritin in all groups of patients in preeclampsia , and premature birth, Where it was found that there is a negative correlation between homocysteine and each of selenium, magnesium, and copper, and a positive correlation between homocysteine and ferritin . also The results shows a significant decrease in the level of antioxidants Ceruloplasmin and Albumin, As well show results decrease in Vitamins B12 and C in preeclampsia and preterm labor groups. Show lipid profile a significant increase in each of the LDL, TG, TC, VLDL and decrease in

HDL in study groups . It was there a significant increase in Malondialdehyde in preeclampsia and preterm labor.

Our study concluded serum homocysteine level was a significantly higher in preeclampsia and preterm birth groups compared to control groups, Also results concluded that homocysteine associated with higher ferritin level , MDA, TC, TG, LDL, VLDL and associated with low selenium level, Mg, Cu, albumin, ceruloplasmin, B12, Vitamin C, HDL.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. Pregnancy and pregnancy complication:

Pregnancy is the time when one or more offspring develop inside a woman's uterus, as in the case of twins or triplets. Childbirth usually occurs after about 38 weeks of pregnancy. Pregnancy is associated with changes. These changes are necessary to help a woman adjust to the state of pregnancy and aid in the development of the fetus. The blood system must adapt in a number of ways, such as providing vitamins and minerals to the fetus (Iron, vitamin B12, and folic acid). The most important blood changes are anemia, thrombocytopenia, increased clotting, and clotting factors remain elevated for up to 8-12 weeks after birth (Mohamed *et al*., 2016) . It's divided the pregnancy into three periods of time known as the first, second and third trimesters. The first trimester starts from the first day of pregnancy until the week (13), and most often the miscarriage occurs at this stage. The second trimester starts from the week (13) to the week (26), during which time it is possible to feel the movement of the fetus through monitoring and evaluation by Ultra sound. The third trimester starts from week (26) until The end of pregnancy, which is usually around week forty (Biswajit *et al*., 2016) .

1.1.1.Preeclampsia:

Is a common pregnancy compilication that affects 3-5% of all pregnancies. Preeclampsia onset is less common but has higher rates of maternal illness and perinatal mortality, The prominent pathological feature of early pre-eclampsia is an abnormal development of the placenta. The placenta in preeclampsia is usually characterized by the remodeling of the abnormal blood vessels that begins in the first trimester of pregnancy where the flexible muscular wall cannot be replaced by fibrous material, as a result of this failure, the spiral arteries fail to

transform into large vessels of low resistance and remain narrow and with high pressure, Local hypoperfusion releases various factors Including inflammatory cytokines and anti-angiogenic proteins (Bokslag *et al*., 2016). Pregnancy-induced hypertension (PIH) occurs on average in 5--10% of pregnant women and increases maternal and fetal morbidity and mortality. This disease includes two main forms: gestational hypertension (GH) and preeclampsia (PE) (Lewandowska et al., 2019). Preeclampsia (PE) is a major condition that affects both the mother and the fetus during the second and third trimesters of pregnancy. Proteinuria, hypertension, and edema are all symptoms of this, quickly progressing disease. Despite advancements in treatment, preeclampsia remains the leading cause of maternal and perinatal death, SODs are metalloenzymes play a role in cellular defense against toxicity caused by oxidative stress (Keshavarz *et al*., 2017) .

1.1.2. Premature birth:

Premature birth is the leading cause of perinatal morbidity and mortality in developing nations, with neonates with a gestational age of fewer than 32 weeks accounting for the bulk of deaths. Annually, fifteen million premature births are registered worldwide, and although the prevalence of preterm labor varies greatly between countries, nearly 90% of these premature births occur in Africa and Asia , low birth weight (LBW) is also linked to an increased risk of death as well as short- and long-term health issues (Stylianou-Riga *et al*., 2018) . The maternal age (40 years or more) was linked to a higher risk of premature birth Mothers aged 30–34 years have the lowest chance of prematurity. Younger women (20–24 years) had more spontaneous preterm births, while women over 40year had more iatrogenic preterm births (Fuchs *et al*., 2018) .

1-2- Homocysteine:

Homocysteine is a sulfhydryl-containing amino acid that is formed when methionine is demethylated and is needed for intravascular metabolism. Homocysteine is thought to be a possible risk factor for the progression of atherosclerotic vascular disease processes that lead to cardiovascular disease (CVD) (Chrysant & Chrysant, 2018) . Hcy that is formed during the conversion of methionine to cysteine has a molar mass of 135.18 g/mol (Rehman *et al*., 2020) .

Physiological levels of Hcy are mainly determined by food intake and vitamin status (Moretti & Caruso, 2019) . Homocysteine levels are mostly regulated by diet, with the consumption of methionine-rich and B-vitamin-family-rich (B6, folic acid, and B12) foods. There is an uptick in overall plasma HCy levels with age, but it stays within a typical range. However, in clinical disorders associated with aging, such as neurogenerative disease, there is an abnormal rise in overall HCy levels in the cerebrospinal fluid (CSF) and plasma (Montecinos-Oliva *et al*., 2020). Homocysteine (Hcy) elevation has been linked to endothelial dysfunction and atherosclerosis in previous study (Esse *et al*., 2019) . Supplementing of folic acid and B12 vitamin lowers elevated Hcy levels in the blood, lowering the risk of cardiovascular disease. Because of the connection between Hcy and hypertension, Hcy levels could be used as a marker for atherosclerosis progression (Obradovic *et al*., 2018). Males have a higher Hcy level than females which may be attributed to gender variations in Hcy metabolism and males' low vitamin B12 and folate levels (Rehman *et al*., 2020) .

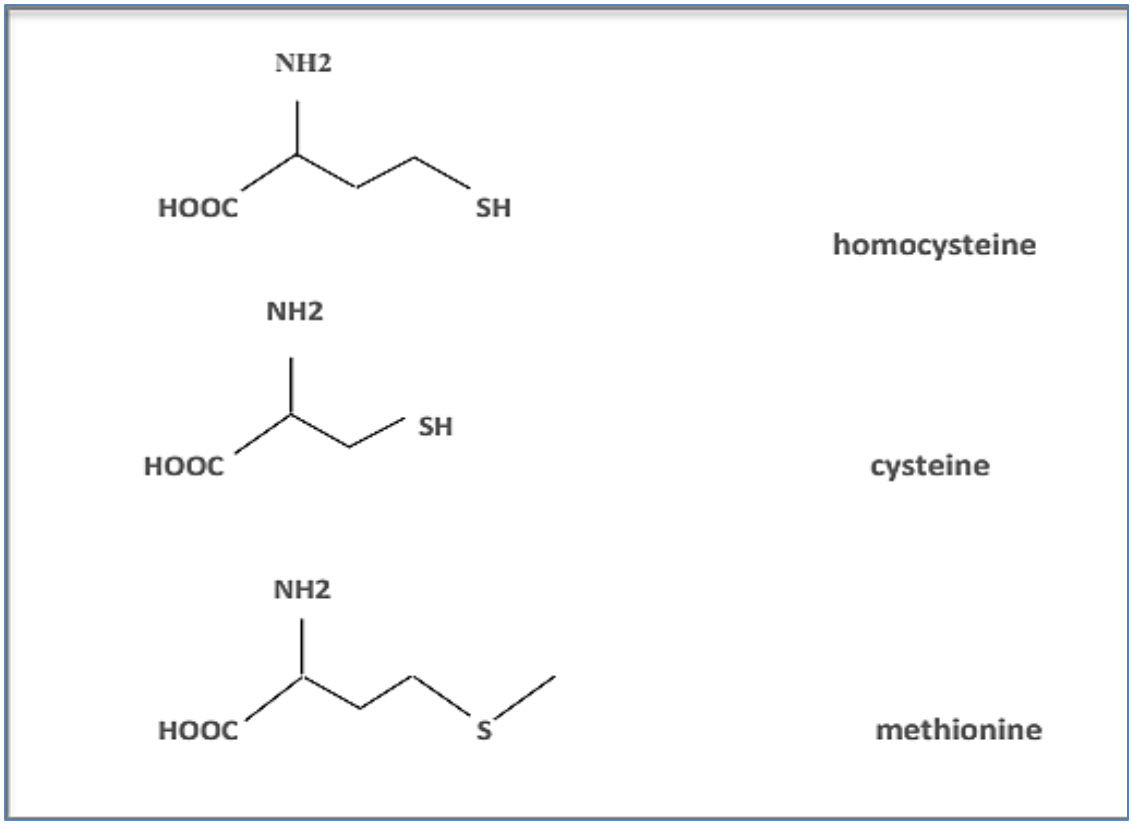

Figure (1-1) Structures of homocysteine ,cysteine ,methionine(Nekrassova et al., 2003)

1.2.1. Metabolism of homocysteine:

The liver is essential in controlling homocysteine levels in the blood (Stead *et al*., 2000), Homocysteine is a sulfur-containing, nonprotein, toxic amino acid found in the pathway for the interconversion of two amino acids: methionine and cysteine. Homocysteine is metabolized via two different pathways: remethylation and trans-sulfuration. When there is an excess of cellular methionine, the trans-sulfuration pathway plays a crucial role in Hcy metabolism, converting Hcy to cystathionine via cystathionine beta synthase (CBS), which requires pyridoxal 5′-phosphate as a co-factor. When the cellular methionine level is low, Hcy is remethylated back to methionine in a betaine- or folate-dependent reaction. In the betaine-dependent pathway, the enzyme betaine-homocysteine S-methyltransferase (BHMT) catalyzes the incorporation

of a methyl group from betaine into homocysteine to form methionine. In the folate-dependent pathway, The two pathways are coordinated by S-adenosylmethionine (SAM), which acts as an allosteric inhibitor of the methylenetetrahydrofolate reductase reaction and as an activator of cystathionine beta-synthase. Hcy acquires a methyl group from N-5-methyltetrahydrofolate with the help of 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) (also known as methionine synthase). Methionine synthase requires vitamin B12 for its functionality, and the reaction also involves recycling of tetrahydrofolate (from N-5-methyltetrahydrofolate), which may eventually be used for nucleotide biosynthesis. Methionine synthase, therefore, couples the folate and Hcy metabolism pathways . Since the generation of tetrahydrofolate depends on the input of exogenous folate for folate metabolism , low folate levels ultimately result in substrate limitation for methionine synthase, thereby affecting the remethylation pathway. Thus, low folate levels result in a high plasma Hcy concentration and vice versa (Hasan et al., 2019; Moretti & Caruso, 2019; Selhub, 1999). as in the figure (1-2):

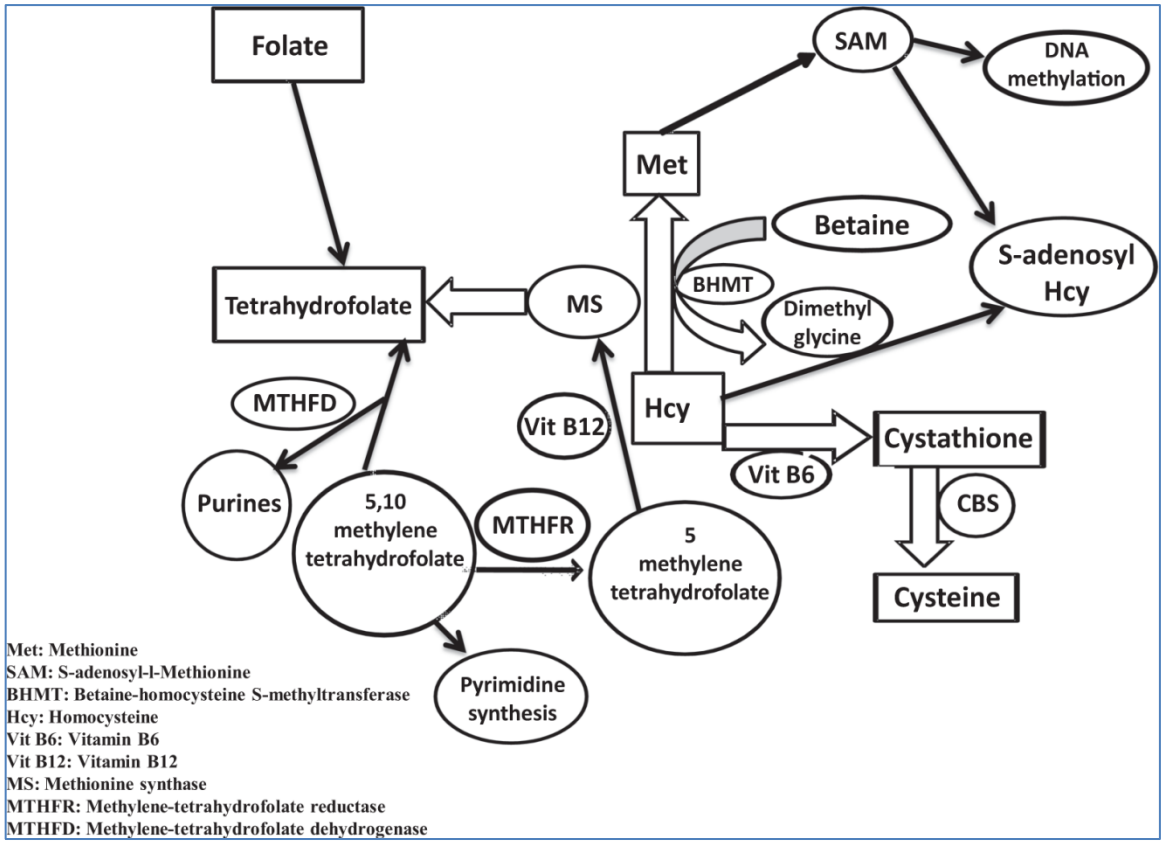

Figure (1-2) Metabolism of homocysteine (Hasan et al., 2019)

1.2.2.Homocysteine in pregnancy:

Plasma homocysteine concentrations decrease normally in pregnancy, as there is a presumed increase in the absorption of homocysteine from the fetus. The most well-known effect of homocysteine metabolism on pregnancy is its association with the problem of neural tube defects (NTD) . Folic acid, can reduce the risk of developing NTD, and for the first time a subsequent systematic review confirmed the benefit of folate before pregnancy and nutritional supplements in preventing NTD without any effect on miscarriage and ectopic pregnancy (Hague, 2003). Homocysteine levels during pregnancy decrease during the first three months of pregnancy, reaching a minimum during the second trimester, and then rise slightly during the third trimester. The rise in homocysteine is associated with preeclampsia and is

also responsible for the death of the fetus in the uterus and repeated miscarriage (Aubard *et al*., 2000) . Complications such as preeclampsia (PE), early pregnancy death, placental abruption (PA), and intrauterine growth restriction (IUGR) have been associated with elevated HCy levels during pregnancy, Clots of blood, etc. These levels are linked to the influence of Hcy on blood vessels endothelial activity, increased pro-oxidative , endothelium metabolic disorders, high Hcy levels were also linked to placental abruption, which increased the risk 5.3-fold (Gaiday et al., 2018). Recurrent pregnancy loss (RPL), preeclampsia (PE), preterm birth, placental abruption, fetal growth restriction (FGR), and gestational diabetes mellitus (GDM) were all linked to Hcy. Furthermore, it was revealed that maternal Hcy levels and newborn birth weight were inversely associated (Dai *et al*., 2021) .

1.2.3. Hyperhomocysteinemia:

Homocysteinemia is caused by mutations in cystathionine beta synthase (CBS), an enzyme found at the junction of the trans-sulfuration and re-methylation pathways. Other genetic (CBS-independent) and environmental factors may cause elevated Hcy serum levels, which is referred to as hyperhomocysteinemia , Hyperhomocystenemia (HHCY) is also recognised as a new independent risk factor for atherosclerotic vascualr disease. Furthermore, HHCY is linked to impaired fibrin network development by slowing the coagulation process and resulting in more closely packed fibrin clots, thereby affecting the spontaneous thrombolysis process (Li *et al*., 2018). Homocysteine (Hcy) upregulation contributes to the progression of age-related diseases such as bone fractures, poor wound healing, lack of regenerative capacity, endothelial impairment, and renal and cognitive deterioration. Surprisingly, a considerable percentage of vegetarians suffer from

hyperhomocysteinemia (Ostrakhovitch & Tabibzadeh, 2019). Hyperhomocysteinemia is also related to kidney failure. Mild HHcy can be caused by changes in our diet, such as smoking, a lack of exercise, the use of nicotine, caffeine, and ageing. A defect in pathways involved in Hcy metabolism is one of the genetic causes. Hcy levels are believed to be affected by gender , with elevated levels of Hcy being seen in Males (Rehman *et al*., 2020). HHcy is associated with endothelial injury, This boosts oxidative stress. During the oxidation process, the production of reactive oxygen species is catalyzed and catalyzed by a mineral cation which reacts to form peroxynitrates with nitrogen oxide. . It activates oxidative stress at the same time. HHcy Increases collagen as well Buildup that contributes to vascular fibrosis (Gaiday *et al*., 2018). Several difficulties have been linked to hyperhomocysteinemia (HHcy) during pregnancy, and high homocysteine (Hcy) levels have been linked to the etiology of preeclampsia, placental abruption, intrauterine growth retardation, and neural tube abnormalities, as well as the neurological effects. that early developmental brain maturation deficits resulted in Long-term problems in learning and memory activities may be caused by prenatal HHcy (Gerasimova *et al*., 2017).

1.3. Oxidative stress:

Oxidative stress is defined as an imbalance between free radical damage (for example, lipid oxidation) and antioxidant protection (Toescu *et al*., 2002) .

Oxidative stress is a pathological condition that results in many cases as a result of the participation of free radicals, and it is usually highly reactive chemical types that possess one free electron, and include two reactive types, reactive oxygen species (ROS) and reactive nitrogen species (RNs) (Mirończuk-Chodakowska *et al*., 2018).

Oxidative stress arises from ROS / RNS Or from the degradation of the antioxidant ability of prevention, which is characterized by a reduced intrinsic capacity , for systems to fight against oxidative attack directed at target biomolecules (Pisoschi & Pop, 2015). Free radicals usually produce free radicals when an individual reaches the age of 40 years or over as enzymes including antioxidants become gradually disrupted due to failure of the antioxidant systems to overcome influx Continuous ROS (Moskovitz *et al*., 2002). Oxidative stress is a prevalent pathophysiological mechanism linked to a variety of disorders, including chronic obstructive pulmonary disease (COPD) and the normal ageing method. Inhalation exposure to air

pollution is known to increase oxidative stress levels in the body *(Cui et al.*, 2018) . It has been suggested that the generation of free radicals in response to decreased placental perfusion may lead to clinical manifestations, the abnormal placenta or perfusion leads to an increase in the inflammatory response and endothelial dysfunction (Roberts *et al*., 2010) .

1.3.1.Lipid peroxidation:

(Lipid peroxidation can generally be described as a process by which oxidizing substances such as free radicals attack fats that contain a double carbon-carbon bond (s), especially polyunsaturated fatty acids) (AAyalantonio *et al*., 2014) . Lipid peroxidation is a chain reactions initiated by the attack of reactive oxygen species (ROS) and reactive nitrogen species (RNS) on polyunsaturated fatty acid (PUFA), leading to cell membrane damage and consequently to cell death (Domijan *et al*., 2015) . The overall process of lipid peroxidation consists of three steps: initiation, propagation, and termination as shown in figure (1-3)

Step 1: During initiation, pro oxidants such as the hydroxyl radical extract the allylic hydrogen, resulting in the formation of a carbon-centered lipid radical (L) .

Step2: During the propagation phase, (L) interacts quickly with oxygen to produce a molecule. The lipid peroxy radical (LOO•) is a radical that takes a hydrogen atom from another molecule. lipid molecule produces a new L (continuing the chain reaction) and hydroperoxide of lipids (LOOH) .

Step 3. In the termination reaction, antioxidants such as vitamin E contribute a hydrogen atom to the LOO• species and produce a matching vitamin E radical, which combines with another LOO• to produce non-radical products (Ayala *et al*., 2014) .

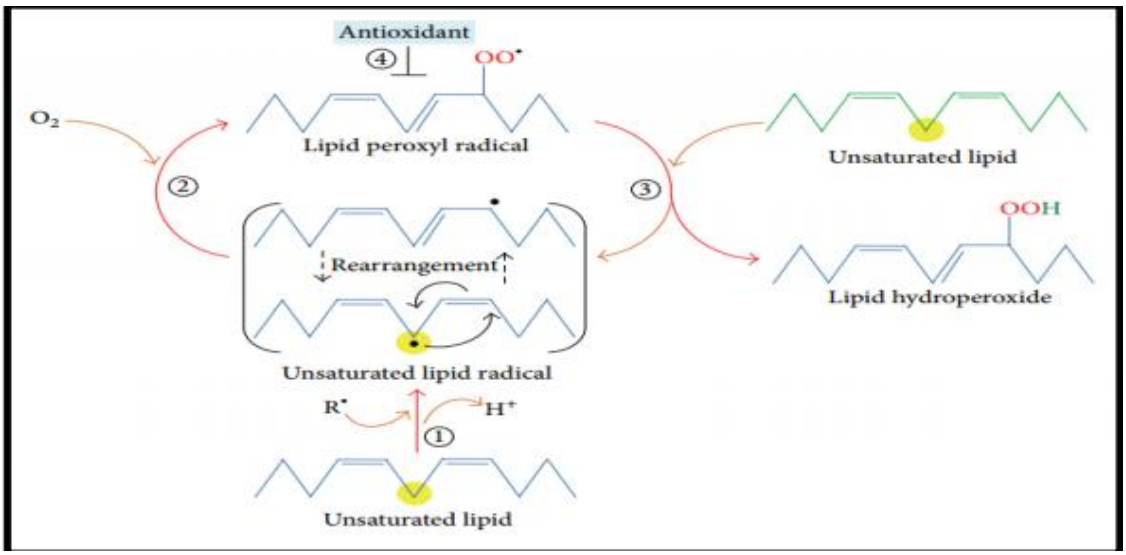

Figure (1-3) Lipid peroxidation process(Ayala et al., 2014)

Primary products of LPO are unstable hydro peroxides that decompose to various secondary products, among which are stabile aldehydes, malondialdehyde, 4-hydroxy-2-nonenal (HNE) and acrolein (Domijan *et al*., 2015).

1.3.2.Malondialdehyde:

MDA is one of the most common and reliable markers in clinical which determined oxidative stress (AAyalantonio *et al.*, 2014), MDA, the most significant end-product of lipid oxidation, has been widely proposed to have genotoxic effects and has also been linked to cardiovascular disease. MDA should therefore be considered more than just a lipid oxidation marker (Ma *et al*., 2019). Malondialdehyde (MDA), a peroxidation product of vegetable oils, has been recognized as toxic; nonetheless, vegetable oils are gradually replacing animal fats in diets (Ma *et al*., 2021) .

MDA has the ability to form bonds via DNA protein, as cross-links between histones and DNA have been observed by MDA. MDA with the protein fraction forms a cross-link. All these activities are potentially genotoxic and lead to mutations (Del Rio *et al*., 2005) . The pathogenesis of many diseases has been linked to lipid peroxidation reactions that release free radicals. Malondialdehyde (MDA), a by-product of lipid peroxidation, is known to induce changes in the composition and function of host cells, Reactive free radicals have the ability to change the responses that take place in tissues, causing deteriorate lipids, proteins, and nucleotides in the tissue (Subramanyam *et al*., 2018). MDA as a significant independent predictor of hypertension/preeclampsia, enterocolitis, and bronchopulmonary dysplasia and IUGR (Parlapani *et al*., 2019) .

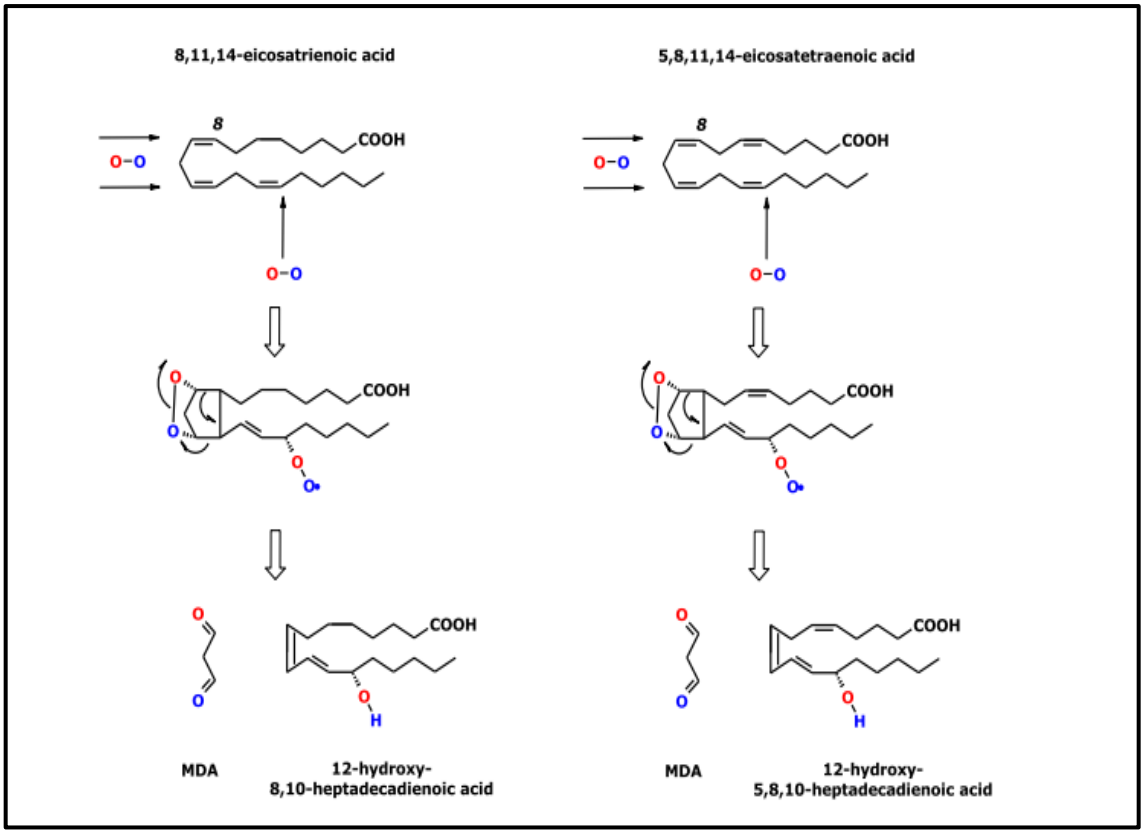

Figure (1-4) Mode of enzymatic formation of malondialdehyde (MDA)(Tsikas, 2017)

1.3.3.Antioxidants:

Antioxidants are defined as "substances that, in limited amounts, can prevent or greatly retard the oxidation of easily oxidisable materials such as fats." The agreed term is "any substance that, when present at low concentrations relative to those of an oxidisable substrate, significantly slows or prevents the oxidation of that substrate. Antioxidant intake improves people's life expectancy and protects them from a variety of deadly diseases. Antioxidants are often used in dietary supplements to promote healthy health and avoid diseases such as cancer and cardiovascular disease. Additionally, they are used as food preservatives (Atta *et al*., 2017) .

Metal-binding proteins (MBPs) are the first endogenous antioxidants, , such as albumin (ALB), Ceruloplasmin (CP) , (Mirończuk-Chodakowska *et al*., 2018).

- Types of antioxidants :

These natural antioxidants from plant materials are mainly carotenoids and vitamins (vitamin E and C) ,polyphenols. Generally, these natural antioxidants, especially carotenoids and polyphenols exhibit a wide range of biological effects, such as anti-aging, and anticancer , anti-inflammatory, antibacterial, antiviral (Xu *et al*., 2017) . often all natural antioxidants delayed primary and secondary oxidation, and increased the oxidative stability index. (Mohanan *et al*., 2018) . reactive oxygen species (ROS) are formed by partial reduction of molecular oxygen. enzymatic and non-enzymatic antioxidants in cells can protect their cells from oxidative damage by scavenging ROS (Shao *et al*., 2008) . Phenols are a group of natural compounds found in a wide range of plants that have a variety of structures and are believed to function as antioxidants in some cases, which have antiviral, anti-inflammatory, and antiatherosclerotic properties, Phenolic antioxidants can scavenge the harmful free radicals and thus inhibit their oxidative reactions with vital biological molecules.It is also known that consumption of natural dietary antioxidants from fruits and vegetables has been shown to enhance the function of the antioxidant enzyme-linked defense response mediated by glutathione, superoxide dismutase, catalase and glutathione-stransferase ((Vattem et al., 2005; De Pinedo et al., 2007) .

Non-enzymatic antioxidative systems are less precise than enzymatic antioxidative systems, but they are the first line of antioxidative protection and therefore play a critical role in the cellular

response to oxidative stress. Vitamin C scavenges radicals and converts them to ascorbyl radicals, which are stable and do no oxidative harm. Vitamin E prevents cell lipid compartments by terminating the lipid peroxidation chain reaction or inactivating ROS, both enzymatic and non-enzymatic, which can be biological response to oxidative stress (Augustyniak *et al*., 2010).

1.3.3.1.Ceruloplasmin:

Human serum ceruloplasmin (hCP) is a glycoprotein containing copper, several functions have been attributed to ceruloplasmin, including the transport of copper, and as an antioxidant in preventing the formation of free radicals in the blood serum. In addition to ceruloplasmin's association with iron metabolism, it is also linked to two genetic disorders of transmission Copper, Menkes disease and Wilson's disease (liver degeneration). In Menkes disease, gene mutations cause the defect in the export and / or intracellular transport of copper associated with deficiency of copper-dependent enzymes (Zaitseva *et al*., 1996) . And it a significant antioxidant protein that is synthesized in many tissues, including the brain, A glycoprotein that transports 95 percent of the copper in the blood (Chauhan *et al*., 2004). Ceruloplasmin which contains copper, stimulates the conversion of ferric ion into ferrous form, favoring iron absorption from the gut. It also plays a role in moving iron into plasma from tissue stores (Pathak & Kapil, 2004) . Oral contraceptives, their use, significantly increases the levels of copper and ceruloplasmin in the blood. During pregnancy, copper is transferred from the maternal tissue reserves, especially in the liver, to the fetus, which stores it for future extra-uterine requirements (Louro *et al*., 2001) . CP is known as a promiscuous enzyme to date, acting Binding to numerous Cu sites (B. Wang & Wang, 2019).

1.3.3.2.Albumin:

Human serum albumin (HSA) is the most abundant protein present in blood. The HSA is made in the liver from a precursor protein called proalbumin, which is produced in the lumen of hepatocytes' endoplasmic reticulum in the form of preproalbumin. Proalbumin is synthesized from a single mutation, which then converts it to preproalbumin and albumin After that, cleavage takes place in the endoplasmic reticulum. Protein has a molecular weight of 66.5 kDa. HSA is the most concentrated protein in human blood, with a normal concentration of 35 to 50 g/l (Rabbani & Ahn, 2019) .

Albumin is a protein that is water soluble and mildly soluble in condensed salt solutions. It is abundantly formed in the liver and accounts for a significant portion (60%) of all plasma protein. Serum albumin serves as a transporter for molecules with poor water solubility, isolating them from the rest of the body lipid soluble hormones, bile salts, unconjugated bilirubin, free fatty acids (Apoprotein), calcium, and ions are hydrophobic (transferrin) Albuminuria is a condition in which albumin is excreted in the urine. At albuminuria occurs as the cells of the kidney called podocytes like a seal, allowing only very simple molecules to exit while preventing red blood cells and larger molecules like albumin from passing As albumin is a protein, albuminuria is a symptom of kidney disease (Al Ghazali *et al*., 2014) .

The liver produces 10 to 15 g of albumin per day, which is then released into the vascular space. It has a half-life of 17 days on average. Serum albumin concentration is physiologically somewhat lower in women than in men and reduces slightly with age. Albumin synthesis is enhanced by insulin and amino acid absorption. Serum albumin has many physiological properties. Serum albumin is a protein that holds a lot of

ions, fatty acids, bilirubin, vitamins, hormones, and steroids . Reduced liver synthesis, increased catabolism, increased artery permeability, and renal and enteral loss are contribute to hypoalbuminemia (Arques, 2018).

1.3.4.Vitamins:

Vitamins are a group of organic molecules essential for proper cellular function which must be obtained almost entirely by the diet. Officially, There are 13 basic vitamins for humans, each of which plays an important part in the body cellular activity that is normal (Combs Jr & McClung, 2016).

Vitamins serve as catalysts in all metabolic reactions that use proteins, fats, and carbohydrates for energy, cell formation, and maintenance. Vitamins are often prescribed as dietary additives and only limited quantities of these essential compounds are absorbed from food. Water-soluble vitamins, such as B complex, C, cannot be preserved if not absorbed, they are expelled. , while fat-soluble vitamins, include A, D, E, and K, can be stored in the liver and fat tissues (Cagetti *et al*., 2020) .

1.3.4.1.Vitamin B12:

Since they contain cobalt, B12 called cobalamins. Vitamin B12 intake of 2.4-2.6 µg per day for pregnant women and 2.8 µg per day for lactating women is usually prescribed. It helps every cell in the human body to function properly, particularly when it comes to DNA synthesis, fatty acid metabolism, and amino acid metabolism. During viral and bacterial infections, B12 deficiency reduced immune response.Animal products, such as beef, fish, poultry, and dairy products, are good sources of vitamin B12 (Aslam *et al*., 2017) . Vitamin B12 is a cofactor for the enzyme methionine synthase, which is involved in the conversion of homocysteine to methionine as well as the conversion of 5-

methyltetrahydrofolate 5-MTHF to tetrahydrofolate THF. When vitamin B12 levels are low, 5-methyltetrahydrofolate accumulates and tetrahydrofolate synthesis fails, resulting in a deficiency in folate metabolism Because the folatepolyglutamate synthase enzyme only consumes tetrahydrofolate, (folate concentrations fall) (Sawant, 2015) .

Vitamin B12 is needed for DNA synthesis, neurological activity, and the development of red blood cells. Methylcobalamin and 5 deoxyadenosylcobalamin are the active forms of B12. Vitamin B12 acts as a cofactor for methionine synthase, affecting the synthesis of approximately 100 substrates such as DNA, RNA, and proteins (Almohanna *et al*., 2019) . Vitamin B12 levels were slightly lower in preeclampsia patients than in healthy women. and maternal vitamin B12 deficiency is linked to negative pregnancy outcomes such as premature abortion, low birth weight in newborns, intrauterine growth restriction and Neural tube defects (Mardali *et al*., 2021) . Homocysteine was significantly elevated in the vitamin B12 deficient states (Lee *et al*., 2019). Figure (1-5) shows the composition of vitamin B12:

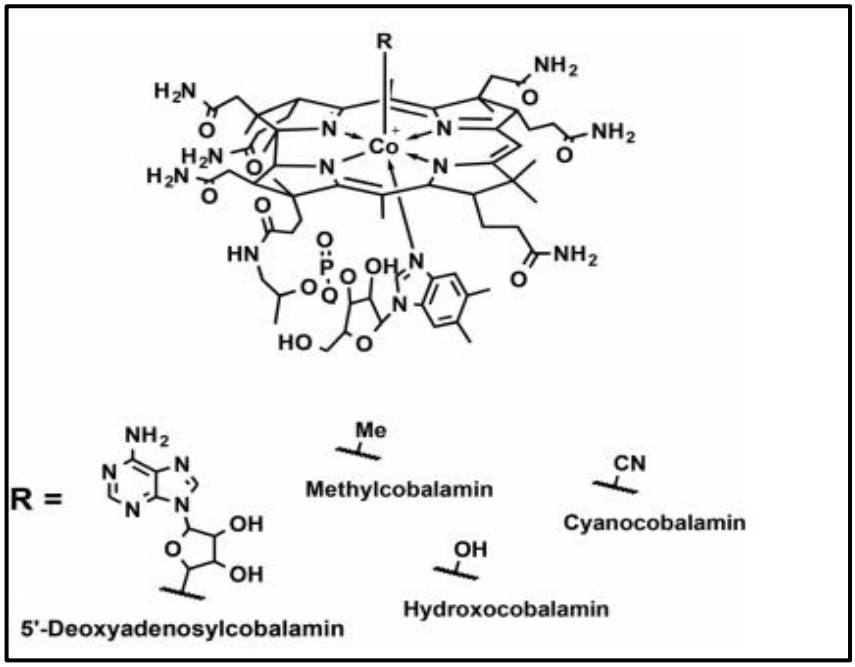

Figure (1-5) Structure of B12 (Jones, 2017)

1.3.4.2. Vitamin C:

It is generally known as L-ascorbic acid or simply ascorbic acid, and it can be detected in nearly any kind of body tissue. However, it is found in higher quantities in the pituitary gland and the central nervous system, up to 400 mg/kg. as well as stimulating iron absorption from the intestine and modulating iron transfer for storage. and the development of collagen Human immunity to diseases and cold ailments is boosted by vitamin C. It neutralizes reactive oxygen species (ROS) produced in immune cells; without vitamin C, reactive oxygen species may not have been eliminated from immune cells, resulting in immune cell destruction; antioxidants such as vitamin C play an important role in this process. Vitamin C is found in high concentrations in leukocytes, Vitamin C concentrations in immune cells (e.g., leukocytes) drop quickly during infection due to its application, and then return to normal after the infection is eradicated (Aslam *et al*., 2017) . By donating electrons to recipient molecules, vitamin C is a powerful reducing agent (oxidativereduction or redox). Vitamin C can serve as an antioxidant and/or an enzyme cofactor in relation to this redox capacity. Vitamin C functions as a nonenzymatic antioxidant in plasma, protecting proteins, lipids, sugars, and nucleic acids (DNA and RNA) from free radicals and other oxidants and reactive oxygen species (ROS), which are generated as by-products of natural metabolic processes, and pollution and toxicity exposure (Alpert, 2017) . Figure (1-6) show Structure of Ascorbic acid .

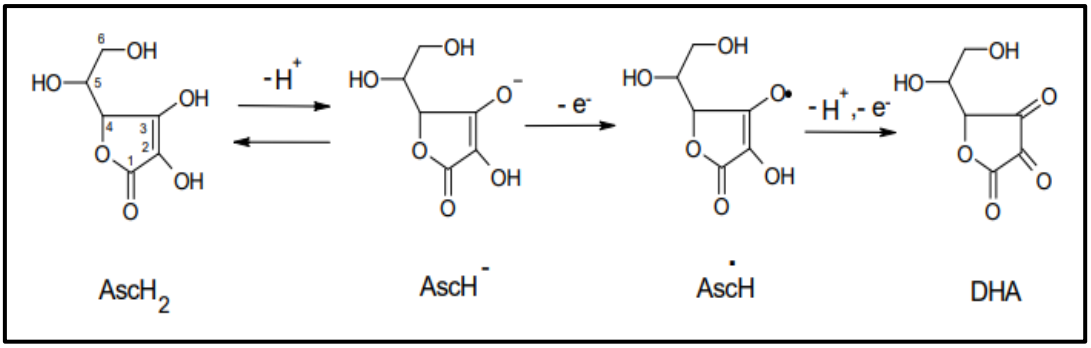

Figure (1-6) Structure of Ascorbic acid (Timoshnikov et al., 2020)

Women with eclampsia and pre-eclampsia have been shown to increase from 3 to 25 times the risk of developing placental abruption, thrombocytopenia, pulmonary edema, and diffuse intravascular thrombosis. Supplements of vitamins C and E may be helpful in preventing preeclampsia (Fu *et al*., 2018) .

1.4.Trace elements:

Trace elements refers to chemical elements present in very small quantities in a natural substance, with an average concentration of <100 µg / g. It is required for the growth and development of the organism, in the biological processes of trace elements of importance, for example iron transports, binds and releases oxygen in the body (Al-Fartusie & Mohssan, 2017) .

Every trace element contributes differently to each part in the body, the several major physiological and biochemical processes, the Interaction in biological processes between trace elements plays a role in The mediation of biological and chemical reactions that could be used for human health management (Rasdi *et al.*, 2013) . Trace elements like zinc, copper, and selenium exhibit antioxidant activity and act as a peroxynitrite scavenger while others like manganese and magnesium are essential micronutrients. The disturbance in the metabolism of these

elements may be a factor contributing to the development of some diseases such as preeclampsia (Akinloye *et al*., 2010) .

1.4.1.Selenium:

Selenium (Se) is an essential micronutrient for the proper functioning of all living organisms, it helps many enzymes such as glutathione peroxidase and is important in protecting against oxidative stress, and when supplementation is lacking, the risk of many diseases increases (Kieliszek, 2019). The absorption of selenium in the form of selenocysteine into an integral group of proteins known as selenoproteins is responsible for the majority of selenium's beneficial effects. Selenocysteine is a chemical and functional analog of cysteine in which a selenium atom replaces one of the atom Sulfur is added to increase catalytic activity (Guillin *et al*., 2019) .

Selenium play an important role in several biological functions of the body, which play an important role in body, such as The oxidative/antioxidant ratio, immune function control and inflammatory response moderation, thyroid hormone metabolism development and regulation, the central nervous system's proper functioning , the regulation of oxidative stress , Selenium compounds protect against cardiovascular problems , With regard to the presence of selenium in the body that battles oxidative stress, it is worth noting the Glutathione-peroxidases, enzymes containing selenium, have an important function. Superoxide, along with superoxidedismutase Catalase protects the cells, together with vitamins E and C, against the harmful effects of reactive oxygen and nitrogen species (Bizerea *et al.*, 2018) . For mammalian redox biology, selenium is a necessary trace ingredient. Numerous

epidemiological studies have linked selenium deficiency to an elevated risk of contracting a variety of diseases, including tumors, neurodegenerative diseases, cardiovascular disorders, and infectious diseases. Selenium is covalently bound to organic molecules, unlike other trace elements that act as cofactors (Guillin *et al*., 2019).

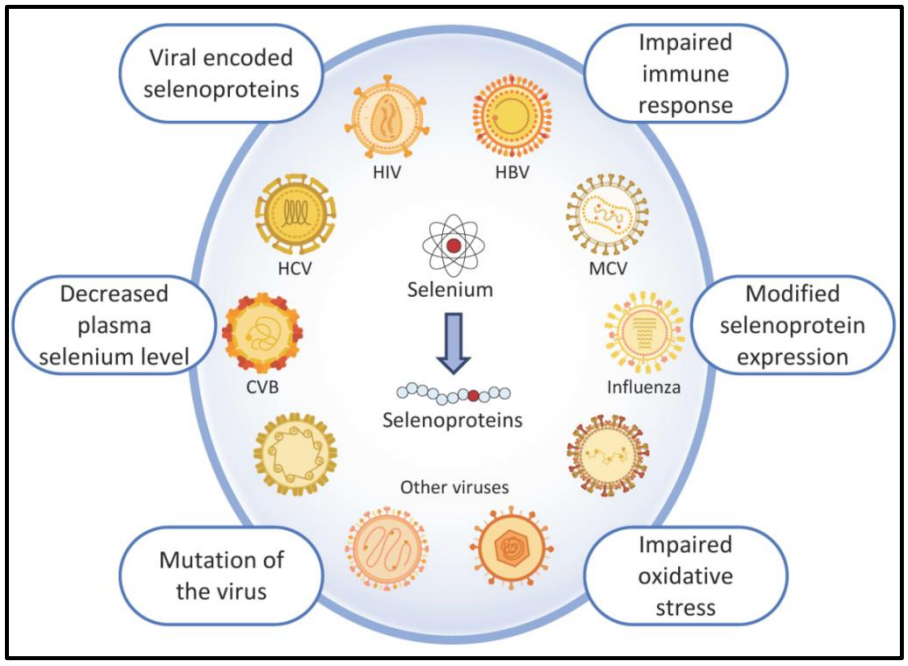

Figure (1-7) Selenium and selenoproteins (Guillin et al., 2019)

Selenium deficiency in pregnant women has been shown to have a detrimental impact on the embryo's development , Selenium deficiency was shown to be slightly more common in preeclamptic women. Selenium in excess can be toxic to the body , Acute selenium toxicity is a very rare occurrence (Eze *et al*., 2020; Kieliszek, 2019).

1.4.2.Magnesium:

The key biological role of Mg^{+2} in mammalian cells is to neutralize anion charge (Kunutsor *et al*., 2017). Magnesium is absorbed primarily in the small intestine but also in the large intestine to some extent. For magnesium absorption, there are two routes: paracellular and

transcellular. Intestinal consumption is not proportional to food intake alone, but rather depends on the body's magnesium status. Magnesium concentrations in the blood do not adequately represent the state of magnesium reserves , Serum magnesium exists in three states: two-thirds is ionized, one-third is protein bound mostly to albumin, and a small amount is complexed with anions (Ahmed & Mohammed, 2019). .Serum and red blood cells are the main sources of magnesium (Mg) , Magnesium is our body's fourth most abundant cation, the second most abundant intracellular cation, and the most abundant intracellular divalent cation. Magnesium is found in the human body in amounts of around 25 g. Magnesium is required for the action of over 300 enzymes in humans , the muscles and bones containing 90% of total body magnesium , 90 percent of which is tied and just 10% of which is unbound. In the serum, 32% of the magnesium is bound to albumin, while the other 55% is free. Maintaining ionic gradients (keeping intracellular sodium and calcium down and potassium high), cellular and tissue integrity, mitochondrial oxidative phosphorylation (ATP synthesis and activation), and DNA and Synthesis of RNA and proteins are only a few of magnesium's key functions (DiNicolantonio *et al*., 2018; Uwitonze & Razzaque, 2018) . In women with preeclampsia, magnesium (Mg^{2+}) levels can be normal or low. However, Mg^{2+} salts, such as Mg^{2+} sulphate, may help to reduce the pathophysiological effects of preeclampsia with extreme features and eclampsia. Although the mechanism of action of this Mg^{2+} salt is unknown, the current data shows that Mg^{2+} is helpful to both the mother and the fetus (Chiarello et al., 2018) .

1.4.3.Copper:

Copper (Cu) is an trace essential element that is a cofactor for antioxidant enzymes, and thus is essential for health and for reducing the

risk of disease . However, excessive copper may be harmful and could generate an oxidizing substance, which thus means an imbalance of antioxidant systems, (Song *et al.*, 2017) , Copper participates in the oxidative balance and immune processes and inhibits or activates many enzymes and is part of the enzyme Cu, Zn-SOD enzyme (superoxide dismutase), which is the enzyme of the first line of antioxidants. Copper's relationship to oxidative balance indicates a possible association with pregnancy-induced hypertension. Copper ions are needed for the health of bone tissue, according to numerous reports. Low serum copper levels are linked to lower bones minerals density (BMD), while high serum copper levels are linked to deformities, hypoplasia, weak bones, and recurrent fractures. Copper may be used as a simple, radiation-free osteoporosis screening tool (Qu *et al*., 2018) . A substantial rise in serum Cu levels is observed in pregnant women. Serum Cu levels were 30% and 35% lower in women who had miscarriages and infertility, respectively, than in pregnant women. The absence of a pregnancy-related rise of metal levels in miscarriage and infertility suggests that metal insufficiency plays at least a partial role in compromised pregnancy and reproductive function in general (Skalnaya *et al*., 2019) .

1.5.Ferritin:

Ferritin is a significant iron storage protein that retains a large iron center in its cavity and has ferroxidase activity. One of ferritin's major properties is its ability to attract iron ions and promote their mineralization by its ferroxidase activity (Arosio et al., 2017) . Ferritin is a significant mediator of immune dysregulation, contributing to a cytokine storm by direct immunosuppressive and inflammatory-stimulating impact (Vargas-Vargas & Cortés-Rojo, 2020) . Inflammation, apoptosis, oxidative stress, lipid transport, and fibrogenesis are all

important pathogenesis processes that ferritin may play a role in it (Kowdley *et al*., 2012) , Due to the expansion of red blood cell mass and the transition of the levels of iron to placental structures and the growing fetus, iron needs to rise during pregnancy. Preterm delivery, small-for-gestational-age birth, and low birth weight are also linked to maternal anemia (Næss-Andresen *et al*., 2019) .

1.6. Lipid profile:

Lipids are a complex and ubiquitous group of compounds that perform a variety of important biological functions, including functioning as structural components of cell membranes, storing energy, and participating in signaling pathways (Fahy *et al*., 2011) . Concentrations of lipids in the blood and plasma lipoproteins increase significantly during pregnancy. Fat storage occurs mainly during mid-pregnancy. Hypercholesterolemia is an important cause of atherosclerosis. Early in the middle of the third trimester, LDL-C levels reach their peak due to the hepatic effect of estradiol and progesterone. (Mankuta *et al*., 2010) . Most cells need lipids such as cholesterol and triglycerides. Cholesterol is a part of cell membranes that also serves as a precursor to bile acids and steroid hormones. Triglycerides are essential energy sources that function as an energy reservoir after being stored. Cholesterol, unlike triglycerides, sugars, and proteins, cannot be broken down by human cells Cholesterol that is freshly synthesized or absorbed through the intestine will thus stay in the body until it is converted to steroid hormones, lost through skin cell removal, or secreted by bile. Plasma has a lipid delivery structure made up of water-soluble lipoproteins though lipids do not mix with water (Nordestgaard, 2017). Elevated total cholesterol (T-Chol), high triglycerides (Tg), low high density lipoprotein cholesterol (HDL-C), and raised amounts of small dense LDL particles describe lipid disorders in

diabetic patients, referred to as "diabetic dyslipidemia " (Bhowmik *et al*., 2018) . Adaptations in maternal lipid metabolism are needed for fetal growth and development during pregnancy. The emergence of maternal hyperlipidemia is one of these adaptations. High levels of remnant cholesterol and an atherogenic lipid profile, known as high total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-c) and/or a low level of high-density lipoprotein cholesterol (HDL-c) are risk factors for the onset and development of atherosclerosis, which may lead to cardiovascular disease (CVD) (Adank *et al*., 2019) .

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Aims of Study

The aims of this research is to evaluate :

1. Homocysteine serum levels in preeclampsia patients and the risk of preterm labor
2. Examine the connection between homocysteine levels and antioxidants .
3. Consider the relation between homocysteine and trace elements
4. Evaluation the relationship between homocysteine and the malondialdehyde oxidative stress index .
5. Consider the association between homocysteine and vitamins .
6. Assessment of the relationship between homocysteine and lipid profile.
7. Finding a link between homocysteine and several parameters used as routine measures of pregnancy to help predict pre-eclampsi and premature birth.

CHAPTER TWO

MATERIALS & METHODS

2.1. Design of Study :

This prospective study was conducted at department chemistry-collage of science- university of Thi-qar, Alshatra general hospital and Maternity and children (Bent Al- Huda) Hospital. The study extended from October (2020) to February (2021). The study included (130) women, Their ages range from (18 - 41)years, were divided into : **Control group** : includes (60) women, divided into two groups, a group of (30) women a healthy pregnancy women, and (30) non-pregnant women . **Preeclampsia group :** Includes (35) women suffering from preeclampsia .**Premature birth :** Includes (35) women at risk of premature birth .

2.1.1.Exclusion Criteria :

the cases that were excluded from the study were those with chronic diseases, as well as those at risk of miscarriages , women who take contraception and women who smoke.

2.1.2.Selection of Criteria :

The women selected in the study, women with pre-eclampsia and women at risk of premature birth, and both groups were diagnosed by a specialist gynecologist .

2.2.Collection of blood Samples:

Venous blood (5 ml) was collected from patients and then divided into two parts, part one It was 1 mL and was added to EDTA containing – Ethylene diamine tetra acetic acid (EDTA) plasma , and gently shaken for use in determining the albumin level . The second part (4 ml) was transferred to a normal tube without the anticoagulant (serum) which recognized the clotting for 20 minutes at room temperature. After the blood clot, it was transferred to a centrifuge at (3000)xg in order to obtain the serum. The collected serum is used to estimate the variables in this study as it was stored in the freeze at (-80 ° C) until use.

2.3. Questionnaire paper form used in this Study:

An interview was used for filling in a questionnaire which designed for matching the study need of the study subjects .

Table (2-1) The clinical chart of the study

	Non pregnant	Healthy pregnant	Preeclampsia	Premature birth
N. of child				
Age				
Weight				
Length				
N. of parity				

2.4.Chemicals and Instruments:

The chemicals, kits and instruments used in this work listed in Tables (2.1), (2.2) and (2.3)

Table (2-2) Chemical compounds with manufactures and Origins

Chemical Compound	Manufacture / Origin
Hydrochloric acid HCL	BDH, England
Nitric acid HNO3	BDH, England
Paraffin oil	BDH, England
Perchloric acid HClO4	BDH, England
Thiobarabituric acid (TBA)	BDH, England
Trichloro acetic acid(TCA)	BDH, England

Table (2-3): Diagnostic kits with their catalogue No., manufactures and Origin

No	Chemicals	Supplied company
1	Albumin	Roch- Germany
2	Ceruloplasmin	Roch- Germany
3	Cholesterol	Roch- Germany
4	Cholesterol HDL	Roch- Germany
5	Cholesterol LDL	Roch- Germany
6	Copper	LTA - Italia
7	Ferritin	Roch- Germany
8	Homocysteine kit	BT LAB – China
9	Magnesium	Biolabo – France
10	Triglyceride	Roch- Germany
11	Vitamin B12	BT LAB – China
12	Vitamin C	Roch- Germany

Table (2-4): Instruments with their models, manufactures and origins

No	Instruments	Manufacturer
1	Atomic apsorption spectrophotometer	PGAA500
2	Centrifuge	Germany
3	Cobas e411	Germany
4	Cobas Integra 400 plus	Germany
5	Full Automated Elisa	Germany
6	Micropipette(0.1 , 0.5, 1.0 ml)	Germany
7	Spectrophotometer	China
8	Test tubes	Pyrex (England)
9	Vortex	Germany
10	Waterbath	Germany

2.5. Biochemical analysis :

Several considerable methods were used to measure the studied parameters. It is notable that all measurements were duplicated for each sample .

2.5.1. Determination of serum homocysteine (nmol /ml) :

The quantitative determination of serum homocysteine by ELISA technique

Principle

HCY ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-HCY antibody and an HCY-HRP conjugate. The assay sample and buffer are incubated together with HCY-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The

wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the HCy concentration since HCY

from samples and HCY-HRP conjugate compete for the anti-HCY antibody binding site. Since the number of sites is limited, as more sites are occupied by HCY from the sample, fewer sites are left to bind HCY-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The HCY concentration in each sample is interpolated from this standard curve.

MATERIALS	SPECIFICATION	QUANTITY
ENZYME CONJUGATE	6.0 mL	1 vial
STANDARD A	0 µmol/L	1 vial
STANDARD B	5.0 µmol/L	1 vial
STANDARD C	10 µmol/L	1 vial
STANDARD D	25 µmol/L	1 vial
STANDARD E	50 µmol/L	1 vial
STANDARD F	100 µmol/L	1 vial
SUBSTRATE A	6 Ml	1 vial
SUBSTRATE B	6 Ml	1 vial
STOP SOLUTION	6 mL	1 vial
WASH SOLUTION (100 x)	10 mL	1 vial
BALANCE SOLUTION	6 Ml	1 vial

Reagent preparation:

All reagents and samples balance to room temperature (18-25 ° C) Before use .

1-Wash Solution

10 mL of Wash Solution concentrate (100×) are diluted with 990 mL of distilled water to prepare 1000 mL of Wash Solution (1x).

Procedure

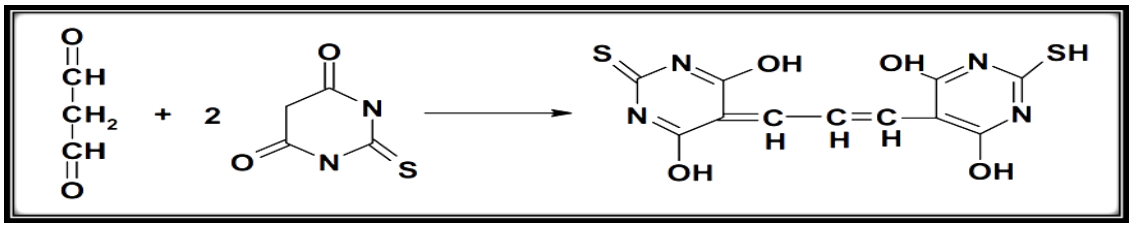
1. The desired numbers of coated wells are secured in the holder then 100 uL of Standards or Samples are added to the appropriate well in the antibody pre-coated Microtiter Plate.100 uL of PBS (pH 7.0-7.2) are added in the blank control Well ..
2. 50 µL of Conjugate is added to each well (NOT blank control well Mixing well in this step is important. The plate is covered and incubated for 1 hour at 37°C.
3. The microtiter plate is washed by using the specified method indicated Below Manual Washing: incubation mixture is removed by aspirating contents of the plate into a proper waste container. each well is filled completely with 1X wash solution, and then contents of the plate are aspirated into a proper waste container. this procedure is repeated five times for a total of FIVE washes. After washing, the plate is inverted, and blotted dry by hitting the plate onto absorbent paper until no moisture appears.
4. 50µL Substrate A and 50 uL Substrate B are added to each well including blank control well, subsequently. Plate is covered and incubated for 10-15 minutes at 20-25°C. (This step is sensitive to sunlight).

5. 50µL of Stop Solution is added to each well including blank control well They are mixed well .
6. The optical density (O.D.) is determined at 450 nm using a microplate reader immediately .

2.5.2. Determination of Serum Malondialdehyde concentration (µmol\l) :

Principle:

Lipid peroxidation was determined by using the thiobarbituric acid method. In this method, Malondialdehyde formed from the breakdown of polyunsaturated fatty acids were identified as the product of LPO that react with thiobarbituric acid, in coexisting trichloro acetic acid, to give a red chromophore absorbing at 532 nm (Fong et al., 1973). MDA concentration were calculated, using the molar extinction coefficient of MDA (ε MDA) equal to 1.56x105 mol^{-1} L cm^{-1} . MDA formed from breakdown(Wills, 1969) of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction



Procedure:

The level of serum MDA was determined spectrophotometrically with a TBA solution. In brief, to 150 µL serum sample the following were added1 mL (17.5%) TCA and 1mL of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One mL of 70% TCA was added and the mixture was allowed to

stand at room temperature for 20 minutes, and centrifuged at 2000 rpm for 15 minutes the supernatant was taken out for scanning spectrophotometrically at 532nm (Muslih *et al*., 2002)

Calculation

The concentration of serum MDA was calculated as follows

Where:

$$\text{MDA Conc.}\left(\frac{\mu\text{mol}}{\text{L}}\right) = \frac{\text{Abs at 532 nm}}{\text{L} \times \boldsymbol{\varepsilon}} \times \text{D} \times 10^6$$

L: Light path (1cm)

ε: Molar Extinction coefficient 1.56×10^5 mol^{-1} L cm^{-1}

D: Dilution factor = 21

2.5.3. Determination of serum Ceruloplasmin concentration(g/l):

Intended use (Halls *et al*., 1981)

In vitro test for the quantitative immunological determination of ceruloplasmin in human serum and plasma on COBAS INTEGRA systems

Test principle

Immunoturbidimetric assay.

Human ceruloplasmin forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.

Reagents - working solutions

R1 Accelerator

Polyethylene glycol (PEG) 50 g/L, in phosphate buffer; preservative

SR Anti-ceruloplasmin T antiserum (rabbit) specific for human ceruloplasmin > 0.42 g/L in phosphate buffer; preservative

R1 is in position B and SR is in position C

COBAS INTEGRA 800 analyzer

Sample size (n) = 73

Passing/Bablok14Linear regression

y = 1.05x - 0.005 g/L y = 1.04x - 0.001 g/L

τ = 0.9330 r = 0.9965

SD (md 95) = 0.015 Sy.x = 0.007

The sample concentrations were between 0.08 and 0.68 g/L (0.597 and 5.07 µmol/L or 8.00 and 68.0 mg/dL).

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factors:8 g/L × 7.46 = µmol/L g/L × 100 = mg/dL

mg/dL × 0.0746 = µmol/L

2.5.4. Determination of plasma Albumin Concentration(mg/dl):

Intended use (Doumas & Peters Jr, 1997)

In vitro test for the quantitative determination of albumin in human serum and plasma on Roche/Hitachi cobas c systems.

Test principle

Colorimetric assay At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{albumin-BCG complex}$$

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer Centrifuge samples containing precipitates before performing the assay .

Stability:

2.5 months at 20 25 °C

4 months at 4 8 °C

4 months at -20 °C

Reagents - working solutions

R1 Citrate buffer: 95 mmol/L, pH 4.1; preservatives, stabilizers-

R2 Citrate buffer: 95 mmol/L, pH 4.1; bromcresol green: 0.66 mmol/L; preservatives, stabilizers

R1 is in position B and R2 is in position C.

cobas c 311 **test definition**

Assay type 2 Point end

Reaction time / Assay10/

6-9 points

Wavelength (sub/main) 505/570

Increase nm Reaction direction

g/L (µmol/L, g/dL) Units

Reagent pipetting Diluent (H_2O)

- 100 µL R1

30 µL R2

Sample	Sample dilution	Sample volumes	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decrease	2 µL	35 µL	70 µL
Increase	2 µL	–	–

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample .

Conversion factors: g/L x 15.2 = µmol/L

µmol/L x 0.0658 = g/L g/L x 0.1 = g/dL

2.5.5. Determination of serum Vitamin B12 concentration(pmol/l):

Intended use

Binding assay for the in vitro quantitative determination of vitamin B12 in human serum and plasma

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers

Test principle

Competition principle. Total duration of assay: 27 minutes **1**st incubation: By incubating the sample (15 µL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is release **2**nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample. **3**rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B12 biotin complex. The entire complex becomes bound to the solid

phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode

Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier

- Results are determined via a calibration curve which is instrument-specifically generated by 2 point calibration and a master curve provided via the reagent barcode or e barcode.

Reagents - working solutions

The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as B12 II. PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5 PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL Streptavidin-coated microparticles 0.72 mg/mL; preservative R1 Intrinsic factor~Ru(bpy) (gray cap), 1 bottle, 10 Ml Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L; cobinamide

dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative R2 Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

Conversion factors: pmol/L x 1.36 = pg/mL pg/mL x 0.738 = pmol/

cobas e 411 analyzer						
				Intermediate precision		
Sample	Mean			SD	CV	
	pmol/L			pmol/L	Repeatability	
Human serum 1	130			9.37	SD	CV
Human serum 2	299			12.9	pmol/L	%
Human serum 3	708			22.9	6.54	5.0
Human serum 4	908			34.2	9.59	3.2
Human serum 5	1432			53.6	14.5	2.1
PreciControl Varia1	330			13.7	20.2	2.2
PreciControl Varia2	689			28.3	30.2	2.1
					9.00	2.7
					14.9	2.2

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean	SD	CV	SD	CV
	pg/Ml	pg/mL	%	pg/mL	%
Human serum 1	176	8.86	5.0	12.7	7.2
Human serum 2	405	13.0	3.2	17.5	4.3
Human serum 3	960	19.7	2.1	31.0	3.2
Human serum 4	1230	27.4	2.2	46.4	3.8
Human serum 5	1940	40.9	2.1	72.6	3.7
PreciControl Varia1	447	12.2	2.7	18.6	4.2
PreciControl Varia2	934	20.2	2.2	38.4	4.1

2.5.6.Determination of serum Vitamin C (ng/ml) :

The quantitative determination of Vit.C concentration in human serum was performed by ELISA technique .

Principle:

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) =

SD/mean x

100

Intra-Assay:

CV<8%

Inter-Assay:

CV<10%

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human VC antibody. VC present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human VC Antibody is added and binds to VC in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated VC antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human VC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

Reagent Provided

Components	Quantity
Standard Solution (320ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human VC Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

- 37°C±0.5°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Reagent Preparation

- All reagents should be brought to room temperature before use.

Standard Reconstitute the 120µl of the standard (320ng/ml) with 120µl of standard diluent to generate a 160ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (160ng/ml) 1:2 with standard diluent to produce

80ng/ml, 40ng/ml, 20ng/ml and 10ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20℃ and used within one month. Dilution of standard solutions suggested are as follows:

160ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
80ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
40ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
20ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
10ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer

1- 20ml of Wash Buffer Concentrate 25x are diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

2- The number of strips required for the assay is determine. the strips is inserted in the frames for use. The unused strips should be stored at 2-8°C.

3- 50µl standard are added to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4- 40µl sample are added to sample wells and then 10µl anti-VC

antibody are added to sample wells, then 50µl streptavidin-HRP are added to sample wells and standard wells (Not blank control well). Mix well. the plate is covered with a sealer and incubated 60 minutes at 37°C

5- the sealer is removed and the plate is washed 5 times with wash buffer. wells are soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, all wells are aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer. the plate is blotted onto paper towels or other absorbent material.

6- 50µl substrate solution A are added to each well and then 50µl substrate solution B are added to each well. plate is incubated and covered with a new sealer for 10 minutes at 37°C in the dark.

7. 50µl Stop Solution are added to each well, the blue color will change into yellow immediately.

8.The optical density (OD value) of each well is determined immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5.7. Determination of Serum Selenium Concentrations ng\ml :

Principle:

Concentrations of element in serum samples were measured by flame atomic absorption method. In this method, light of hollow cathode (HC) lamp is absorbed by ground state atoms in a lean air-acetylene flame.The amount of light absorbed is directly proportional to the

concentration of atoms in the gaseous state in the light path and hence to the concentration of selenium in the solution.

Digestion of samples

The serum samples were digested by adding 2 mL of conc. nitric acid and 1 mL of conc. Perchloric acid to 0.5 mL serum in Pyrex tube. Heated for 1 hour at 160 ˚C using oil bath, then samples were cooled, and the volume was completed to10 mL by 0.3 N hydrochloric acid .

2.5.8. Determination of Serum Magnesium concentration mg/dl:

Intended use (Ferguson *et al*., 1964)

In vitro test for the quantitative determination of magnesium in human serum, plasma, and urine on COBAS INTEGRA systems.

Test principle

In vitro test for the quantitative determination of magnesium in human serum, plasma, and urine on COBAS INTEGRA systems

Test principle

Colorimetric method with Chlorophosphonazo III.5

Chlorophosphonazo III (CPZ III) binds to magnesium and causes an absorbance increase at 659 nm. EGTA (ethylenebis(oxyethylenenitrilo) tetra-acetic acid) is used to inhibit calcium binding to CPZ III.

$$Mg^{++} + \text{CPZ III} \xrightarrow{\text{PH7.5}} \text{Mg-CPZ III complex}$$

Nonspecific absorbance interferences are reduced by the addition of EDTA (ethylenediaminetetra-acetic acid), which removes magnesium from the magnesium-CPZ III complex and allows for an accurate sample blank measurement

PH7.5

Mg-CPZ III complex + EDTA → Mg-EDTA + CPZ III

The difference in absorbance between the magnesium-CPZ III complex and the EDTA treated complex is the absorbance due to magnesium alone.

Reagents - working solutions

R1 TESa): 145 mmol/L; pH 7.5; Chlorophosphonazo III: 0.2 mmol/L; EGTA: 10 mmol/L; preservative

SR TESa): 100 mmol/L; pH 7.5; EDTA: 16 mmol/L; preservative

a) N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid

R1 is in position B and SR is in position C

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs.calculation mode	Endpoint
Reaction direction	Decrease

Wavelength A	659 nm
Calc. first/last	43/49
Unit	mmol/L
Serum ,Plasma	
Reaction mode	R1-S-SR
Urine	
Reaction mode	D-R1-S-SR
Predilution factor	5

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers)

Conversion factors: mmol/L × 2.43 = mg/dL mEq/L × 0.5 = mmol/L mEq/L × 1.22 = mg/dL

2.5.9. Determination of serum copper concentration µg/dl :

Test summary

The cupric ions react with the chromogen Di-Br- PAESA forming a blue compound which intensity is proportional to the copper concentration present in the sample.

Reagents

Reagent A: Acetate buffer 0.1 M PH 4.9 : reducing Agents and preservatives.

Reagent B: 3.5 Di-Br-PAESA.

Standard: Ion copper 200 µg\dl; preservatives.

REAGENTS PREPARATION

Prepare the work reagent mixing in equal quantity the Reagent A with Reagent B.

Reagents are stored at 2-8 C and are stable until expiration date on label.

Work Reagent is stable 20 days at room temperature.

PROCEDURE SERUM\PLASMA

Kind of analysis: End point

Reading time: 10 minutes

Colour stability: 30 minutes

Wave length: 580 nm|(570-590)

Temperature: 20-25 C

Light path 1 cm

Zero: Blank Reagent

Mix and wait for 10 minutes then read the absorbances against the blank at 580 nm. the colour is stable for 30 minutes.

Reagents	Blank	Standard	Sample
Work Reagent	1 ml	1 ml	1ml
Distilled water	66 µl	--	--
Standard	--	66 µl	--
Sample	--	--	66 µl

Calculation

$$\text{Copper µg/l} = \frac{A\,(sample)}{A\,(standard)} \times 200$$

$$\text{Copper µmol/l} = \frac{A\,(sample)}{A\,(STANDARD)} \times 31.47$$

2.5.10.Determination of serum ferritin concentration ng/ml :

Intended use

Immunoassay for the in vitro quantitative determination of ferritin in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complexa) form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When

processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Reagents - working solutions

The reagent rackpack is labeled as FERR.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-Ferritin-Ab~biotin (gray cap), 1 bottle, 10 mL:

Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-ferritin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:

Monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
7.0	1.45	0.101		0.168	11.6
3.5	11.9	0.411		0.798	6.7
4.1	19.2	0.780		1.47	7.7
Human serum 4	376	10.8	2.9	17.2	4.6
Human serum 5	1361	26.5	1.9	84.4	6.2
PreciControl Varia 1	134	1.96	1.5	2.75	2.1
PreciControl Varia 2	858	15.1	1.8	21.7	2.5

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	1.45	0.101	7.0	0.168	11.6
Human serum 2	11.9	0.411	3.5	0.798	6.7
Human serum 3	19.2	0.780	4.1	1.47	7.7
Human serum 4	376	10.8	2.9	17.2	4.6
Human serum 5	1361	26.5	1.9	84.4	6.2
PreciControl Varia 1	134	1.96	1.5	2.75	2.1
PreciControl Varia 2	858	15.1	1.8	21.7	2.5

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

2.5.11. Determination of Serum Lipid Profile:

2.5.11.1. Determination of Serum Total Cholesterol Concentration mg/dl :

Intended use

In vitro test for the quantitative determination of total cholesterol in serum andplasma on COBAS INTEGRA systems.

Test principle

Enzymatic, colorimetric method, Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye(Allain *et al*., 1974)

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye} + 4\,H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

Reagents - working solutions

R PIPES[a]) buffer: 225 mmol/L, pH 6.8; Mg2+: 10 mmol/L; sodiumcholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %;

cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L

(≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

PIPES = Piperazine-1,4-bis(2-ethanesulfonic acid)

R is in position B.

Application for serum and plasma COBAS INTEGRA 400 plus test definition

Test definition	
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R-S
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	17/69
Unit	mmol/L

Pipetting parameter		Diluent (H2O)
R1	47 Ml	70 µL
Sample	2 Ml	23 µL
Total volume	142µL	

Calculation

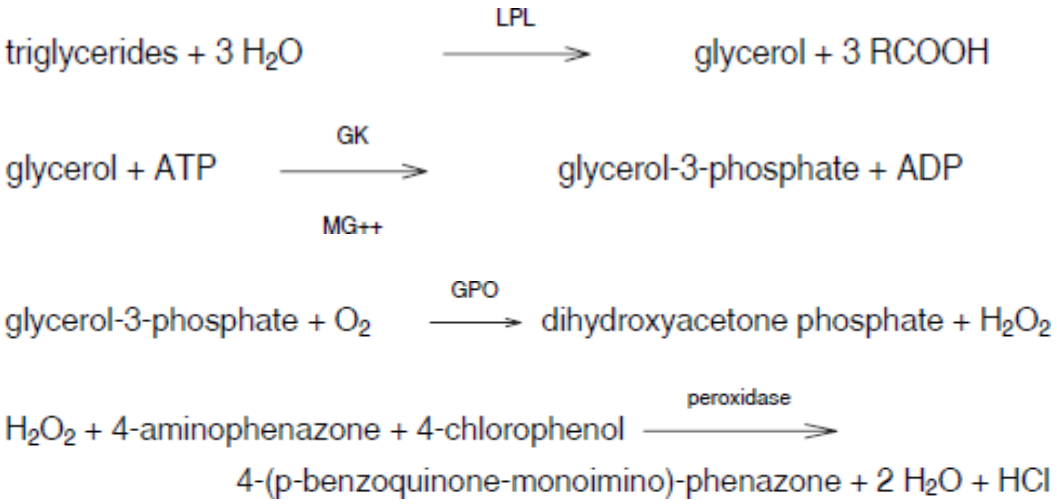
COBAS INTEGRA analyzers automatically calculate the analyte concentration ofeach sample .

2.5.11.2.Determination of Serum Triglyceride Concentration mg/dl :

Serum TG was determined by quantitative determination of the triglycerides concentration in human serum and plasma on COBAS INTEGRA systems.

Principle (Siedel *et al*., 1993)

Enzymatic colorimetric test (Siedel *et al*., 1993)



Reagents - working solutions

R PIPES buffer: 50 mmol/L, pH 6.8; Mg2+: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; LPL (microbial): ≥ 83 µkat/L; GK (microbial): ≥ 3 µkat/L; GPO (microbial): ≥ 41 µkat/L; POD (horseradish): ≥ 1.6 µkat/L; preservative; stabilizers

R is in position **B.**

COBAS INTEGRA 400 plus test definition

Test definition	
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R-S
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	17/42
Unit	mmol/L

Pipetting parameter		Diluent (H2O)
R1	120 µL	
Sample	2 µL	28 µL
Total volume	150 µL	

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration ofeach sample

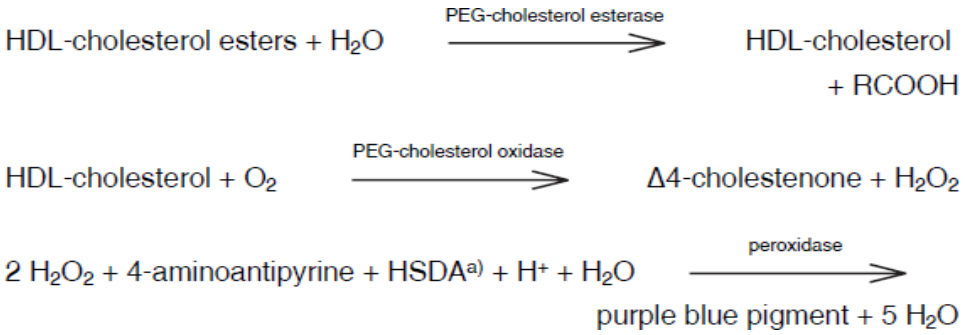
2.5.11.3. Determination of Serum HDL-c Concentration mg/dl :

Intended use

In vitro test for the quantitative determination of HDL-cholesterol concentration in serum and plasma on COBAS INTEGRA systems.

Test principle

Homogeneous enzymatic colorimetric assay (Matsuzaki *et al*., 1996). In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDLcholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4- cholestenone and hydrogen peroxide



a) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL- cholesterol concentration. It is determined by measuring the increase in absorbanceat 583 nm.

Reagents - working solutions

R1 HEPES buffer: 10.07 mmol/L; CHES: 96.95 mmol/L, pH 7.4; dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate:
> 11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): > 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative

SR HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L;
Preservative

R1 is in position B and SR is in position C. **Application for serum and plasma COBAS INTEGRA 400 plus test**

Definition

Test definition	
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1−S−SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	33/69
Unit	mmol/L

Pipetting parameter		Diluent (H2O)
R1	150 µL	
Sample	2.5 µL	7.0 µL
SR	50 Ml	
Total volume	209.5 µL	

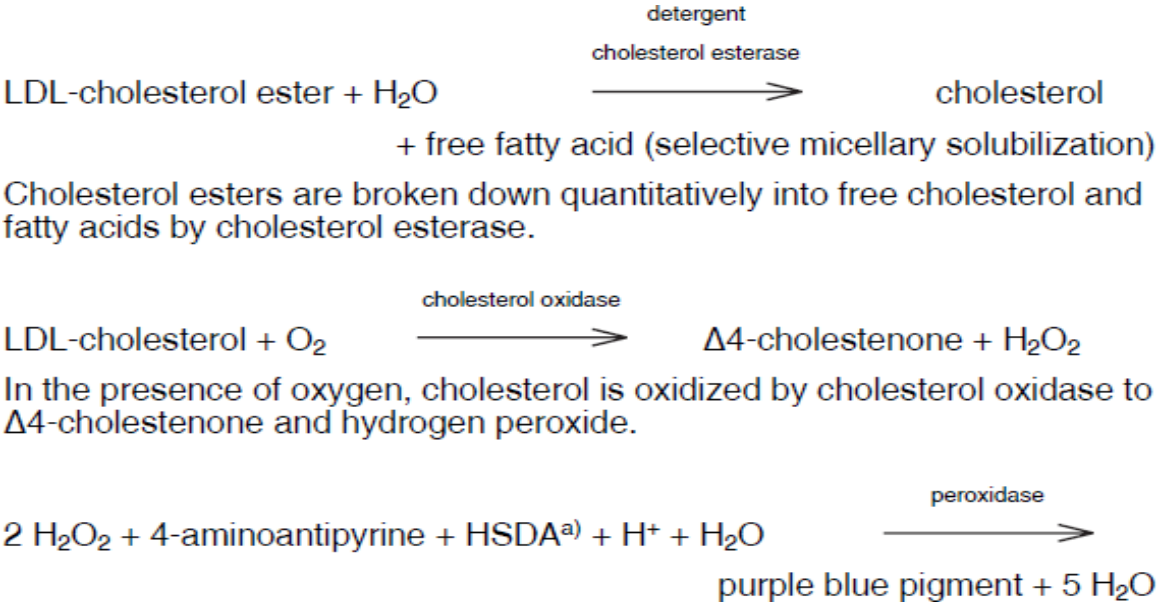
Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration ofeach sample.

2.5.11.4. Determination of LDL level Test mg/dl:

principle

Homogeneous enzymatic colorimetric assay.



a) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents - working solutions

R1 MOPSb): 20.1 mmol/L, pH 6.5; HSDA: 0.958 mmol/L; ascorbate oxidase (recombinant): ≥ 50 µkat/L; peroxidase (horseradish): ≥167 µkat/L; stabilizers, preservative

SR MOPS: 20.1 mmol/L, pH 6.8; MgSO4·7H2O: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (microbial): ≥ 50 µkat/L; cholesterol oxidase (microbial): ≥ 33 µkat/L; peroxidase (horseradish): ≥ 334 µkat/L; stabilizers; preservative

b) 3-morpholinopropane sulfonic acid buffer

R1 is in position B and SR is in position C.

Application for serum and plasma COBAS INTEGRA 400 plus test definition

Test definition	
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1−S−SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	33/69
Unit	mmol/L

Pipetting parameter		Diluent (H2O)
R1	150 Ml	
Sample	2 Ml	26 µL
SR	50 µL	
Total volume	209 Ml	

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration ofeach sample.

Measurement of VLDL level mg/dl

VLDL(mg/dl) = serum TG /5

2.6. Statistical Analysis:

The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using one way ANOVA (analysis of variations) test with LSD (least significant difference) were performed using mean and standard deviations (SDs) for continuous variables (p_ value ≤ 0.05) was considered to be significant and correlations coefficient(r) test.

All analyses were performed with statistical Package for the social sciences SPSS for Windows (version 23.0 SPSS Inc, Chicago, 111).

CHAPTER THREE

RESULTS & DISCUSSION

3.1. Clinical and Characteristic Features of the Study Groups

There are (130) women included in the current study, the group of patients (70) divided into two groups (preeclampsia and premature birth) were compared with the tow control group of healthy subjects (60). Characteristic data for all studied groups shown in Table (3-1) .

Table (3-1):- Characteristic data for studied groups

Parameters / Groups	NO.	Age (Year)	BMI
Non pregnant	30	32.35 ± 7.58	30.41 ± 4.70 [a]
Healthy pregnancy	30	26.25 ± 6.01	30.52 ± 4.09 [a]
Premature birth	35	31.05 ± 11.36	26.90 ± 3.35 [b]
Preeclampsia	35	30.85 ± 5.15	30.25 ± 5.23 [a]
L.S.D		4.15	1.87

3.2. General Comparison for all Studied Parameters:

3.2.1.Serum Homocystiene Concentration:

Table (3-2) shows a significant increase in the level of homocysteine in the preeclampsia and premature birth groups compared to the healthy groups at ($P < 0.05$), as previous studies indicated an increase in homocysteine in women exposed to preeclampsia and at risk of premature birth (Dymara-Konopka & Laskowska, 2019; Pisal *et al*., 2019) . Hyperhomocysteinemia can damage the vascular system that supports placental function during pregnancy, which can lead to miscarriage and other negative pregnancy outcomes. In women who have experienced

repeated pregnancy loss, lowering homocysteine levels will increase pregnancy outcome (live birth) (Humadi, 2018). Hyperhomocysteinemia has been linked to a number of disorders, including cardiovascular and neurological problems. Homocysteine (HCys) is a crucial metabolite involved in the biosynthesis and metabolism of methionine (Met), which is important for the life cycle of physiological cells. Several enzymes that control HCys concentration finely regulate Met's biochemistry. Indeed, the cell's well-being depends on controlled enzyme activity, and its failure could result in an increase in HCys concentration, which could lead to the onset of a variety of pathological conditions (Kim et al., 2018; Tinelli *et al*., 2019) . Patients with hyperhomocysteinemia and MTHFR (methylenetetrahydrofolate reductase) mutations have trouble getting pregnant. High concentration methylfolate, vitamins B6 and B12 supplementation improves pregnancy outcomes in women with MTHFR mutations (Serapinas *et al*., 2017) .

Table (3-2): Serum homocystiene levels of control and patients groups

Parameters / Groups	NO.	Homocysteine (µmol /L) Mean ±SD
Non pregnant	30	8.92 ± 0.64^{b}
Healthy pregnancy	30	9.13 ± 0.40^{b}
Premature birth	35	13.03 ± 0.95^{a}
Preeclampsia	35	13.27 ± 2.49^{a}
L.S.D		0.73

No: Number of subjects.

SD : Standard deviation.

LSD: Least Significant Difference

(a, b, c) Means having different letters in the same column differed significantly (P <0.05) .

3.2.2. Comparison of Homocysteine Levels of Oxidative Stress Index of Malondialdehyde:

3-2.2.1-Serum malondialdehyde concentration:

We note in Table (3-3) and Fig. (3-1) there is a clear increase in MDA Levels in the groups of preeclampsia and risk of premature birth compared with the two groups of controls. Healthy and non-pregnant pregnancy at ($p < 0.05$) . The results were more consistent than a previous study (Al-Maiahy et al., 2021; Asiltas *et al*., 2018; İlhan et al., 2017; Micle *et al*., 2012) .Since MDA is a by-product of lipid peroxide, elevated MDA levels may indicate a weakened antioxidant protection system and/or an excess of lipid peroxides. Peroxides of these fats are primarily formed in the placenta as a result of reactive oxygen species peroxiding the membrane (ROS) (Laloo, 2019) . Oxidative stress arises as free radical synthesis exceeds the ability of cellular defense mechanisms to buffer them. The pathogenesis of preeclampsia is characterized by an accumulation in free radicals , as well as a reduction in antioxidants. Several enzymatic buffers, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase GPX help to counteract oxidative stress (Haram *et al*., 2019) .

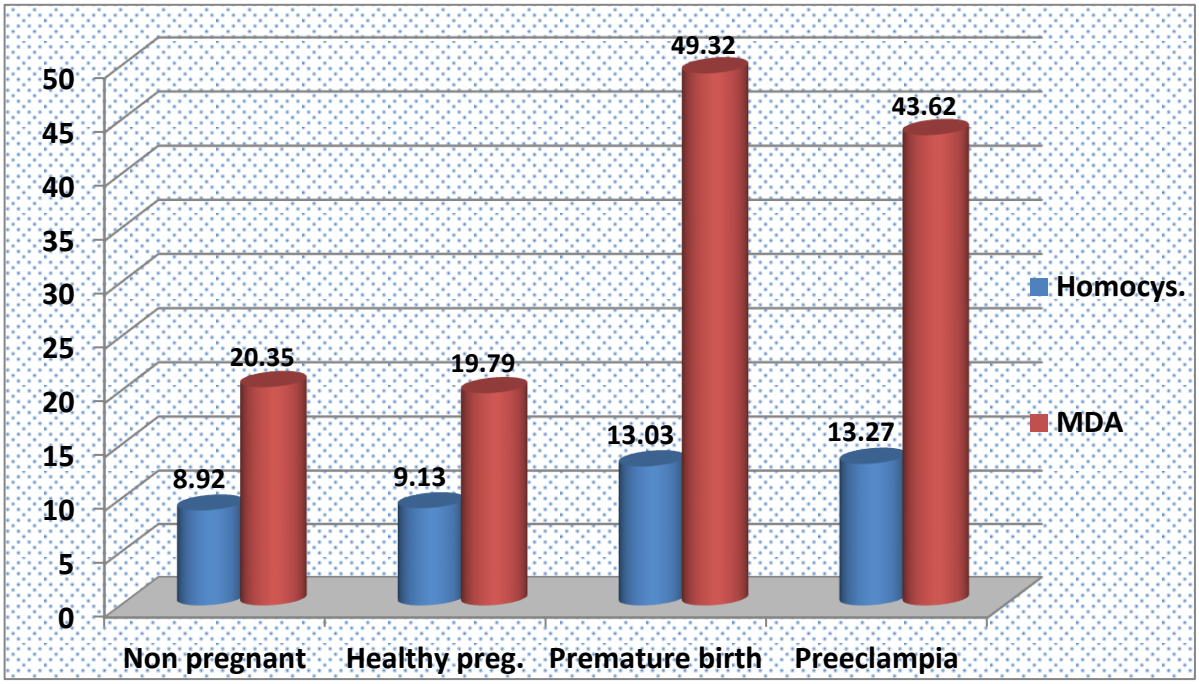
Increased free radicals cause cellular dysfunction, oxidative damage to macromolecules, and endothelial dysfunction, which may play a role in the pathogenesis of preeclampsia .Higher systolic and diastolic blood pressure, decreased weight, increased oxidative stress markers (plasma

homocysteine and malondialdehyde (MDA) levels), and reduced placental weight were all seen in PIH-induced , homocysteine was found to impact angiogenesis, contribute to preeclamptic pathology, and have negative impacts on fetal development measurements in PE women. (Kemse *et al*., 2014) .

Table (3-3) Serum Homocysteine and MDA Tests for study groups

Parameters / Groups	Homocysteine µmol /L	MDA µmol\l
Non pregnant	8.92 ± 0.64 [b]	20.35 ± 5.54 [c]
Healthy pregnancy	9.13 ± 0.40 [b]	19.79 ± 4.21 [c]
Premature birth	13.03 ± 0.95 [a]	49.32 ± 11.05 [a]
Preeclampsia	13.27 ± 2.49 [a]	43.62 ± 6.78 [b]
L.S.D	0.73	3.87

Legend as in table (3-2)



Figure(3-1) Homocysteine and MDA levels of control and Patients groups

Figure (3-2) shows positive correlation between serum homocysteine and MDA in premature birth group with correlation coefficient (r= 0.17) and positive correlation in preeclampsia group with correlation coefficient (r= 0.16). This positive association between homocysteine and malondialdehyde was also reported in this study (Khosrowbeygi *et al*., 2011) .

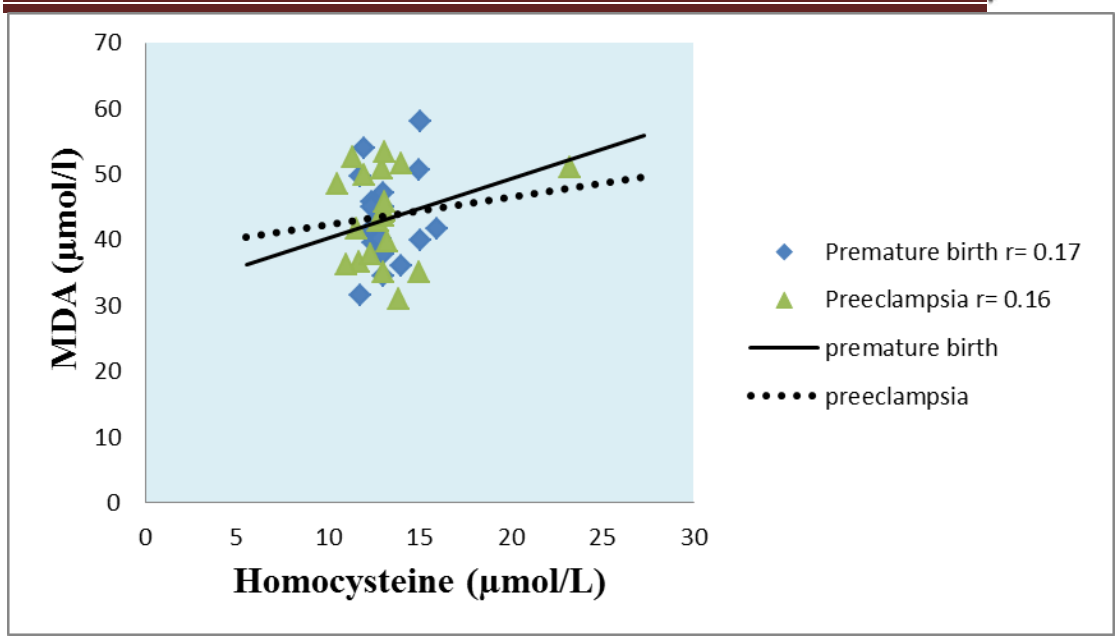


Figure (3-2): Correlation between serum Hcy and MDA in preeclampsia and premature birth groups

*Correlation is significant at the 0.01 level .

**Correlation is significant at the 0.05 level .

R=0 No correlation

0.00< R<0.25 weak extreme

0.25<R<0.75 moderate extreme

0.75<R<1 strong extreme

R=1 Perfect

Negative value inversely

3.2.3. Comparison of homocysteine levels with antioxidants:

3.2.3.1. Serum Ceruloplasmin and Albumin Concentrations:

As it is shown in Table (3-4) and Fig. (3-3) there is a significant decrease in the level of ceruloplasmin in the groups of preeclampsia and the risk of premature birth compared with the two groups of healthy and non-pregnant controls at ($p < 0.05$) . These results are consistent with previous study (Rathore *et al*., 2011) .

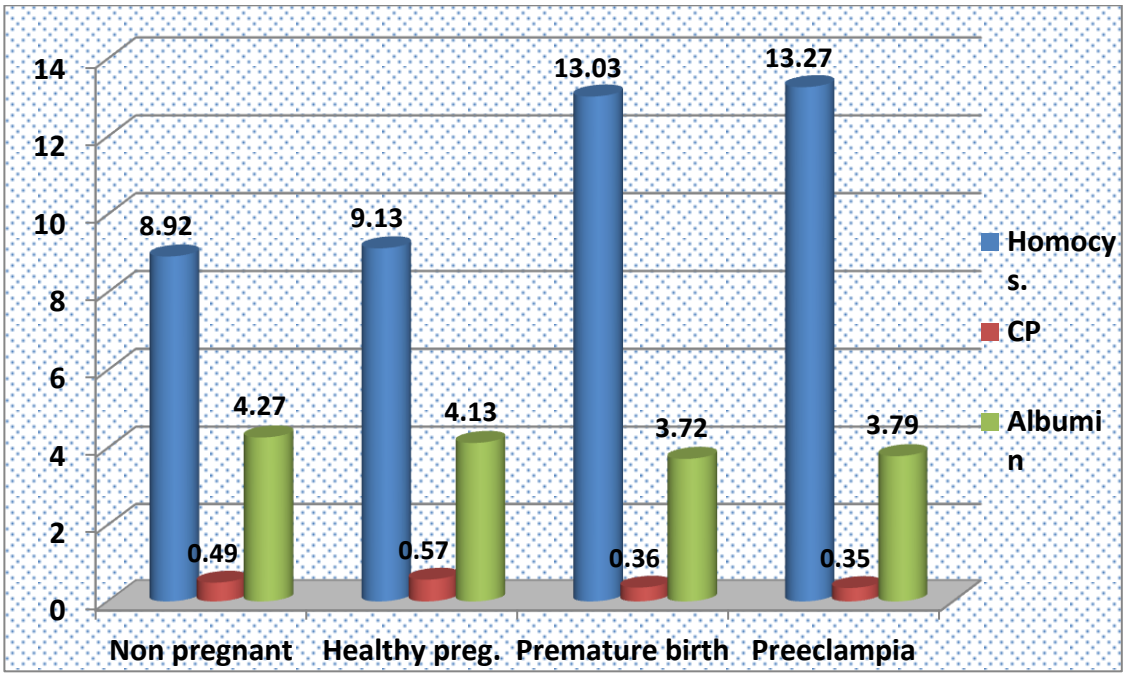
Progesterone causes the liver to produce more ceruloplasmin, which may help with fetal growth and development health and immune system bolstering (Shen et al., 2015) , Progesterone was shown to lower the risk of early premature birth PB in women (Tita & Rouse, 2009). progesterone levels in women with preeclampsia were few. The deficiency in placental control in preeclampsia may be to blame for the decrease (Wan *et al*., 2018) , As a result, the lack of progesterone hormone results in a deficiency of ceruloplasmin in the case of preeclampsia and the risk of premature birth . Women with preeclampsia had considerably lower amounts of, progesterone, and estrogen in their blood. Estrogen and ceruloplasmin have a positive relationship, and medicines containing estrogen hormones may raise ceruloplasmin levels in the blood. The precise method through which estradiol boosted ceruloplasmin levels is yet unknown, However, there are a mass of data demonstrating that estradiol might increase the creation of numerous enzymes in the liver, So the decrease in estrogen during preeclampsia may be the cause of the deficiency of ceruloplasmin by reducing the production of it by the liver (Ani & Moshtaghie, 1990; Wan *et al*., 2018). Cp level was significantly increased when pre-treated with vitamin C (Vinodini *et al*., 2012), and this may be one of the reasons for the

decrease in ceruloplasmin, as its decrease in the study groups was associated with a decrease in ceruloplasmin.

Table (3-4) Serum Homocysteine and Antioxidants Tests for study groups

Parameters / Groups	Homocysteine µmol /L	CP g/L	Albumin mg/dl
Non pregnant	8.92 ± 0.64 [b]	0.49 ± 0.03 [b]	4.27 ± 0.39 [a]
Healthy pregnancy	9.13 ± 0.40 [b]	0.57 ± 0.07 [a]	4.13 ± 0.53 [a]
Premature birth	13.03 ± 0.95 [a]	0.36 ± 0.08 [c]	3.72 ± 0.30 [b]
Preeclampsia	13.27 ± 2.49 [a]	0.35 ± 0.06 [c]	3.79 ± 0.46 [b]
L.S.D	0.73	0.03	0.23

Legend as in table (3-2)



Figure(3-3) Homocysteine and Antioxidants levels of control and Patients groups

Figure (3-4) shows negative correlation between serum homocysteine and ceruloplasmin in premature group with correlation coefficient (r= - 0.16) and positive correlation in preeclampsia group with correlation coefficient (r=- 0.14).

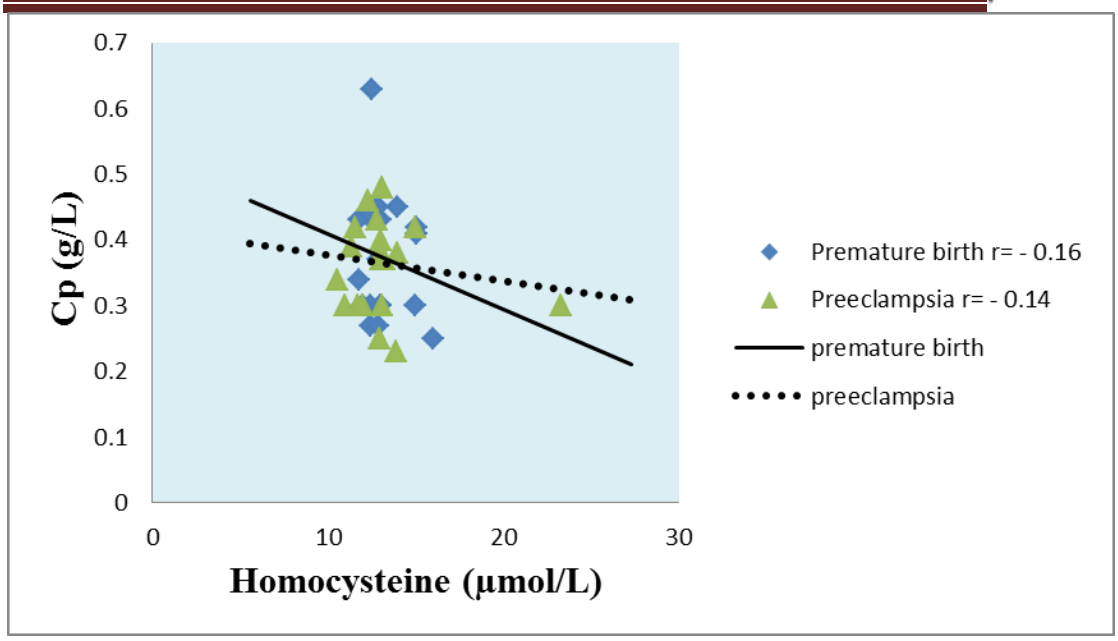


Figure (3-4): Correlation between serum Hcy and Ceruloplasmin in preeclampsia and premature birth groups

Our results are shown in (3-4) and Fig. (3-3) there is a significant decrease in the level of albumin in the groups of preeclampsia and the risk of premature birth compared with the two groups of healthy and non-pregnant controls ($p < 0.05$) .

that serum albumin level had statistically reduction in preeclampsia .that serum albumin level in preeclampsia may be used as a significant determinant of disease seriousness and can be used as a valuable marker for predicting time of birth or termination of pregnancy and pregnancy outcomes (Al Ghazali *et al*., 2014). Our results matched several previous

studies (D.-M. Dai et al., 2017; Özdemir et al., 2018; Rzepka et al., 2019; Yang et al., 2017) ,

In women with pre-eclampsia, the severity of urinary protein, predicts maternal or fetal complications. Arbitration of systemic small arteries and capillary endothelial cells, which contributes to increased vascular permeability and therefore blood concentration, edema, and hypoalbuminemia , is the pathophysiological cause of preeclampsia. As a result, in patients with preeclampsia and eclampsia , there could be a drop in blood albumin (ALB), During pregnancy, a physiological rise in plasma volume can lead to changes in maternal blood components such as decreased hemoglobin (HB) and ALB. Plasma albumin is well known to decrease in response to inflammation. Therefore, albumin synthesis measured in the plasma is a good indicator of liver albumin synthesis (García-Tevijano *et al*., 2001; Uwitonze & Razzaque, 2018) .

Figure (3-5) shows negative correlation between serum homocysteine and albumin in premature group with correlation coefficient (r= -0.15) and positive correlation in preeclampsia group with correlation coefficient (r=- 0.45).

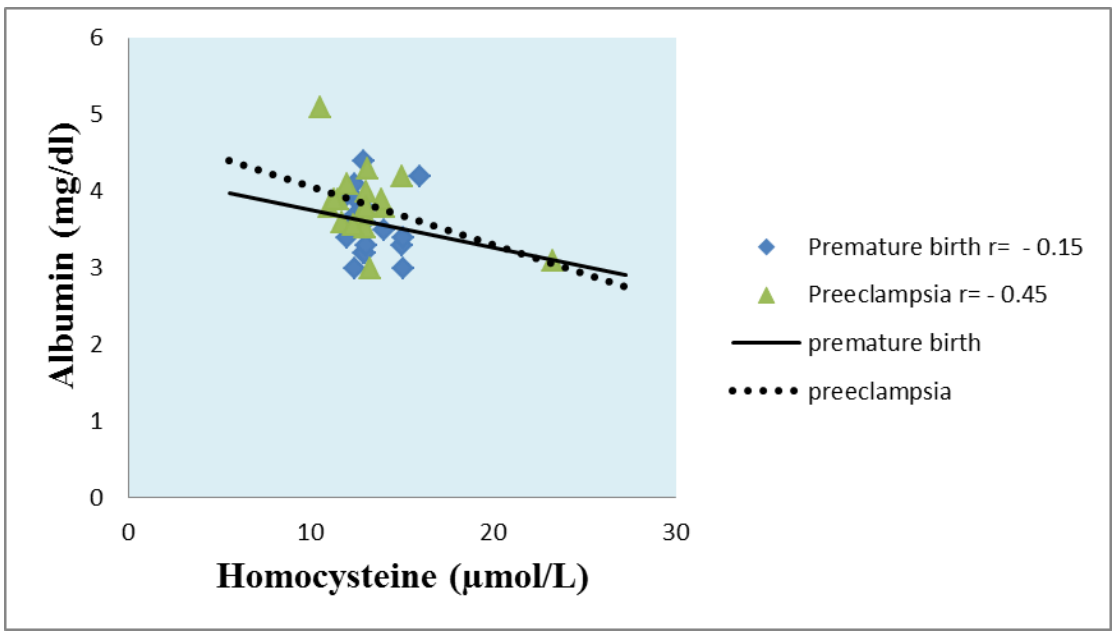


Figure (3-5): Correlation between serum Hcy and Albumin in preeclampsia and premature birth groups

3.2.4. Comparison of homocysteine levels with Vitamins concentration:

3.2.4.1. Serum Vitamins B12 and C concentrations:

Table (3-5) and Fig. (3-6) Shows there is a significant decrease in the level of vitamin B12 in the groups of preeclampsia and the risk of premature birth compared with the two groups of healthy and non-pregnant controls at ($p < 0.05$).

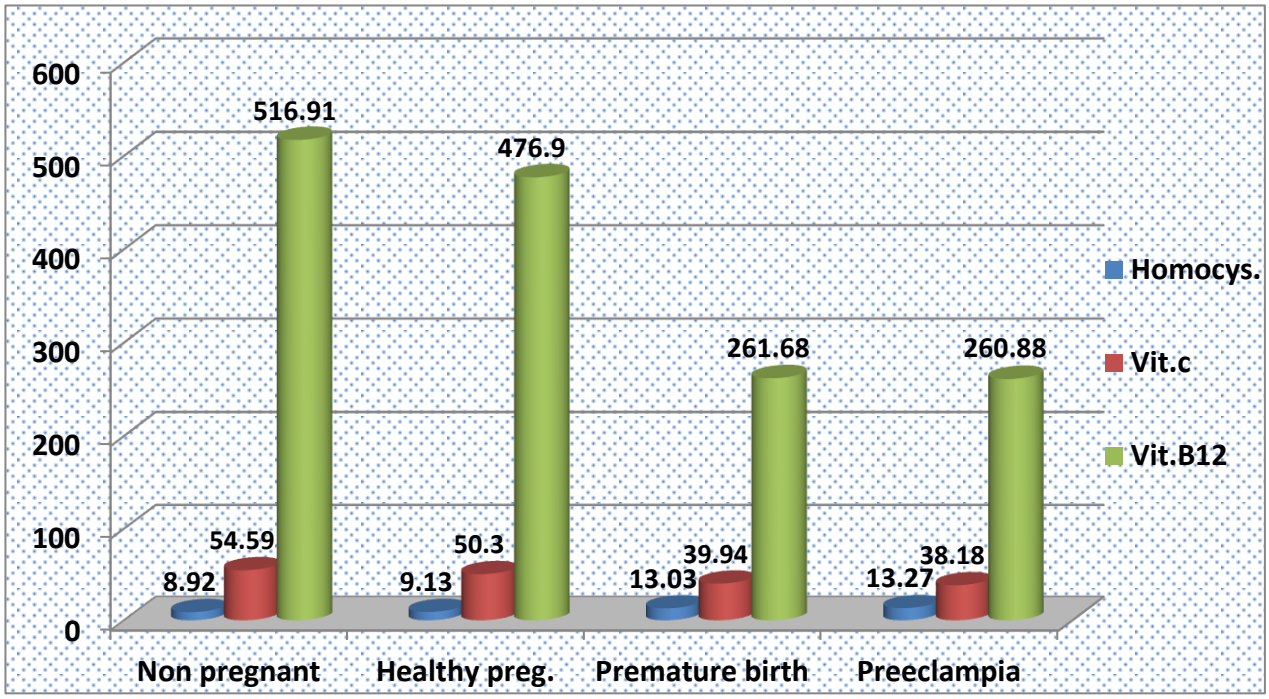
The results were identical to more than one study (Kadhim & Mater, 2019; Mardali *et al*., 2021; Rogne *et al.*, 2017) .

B12 is an important cofactor in the transition of methyl groups in chemical reactions in which enzymes such as methionine synthetase. If a vitamin B12 deficiency The transfer of homocysteine to its metabolites is impair , resulting in homocysteine aggregation. As a result of the rise of homocysteine levels,Homocysteine in endothelial cells can cause preeclampsia, and there would be further oxidative stress (Mardali *et al*., 2021). Homocysteine levels that are too high may be caused by a genetic or dietary problem, or a mixture of the two. Folic acid and vitamin B12 deficiency are examples of nutritional deficiencies (Kannayan *et al*., 2020) . We also note that the level of vitamin B12 is lower in the healthy pregnancy group than in the non-pregnant group, and this may be due to the pregnant woman’s consumption of a greater amount of vitamins in feeding the fetus .

Table (3-5) Serum Homocysteine and Vitamins conc. for study groups

Parameters / Groups	Homocysteine µmol /L	Vit.c ng/ml	Vit.B12 pmol/l
Non pregnant	8.92 ± 0.64 [b]	54.59 ± 12.14 [a]	516.91 ± 36.06 [a]
Healthy pregnancy	9.13 ± 0.40 [b]	50.30 ± 1.67 [b]	476.90 ± 44.40 [b]
Premature birth	13.03 ± 0.95 [a]	39.94 ± 3.69 [c]	261.68 ± 32.92 [c]
Preeclampsia	13.27 ± 2.49 [a]	38.18 ± 8.58 [c]	260.88 ± 35.16 [c]
L.S.D	0.73	4.05	19.67

Legend as in table (3-2)



Figure(3-6) Homocysteine and Vitamins levels of control and Patients groups

Figure (3-7) shows negative correlation between serum homocysteine and B12 in premature group with correlation coefficient (r= -0.23) and positive correlation in preeclampsia group with correlation coefficient (r= -0.14). (Mujawar *et al*., 2011)

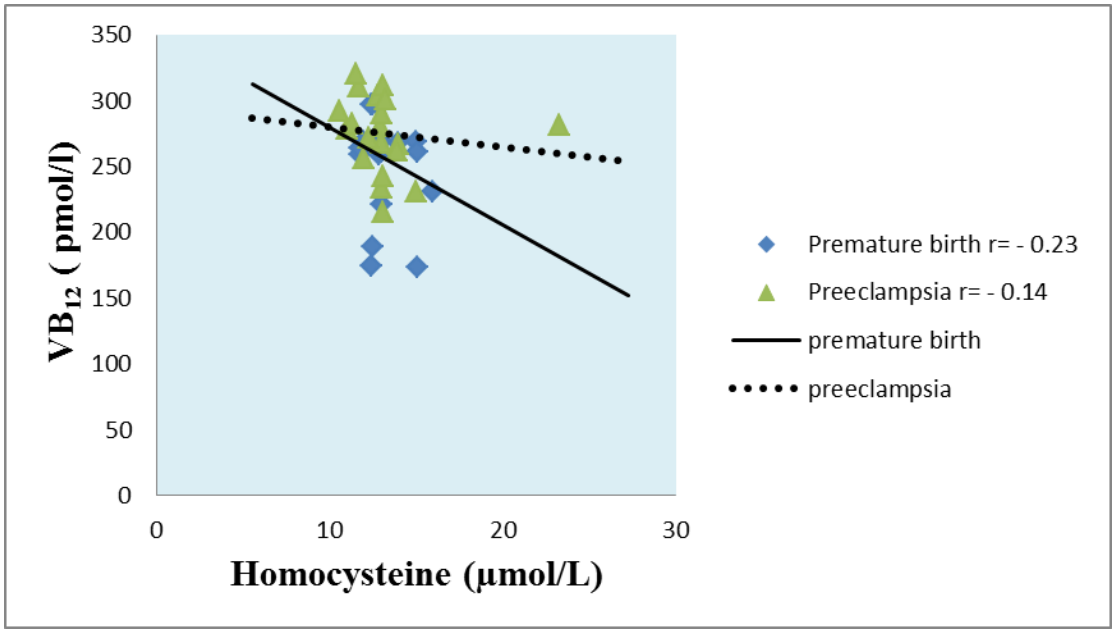


Figure (3-7): Correlation between serum Hcy and Vitamin B12 in preeclampsia and premature birth groups

As noted in Table (3-5) and Figure (3-6), there is a significant decrease in vitamin C values in the main groups, preeclampsia and risk of premature birth when compared with the two control groups from healthy and non-pregnant pregnancy at ($p < 0.05$). the current public health initiatives to increase consumption of vitamin C and other antioxidant-rich fruits and vegetables can reduce the risk of preeclampsia (Zhang et al., 2002) .

The results were consistent with (Casanueva et al., 2005; Gupta et al., 2020; Shetty et al., 2018; Siega-Riz et al., 2003) . Vitamin C is a carbohydrate with a low molecular weight and an enediol structure. As a

result, it is a powerful electron donor that may provide antioxidant protection against oxidative stress caused by preeclampsia (Dewi *et al*., 2021) . antioxidant vitamins like vitamin C and E may be useful in reducing Hcy-mediated oxidative vascular damage. Hcy levels were negatively related to folate, vitamin C and vitamin B12 (Krajcovicova-Kudlackova *et al*., 2002). that an acute elevation in homocysteine concentration is associated with rapid onset endothelial dysfunction, which can be prevented by pretreatment with vitamin C . that oxidative stress mechanisms mediate endothelial dysfunction during hyperhomocysteinemia. Oxidative stress is a key factor in atherosclerosis. Generation of free-radical superoxide anions deactivates nitric oxide. Deactivation of nitric oxide, the major endothelium derived vasodilator, may lead to vasoconstriction, platelet aggregation, and all of which promote atherosclerosis. that an elevation in homocysteine concentration is associated with an acute impairment of endothelial function that can be prevented by pretreatment with vitamin C. the hypothesis that the adverse effects of homocysteine on vascular endothelial cells are mediated through oxidative stress mechanisms (Chambers *et al*., 1999) . There is also a decrease in the level of vitamin C in the healthy pregnancy group relative to non-pregnant women .

Figure (3-8) there is a negative correlation between homocysteine and vitamin C in premature group with correlation coefficient (r= -0.09) and positive correlation in preeclampsia group with correlation coefficient (r= -0.16).

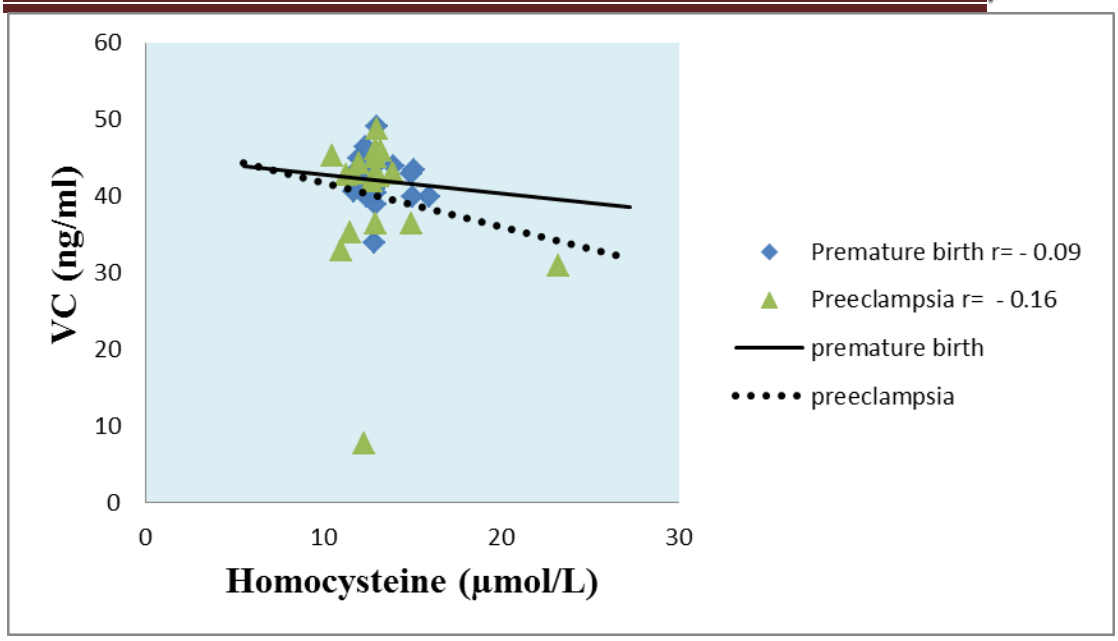


Figure (3-8): Correlation between serum Hcy and Vitamin C in preeclampsia and premature birth groups

3.2.5 Comparison of homocysteine levels with Trace Elements(Se, Mg, Cu) Concentration:

Table (3-6) and figure (3-8) shows a significant decrease in the level of trace elements, selenium, copper and magnesium in the preeclampsia and premature birth groups compared to the control groups, while there is a significant increase in the ferritin values of the same groups .

Shows the Table (3-6) and Figure (3-9) there is a decrease in selenium values for the groups of preeclampsia and risk of premature birth compared to the two control groups, healthy and non-pregnant pregnancy ($p < 0.05$) . Our results matched more than a previous studies (Duntas, 2020; Eze et al., 2020; Mazloomi et al., 2020; Vanderlelie & Perkins, 2011; Yıldırım et al., 2019).

Remethylation and transulfuration are two important homocysteine metabolic processes. tHcy is transformed to methionine by gaining a methyl group from either N5 -methyltetrahydrofolate or betaine during remethylation. Methionine synthase (MS) and betaine homocysteine methyltransferase (BHMT) are the enzymes that catalyze this reaction.In selenium insufficiency, BHMT activity is greatly reduced, resulting in less homocysteine being remethylated to methionine (Gonzalez *et al*., 2004) . Hcy-induced endothelia damage and impairment of endothelium-dependent relaxation were both mitigated by Se. Hcy-induced apoptosis was reduced by Se. Furthermore, Se administration reversed the Hcy-induced downregulation of $NO^{\cdot}$ and NOS (Ren *et al*., 2016) .

Table (3-6) Serum Homocysteine and Trace elements Tests for study groups

Parameters / Groups	Hcy µmol/L	Se ng\ml	Mg mg/dl	Cu µg/dl
Non pregnant	8.92 ± 0.64 b	81.26 ± 7.21 a	2.31 ± 0.49 a	154.44 ± 21.79 a
Healthy pregnancy	9.13 ± 0.40 b	82.65 ± 10.41 a	2.42 ± 0.58 a	156.16 ± 20.13 a
Premature birth	13.03 ± 0.95 a	62.78 ± 12.37 b	1.65 ± 0.24 b	136.52 ± 30.34 b
Preeclampsia	13.27 ± 2.49 a	61.20 ± 19.09 b	1.82 ± 0.31 b	139.82 ± 33.63 b
L.S.D	0.73	6.85	0.22	14.24

Legend as in table (3-2)

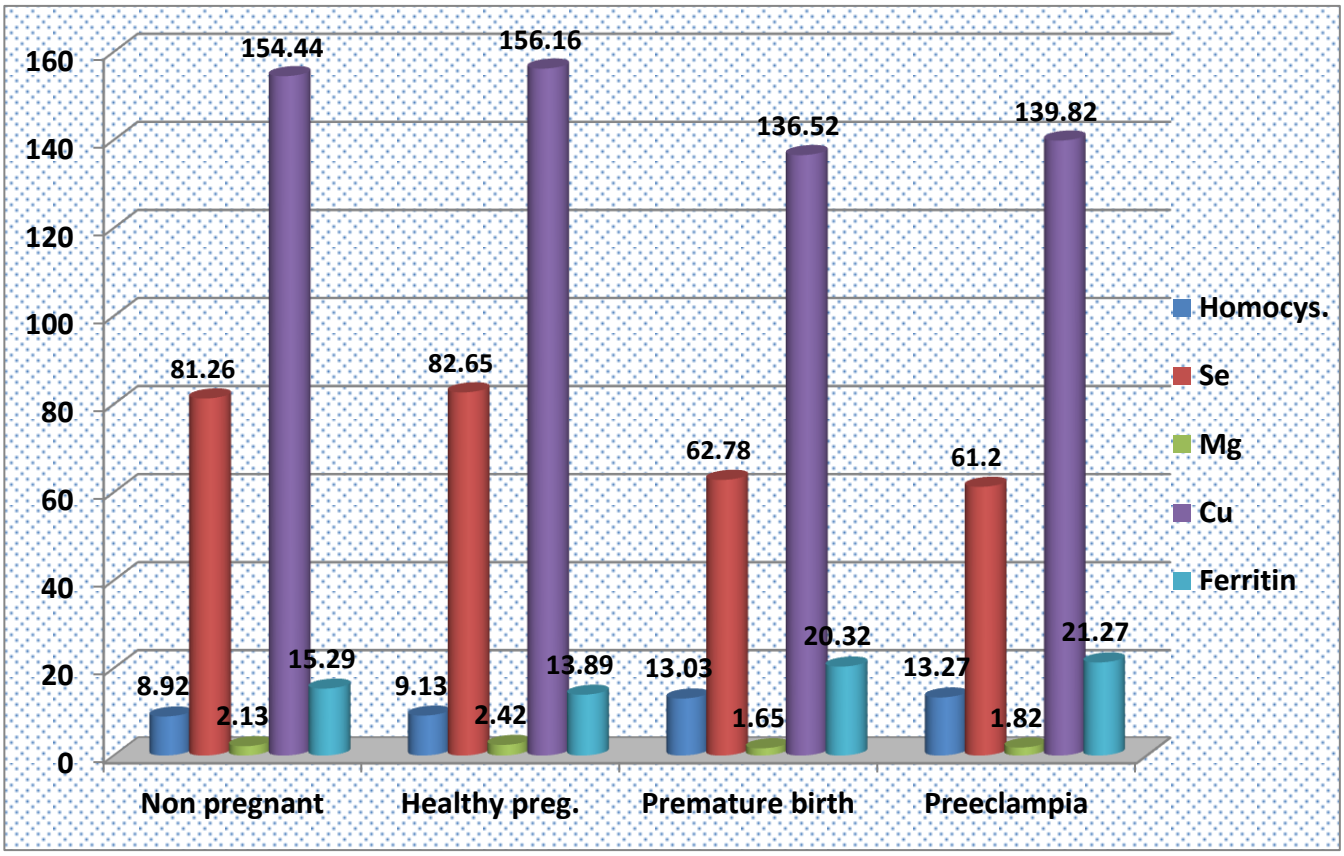


Figure (3-9) Homocysteine and Trace elements levels of control and patients groups

Figure (3-10) shows there is a negative association between homocysteine and selenium in premature group with correlation coefficient (r= -0.13) and positive correlation in preeclampsia group with correlation coefficient (r= -0.07)..

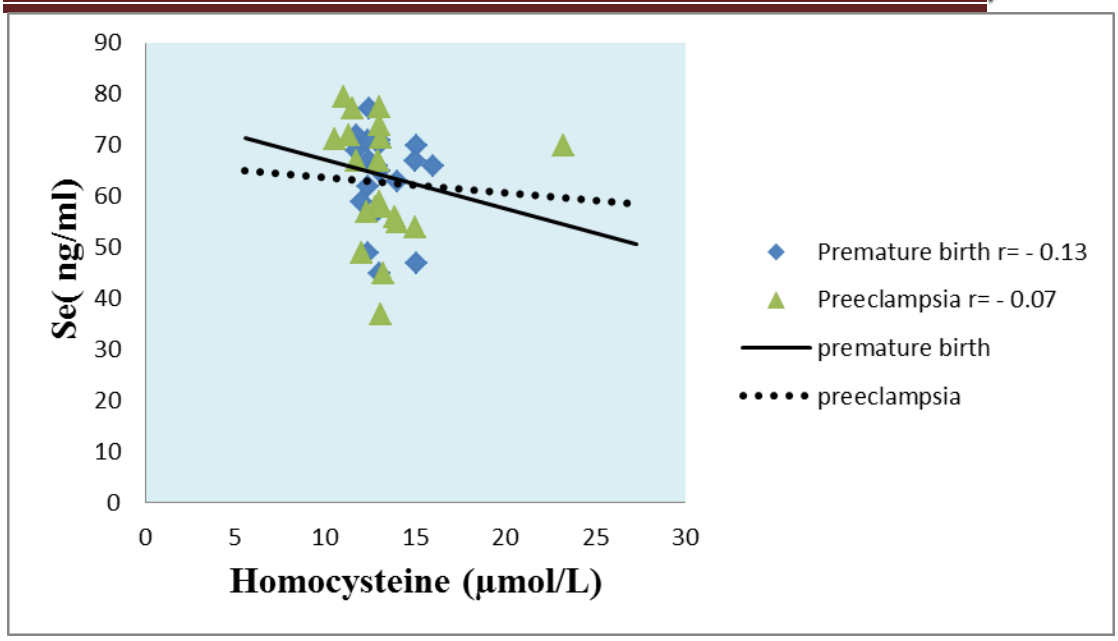


Figure (3-10): Correlation between serum Hcy and Selenium in preeclampsia and premature birth groups

As we note in Table (3-6) and Figure (3-9) there is a significant decrease in the magnesium values in the groups of preeclampsia and the risk of premature birth ($p < 0.05$) . Mg deficiency may be a contributing factor in the development of preeclampsia (Kharb et al., 2018) .

These results were consistent with (Darkwa et al., 2017; Rossokha et al., 2020; Saputri et al., 2020; Sukonpan & Phupong, 2005) .

Results of some studies show that there is low levels of magnesium and calcium in women with preeclampsia. Low magnesium has consequences as there is a decrease in cerebral blood flow, an increase in the bursting of nerve cells, and a spasm of blood vessels. Magnesium has a protective effect on vessels. Clinical implications of low magnesium may be a constriction of blood vessels and a decrease in cerebral blood flow and thus the tendency to eclampsia. Doctors now agree that the drug for preeclampsia is magnesium sulfate. Its effects include increased blood flow, vasodilation, which prevents preeclampsia by selectively expanding cerebral blood vessels and relieving cerebral spasm associated with

preeclampsia. It will prevent recurrent seizures in the eclampsia patient, and it is beneficial in reducing mortality and morbidity in both the mother and the fetus magnesium is capable shortening the hypertensive episode in preeclampsia (Kreepala *et al*., 2018) , In women with preeclampsia, magnesium (Mg2+) levels may be normal or low. However, Mg2+ salts, such as Mg2+ sulphate, may help to reduce the pathophysiological effects of preeclampsia and eclampsia. Although the mechanism of action of this Mg2+ salt is unknown (Chiarello *et al*., 2018).

Magnesium regulates blood pressure by influencing vascular . It works as a calcium channel blocker and promotes the synthesis of the vasodilator prostacyclins and nitric oxide (Sontia & Touyz, 2007) . Hypomagnesemia and pregnancy-induced hypertension have a link. that magnesium improves vascular muscle relaxation, despite the fact that the mechanism for this effect is unclear (Sukonpan & Phupong, 2005) .

shows in Figure (3-11) there is a negative correlation between homocysteine and magnesium in premature group with correlation coefficient (r= -0.15) and positive correlation in preeclampsia group with correlation coefficient (r= -0.25).

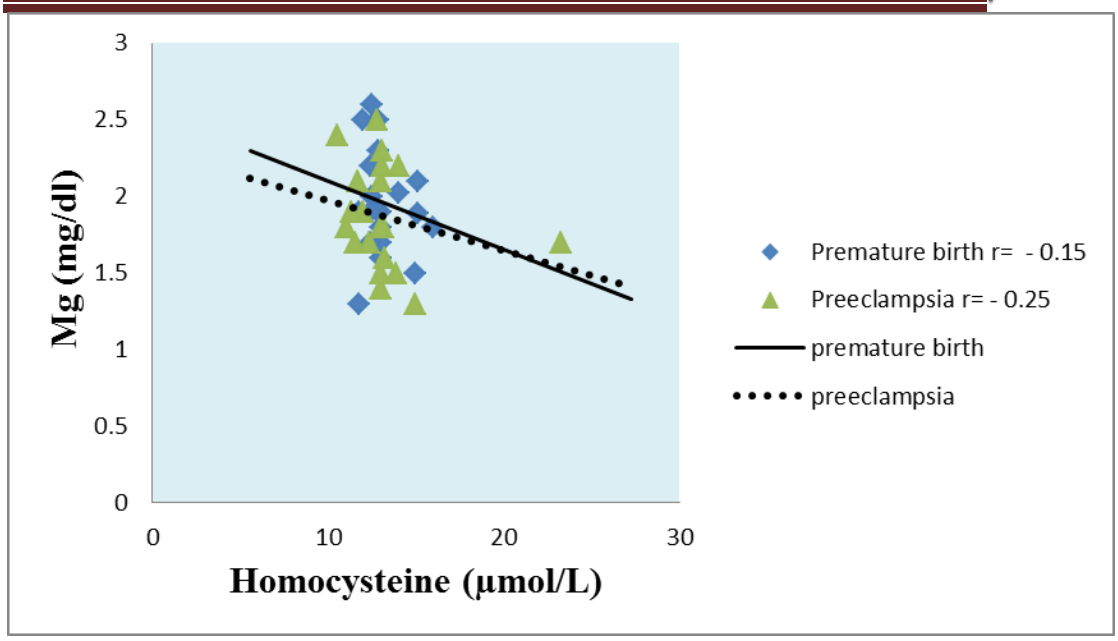


Figure (3-11): Correlation between serum Hcy and Magnesium in preeclampsia and premature birth groups

In Table (3-6) and Figure (3-9) we note there is a significant decrease in the copper values in the groups of preeclampsia and premature birth compared to the two non-pregnant and healthy pregnancy groups ($p < 0.05$) .

Low maternal copper levels are linked to preeclampsia and can play a role in the disease's development (Kanagal et al., 2014) . There are several studies matching our results (Vukelić *et al*., 2012; Al-Jameil *et al*., 2014; Keshavarz *et al*., 2017) .

Copper deficiency is characterized by a decrease in Cp, which is a sensitive biomarker (Broderius *et al.*, 2010). that women with a hyperactive placenta have higher serum Cu levels, whereas women with placental insufficiency have lower values (Kumru *et al.*, 2003) . diminished levels of copper could be due to either nutritional deficiencies (low Cu diet) or hemodilution. Other disorders associated with hypocupremia in PE patients may be because of malabsorption .Increased

urinary and decreased serum Cu levels along with reduced serum ceruloplasmin concentrations are observed (Keshavarz *et al*., 2017)

Figure (3-12) there is a negative correlation between homocysteine and copper in premature group with correlation coefficient (r= - 0.09) and positive correlation in preeclampsia group with correlation coefficient (r= -0.15).

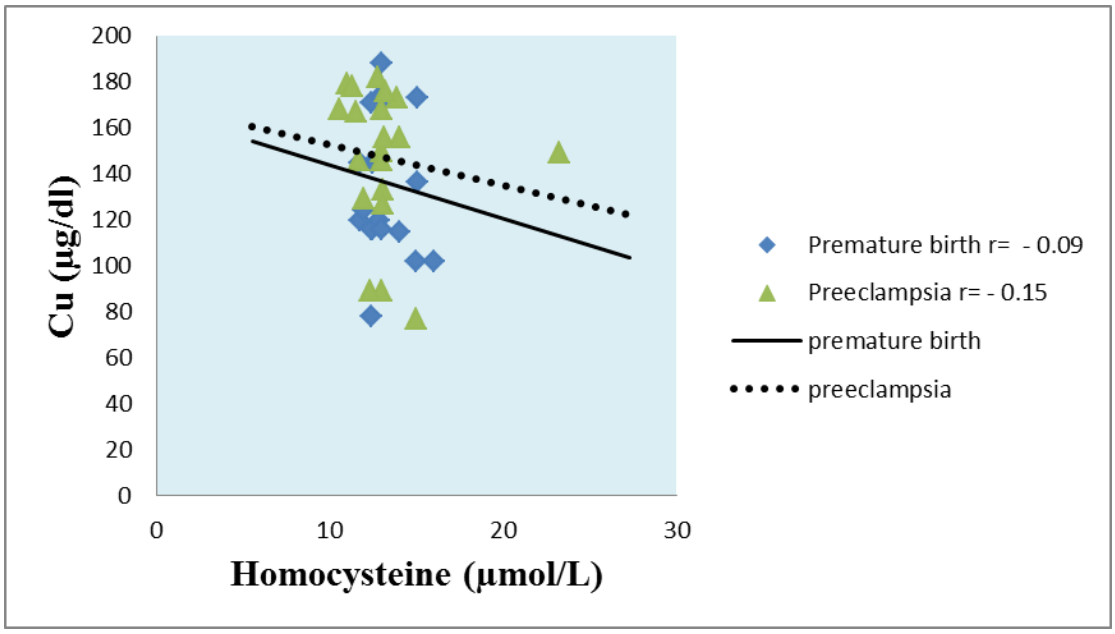


Figure (3-12): Correlation between serum Hcy and Copper in preeclampsia and premature birth groups

3.2.6.Serum Ferritin Concentration :

As shown in the table (3-7) and figure (3-9), there is a significant increase in the level of ferritin in the basic groups compared to the control groups ($p<0.05$). Serum ferritin levels are significantly higher in pregnant women with pre eclampsia. Excess iron may be a cause of oxidative stress, which may play a role in the pathogenesis of preeclampsia (Maitra *et al.*, 2019) .

Results match several previous studies (Abdel-Malek *et al.*, 2018; ElShahat *et al*., n.d.; Shaji Geetha *et al*., 2020; Ahn *et al*., 2021).

Elevated serum ferritin occurs in a number of clinical conditions involving non-utilization of iron and tissue destruction, such as hemolytic anemia, hepatic damage, or suppression of erythropoiesis leading to storage iron accumulation, low ferritin levels were linked to a lower risk of pre-eclampsia and pre-labor rupture of membranes, whereas higher ferritin levels were linked to a higher risk of premature birth and neonatal asphyxia (Maitra *et al*., 2019) . Ferritin is a protein that can rise in response to a variety of inflammatory or infectious stimuli or simply due to insufficient plasma volume expansion during pregnancy (Ng *et al.*, 2019) . oxidative stress caused by iron excess, which affects duodenal and placental iron transporters, preventing iron absorption from the gut , and delivery to the fetus Many studies have shown that when the body is overloaded with iron, serum ferritin levels rise but transferrin and transferrin receptor levels low. This is because the cells don't need any more iron and are attempting to store what they currently have , Because increasing blood iron and ferritin, An rise in serum iron levels of this magnitude is dangerous. Large amounts of supplementary iron may also raise blood viscosity, impairing uteroplacental blood flow. (ElShahat et al., n.d.; Shaji Geetha et al., 2020) . High homocysteine in the blood can cause impaired liver function and also has a role in the development of cirrhosis of the liver , Serum ferritin was commonly elevated in patients with all forms of liver disease It was apparent that in patients with liver disease, the serum ferritin level did not simply reflect the storage iron content the values showed no correlation with liver iron concentration and were generally higher than expected for the liver iron concentrations ,

that inflammatory states are also accompanied by elevated serum ferritin levels. (Prieto *et al*., 1975; García-Tevijano *et al*., 2001) .

Table (3-7) Serum Homocysteine and Ferritin Tests for study groups

Parameters / Groups	Hcy µmol/L	Ferritin ng/ml
Non pregnant	8.92 ± 0.64 [b]	15.29 ± 2.16 [b]
Healthy pregnancy	9.13 ± 0.40 [b]	13.89 ± 1.72 [b]
Premature birth	13.03 ± 0.95 [a]	20.32 ± 6.66 [a]
Preeclampsia	13.27 ± 2.49 [a]	21.27 ± 7.57 [a]
L.S.D	0.73	2.75

Legend as in table (3-2)

Figure (3-13) shows the positive correlation between homocysteine and ferritin in premature group with correlation coefficient (r= 0.11) and positive correlation in preeclampsia group with correlation coefficient (r= 0.03). . Although iron supplementation can improve pregnancy outcomes when the mother is anemic, prophylactic supplementation may increase risk when the mother is not anemic. Estimates of gestational iron needs and the proportion of iron consumed from various iron supplements indicate that current supplementation schemes are causing the intestinal mucosa to become irritated . (Maitra *et al*., 2019) .

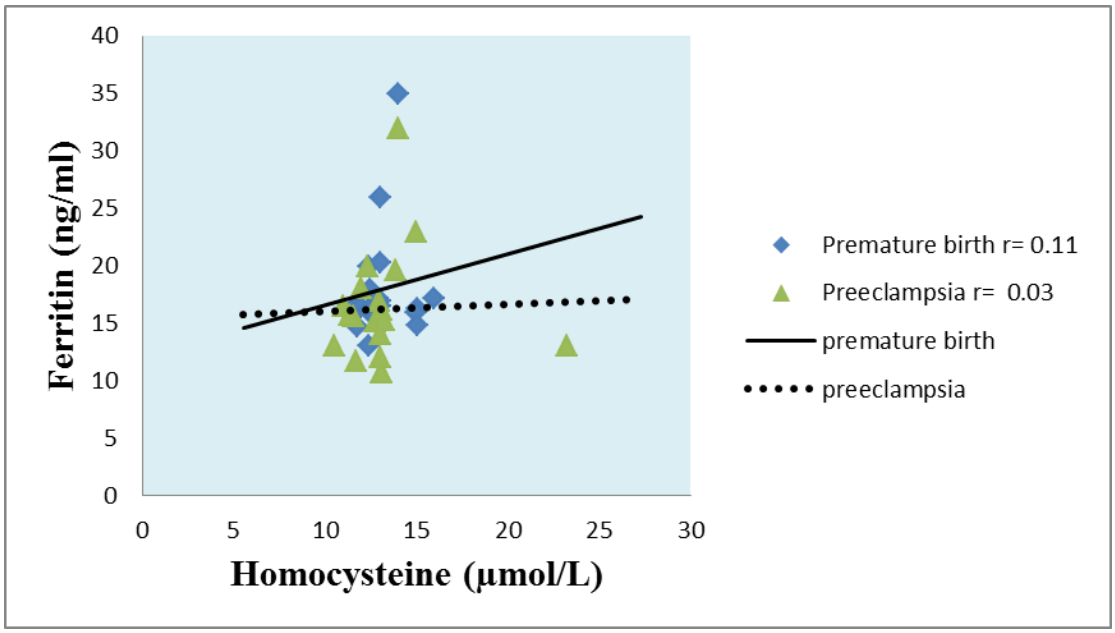


Figure (3-13): Correlation between serum Hcy and Ferritin in preeclampsia and premature birth groups

3.2.7. Comparison of homocysteine levels with Lipid Profile:

Our results are shown in Table (3-8) and Figure (3-14) there is a significant increase in the level of cholesterol in the basic groups of preeclampsia and risk of premature birth, compared with the two groups of controls, healthy and non-pregnant pregnancy ($P < 0.05$).

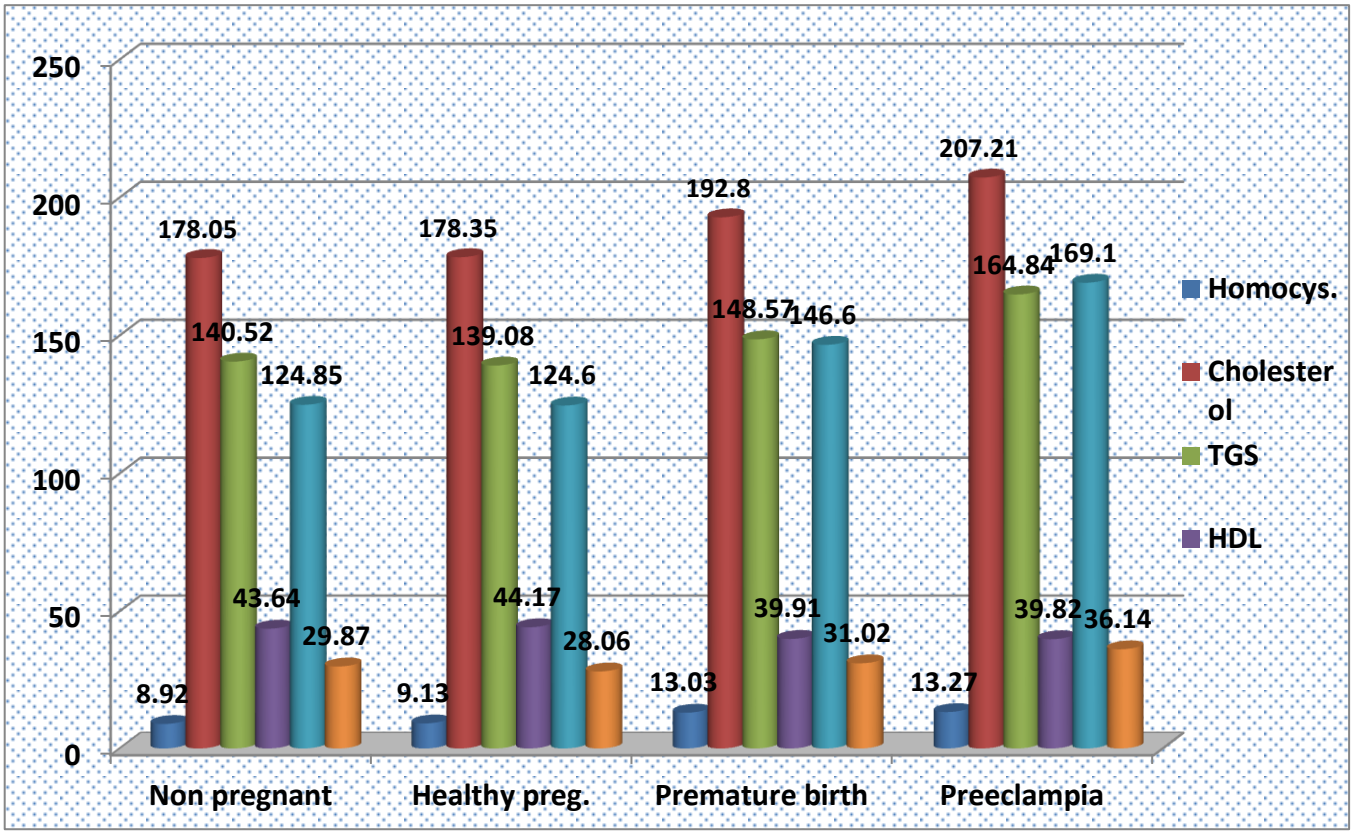
The results were in agreement with several studies (Gohil et al., 2011; Olalere et al., 2018) .

Preterm delivery is linked to hypercholesterolemia. (Wang .H.*et al*., 2020) . Serum lipid levels, especially TG, are higher in preeclamptic pregnant women than in normal pregnant women during normal pregnancies. Hyperlipidemia seems to raise the risk of artery problems such as endothelial disease, atherosclerosis, and thrombosis. Estrogen is released predominantly in the placenta during pregnancy by the conversion of androgen precursors derived from maternal and fetal, adrenal glands. These processes result in higher plasma estrogen concentrations than in non-pregnant women, and these hormones decrease hepatic lipase (HL) production in women , As a result, elevated lipid levels can be caused by low HL activity , where Hepatic lipase facilitates the clearance of TG from the low-density lipoprotein (VLDL) pool, and this function is governed by the composition and quality of the high-density lipoprotein (HDL) particles. HDL regulates the release of HL from the liver and the structure of HDL controls the transport and activation of HL in the circulation. And since HDL is lower in the basic groups, this may explain the increase in fat as a result of a defect in hepatic lipase activity (Chatterjee & Sparks, 2011;Berkane *et al*., 2017; Keshavarz *et al*., 2017) .

Table (3-7) Serum Homocysteine and Lipid profile Tests for study groups

Parametes / Groups	Homocysteine µmol /L	Cholesterol Mg/dl	TGS Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl
Non pregnant	8.92 [b] ± 0.64	178.05 [b] ± 25.20	140.52 [c] ± 7.46	43.64 [a] ± 5.44	124.85 [c] ± 34.40	29.87 [b] ± 2.95
Healthy pregnancy	9.13 [b] ± 0.40	178.35 [b] ± 32.85	139.08 [c] ± 8.51	44.17 [a] ± 6.38	124.60 [c] ± 47.20	28.06 [b c] ± 6.05
Premature birth	13.03 [a] ± 0.95	192.80 [ab] ± 20.75	148.57 [b] ± 4.10	39.91 [b] ± 1.14	146.60 [b] ± 18.34	31.02 [b] ± 1.04
Preeclampsia	13.27 [a] ± 2.49	207.21 [a] ± 44.84	164.84 [a] ± 18.23	39.82 [b] ± 2.38	169.10 [a] ± 44.44	36.14 [a] ± 2.67
L.S.D	0.73	16.95	5.74	2.31	19.90	1.92

Legend as in table (3-2)



Figure(3-14) Homocysteine and Lipid profile levels of control and Patients groups

Figure (3-15) there is a positive correlation between homocysteine and total cholesterol in premature group with correlation coefficient (r= 0.11) and positive correlation in preeclampsia group with correlation coefficient (r= 0.50).

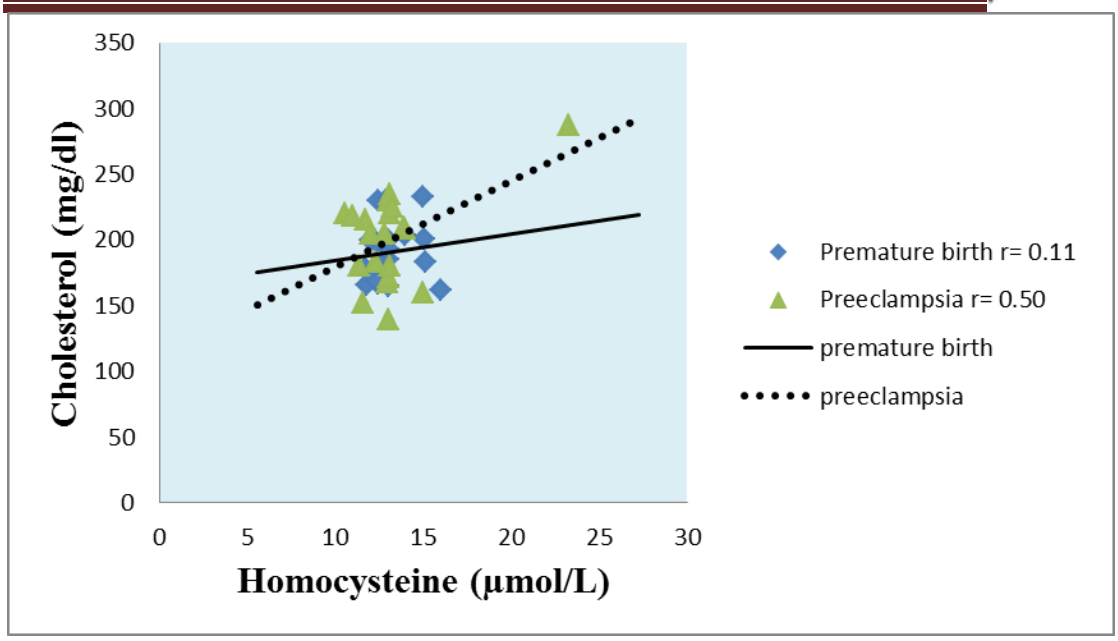


Figure (3-15): Correlation between serum Hcy and total cholesterol in preeclampsia and premature birth groups

As we note in Table (3-7) and Fig. (3-14), there is a significant increase in triglyceride values in the basic groups of preeclampsia and risk of premature birth, compared with the two groups of controls, healthy and non-pregnant pregnancy ($p < 0.05$) .

Our results are consistent with (Gohil *et al*., 2011; Olalere *et al*., 2018 ; Kayastha *et al*., 2020 ;Olalere *et al*.,2020)

Dyslipidemia and PE were found to have a positive correlation , Deregulations of feto-maternal lipid metabolism are related to the pathogenesis of PE, according to new data. However, not all preeclamptic pregnant women experience dyslipidemia, and some preeclamptic pregnant women with severe dyslipidemia have had healthy pregnancies. High TG serum levels, on the other hand, cause vasoconstriction and endothelial dysfunction by suppressing endothelial prostacyclin and nitric oxide (NO) (Al-Kuraishy *et al*., 2018). Increased amounts of serum

triglycerides, very low-density lipoproteins are linked to an increased risk of preeclampsia and premature birth. This lipid changes facilitate oxidative stress, which contributes to endothelial cell dysfunction, which is caused by the ischemia–reperfusion pathway. Dyslipidemia can also lead to atherothrombosis (Saputra *et al*., 2017) .

Increased triglyceride found in preeclampsia is likely accumulated in predisposed vessels, such as the uterine spiral arteries, and leads to endothelial dysfunction both directly and indirectly by producing thin, dense low density lipoprotein cholesterol. hepatic lipase causes increased triglyceride production, whereas low lipoprotein lipase activity causes reduced catabolism in the adipose tissue. Estrogen raises VLDL activity while decreasing lipolysis in late pregnancy. VLDL receptors in the placenta are upregulated, creating a concerted rerouting of blood flow .

To satisfy the nutritional demands of the developing fetus, the mother sends TG-rich lipoproteins to the fetoplacental unit (Lokhande Manoj *et al*., n.d.2016) . Elevated plasma lipid levels are believed to be the probable cause of endothelial cell dysfunction. In the endothelial cell, During pregnancy, serum lipoprotein levels increase considerably and are two times higher in PIH. Alterations that take place during pregnancy include insulin resistance, hyperlipidemia, and upregulation of inflammatory markers.serum VLDL levels were high in which may be due to hypertriglyceridemia leading to increased entry of VLDL that carries the endogenous TGs into the circulation , the principal modulator of hypertriglyceridemia is estrogen because pregnancy is associated with hyper-estrogenemia. This explains elevated TG in both preeclamptic and normotensive women (Olalere et al., 2020; Yadav et al., 2018) .

Figure (3-15) there is a positive correlation between homocysteine and Triglyceride in premature group with correlation coefficient (r= 0.17) and positive correlation in preeclampsia group with correlation coefficient (r= 0.29).

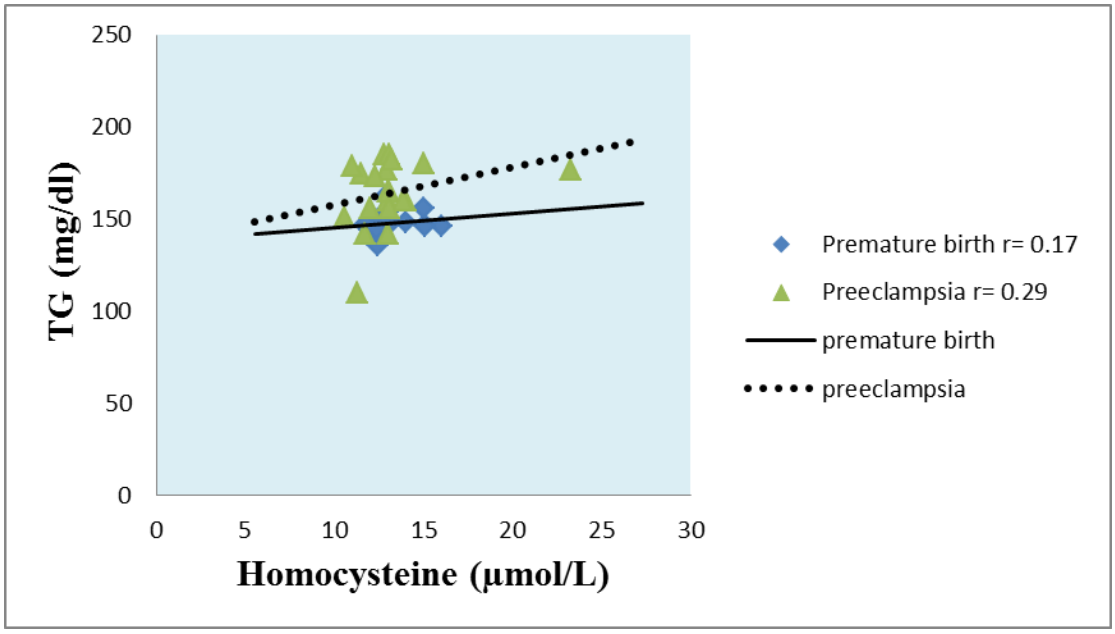


Figure (3-16): Correlation between serum Hcy and Triglyceride in preeclampsia and premature birth groups

As noted in Table (3-7) and Figure (3-14), there is a significant decrease in HDL values in the basic groups of preeclampsia and risk of premature birth, compared with the two groups of controls, healthy and non-pregnant pregnancy ($P<0.05$). Early pregnancy high TC, TG, LDL-C, and low HDL-C levels may be predictive biomarkers for poor pregnancy results, while early pregnancy low TC, TG, LDL-C, and high HDL-C levels may be protective (C. Wang et al., 2017) .

These results are consistent with several previous studies (Gohil et al., 2011; Olalere et al., 2018; Zhou et al., 2012) .

high-density lipoprotein cholesterol (HDLc) protects against endothelium-dependent vasodilation (Zhou *et al*., 2012) .People with PE have low levels of HDL (high-density lipoprotein) ,women with PE at a higher risk of cardiovascular disease (Einbinder *et al*., 2018).

Hypooestrogenaemia causes low HDL levels in pre-eclampsia, Dyslipidemia can play a role in the pathogenesis of pregnancy-induced hypertension by activating endothelial cells in response to placental-derived endothelial disturbing factors such as lipid peroxides and lipoproteins. Metabolic syndrome is characterized by high triglyceride levels, low HDL levels, and hypertension (Lokhande Manoj et al., n.d.). In the absence of vitamin C, lipid oxidation in HDL began immediately and proceeded rapidly. vitamin C inhibits lipid oxidation in HDL and preserves the antioxidant activity associated with this lipoprotein fraction (Hillstrom *et al.*, 2003) . Low levels of HDL in PIH are not only because of hypoestrogenemia but also are due to insulin resistance (Olalere *et al*., 2018) . The mechanism by which an accumulation of homocysteine might lead to a decrease in the HDL cholesterol level is unknown. It is conceivable that that homocysteine reacts with HDL-cholesterol and thereby increases its rate of catabolism , it is possible that Hcy acts directly on the liver to reduce in a selective manner the synthesis and secretion of HDL particles without affecting the production of VLDL which is the precursor of LDL-cholesterol (Vanderjagt *et al*., 2004) .

Figure (3-17) there is a negative correlation between homocysteine and high density lipoprotein in premature group with correlation coefficient (r= -0.05) and positive correlation in preeclampsia group with correlation coefficient (r= -0.26).

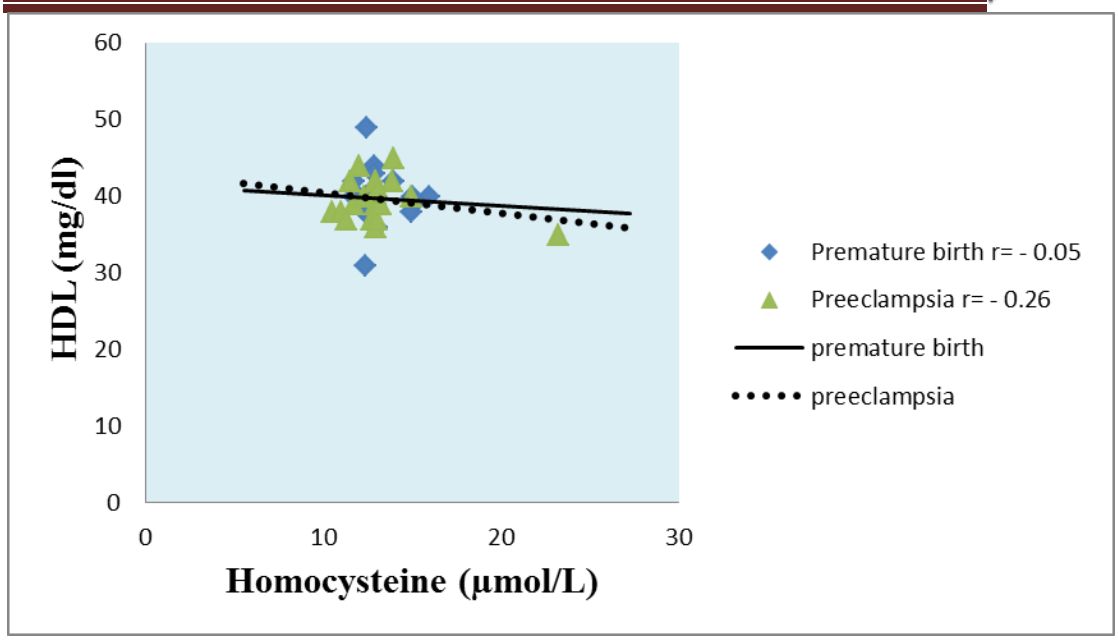


Figure (3-17): Correlation between serum Hcy and high density lipoprotein HDL in preeclampsia and premature birth groups

Show table (3-7) and Figure (3-14), there is a significant increase in LDL values in the basic groups of preeclampsia and risk of premature birth, compared with the two groups of controls, healthy and non-pregnant pregnancy ($P<0.05$).

These results matched several studies (Gohil et al., 2011; Olalere et al., 2018, 2020) .

Abnormal lipid profiles can play a role in the development of Preeclampsia causes oxidative stress and artery impairment. In preeclampsia, atherogenic small low density lipoproteins (LDL) and Vascular Cell Adhesion Molecules (VCAM) are primarily elevated with hyperlipidemia (Gohil *et al*., 2011) .

Preeclampsia is associated with significantly higher LDL-C levels due to reduced oestrogen levels (Lokhande Manoj et al., n.d.) . that oxidation of lipids in HDL lowered the ability to protect LDL from oxidative (i.e., atherogenic) modification. However,prevention of lipid

oxidation by physiologic concentrations of vitamin C attenuated this loss of HDL antioxidant activity ,that vitamin C prevents loss of the physiologic antioxidant activity associated with HDL during oxidation, The ability of HDL to protect LDL activity, which catalyzes the breakdown of lipid peroxides in LDL . Homocysteine, has been shown to promote LDL oxidation (Alul *et al.*, 2003; AnandBabu *et al.*, 2019) . Homocysteine can also non-enzymatically form homocysteine thiolactone which is highly reactive, This in turn can cause LDL to aggregate, leading to lipid deposits that can then form atheromas. Homocysteine can also cause lipid oxidation and platelet aggregation, which can lead to fibrosis and calcification of atherosclerotic plaques (Vanderjagt *et al*., 2004) .

Figure (3-18) there is a positive correlation between homocysteine and low density lipoprotein in premature group with correlation coefficient (r= 0.15) and positive correlation in preeclampsia group with correlation coefficient (r= 0.14).

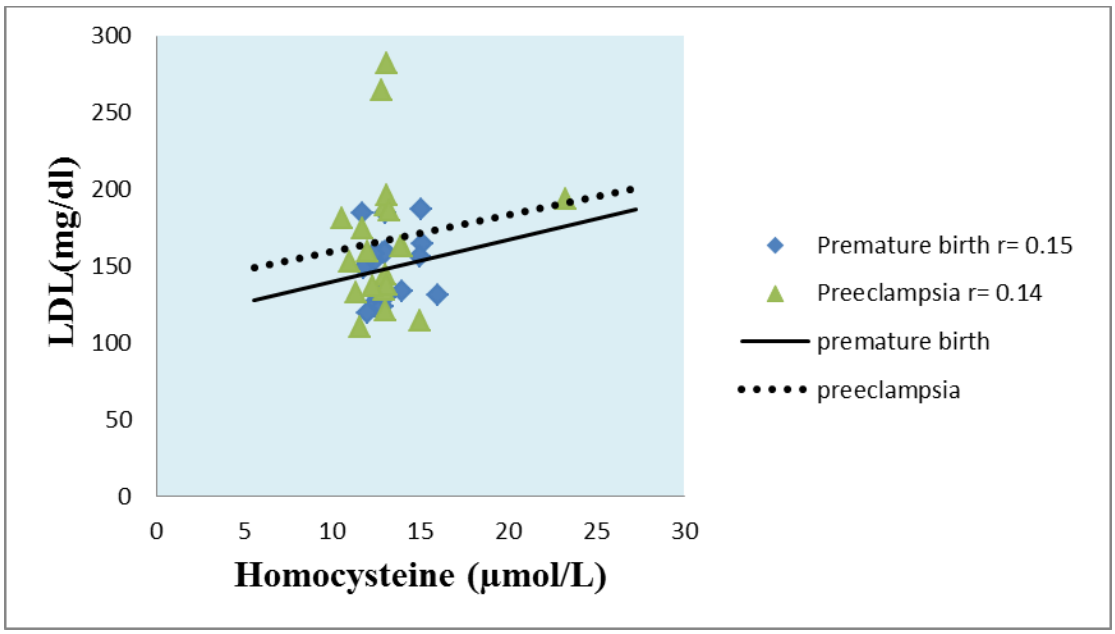


Figure (3-18): Correlation between serum Hcy and low density lipoprotein LDL in preeclampsia and premature birth groups

According to our results in the table (3-7) and Figure (3-14), there is a significant increase in VLDL values in the basic groups of preeclampsia and risk of premature birth, compared with the two groups of controls, healthy and non-pregnant pregnancy ($P<0.05$).

These results are consistent with several previous studies (Gohil *et al*., 2011; Kayastha *et al*., 2020; Olalere *et al*., 2020).

Serum lipid profile parameters during pregnancy and after birth are linked to extreme preeclampsia. In acute preeclampsia, total cholesterol, LDL, triglyceride, and VLDL all associated with blood pressure (White *et al*., 2019), Increased VLDL in preeclampsia indicate a problem with LDL conversion. It's worth noting that the role of IUGR in hypertensive disorders affects lipid profiles even further (Contini *et al*., 2019). As a result of early pregnancy, metabolic changes occur that encourage the formation and storage of fats in preparation for the phase of undermining a late pregnancy, when there is rapid fetal growth. Insulin resistance during pregnancy (in this case there are certain hormones in the placenta that cause insulin resistance) increases the breakdown of fats in the tissues, which leads to an enhanced flow of fatty acids to the liver. This promotes low-density lipoprotein (VLDL) synthesis and as a result, triglyceride concentrations have increased. In addition, insulin resistance reduces the activity of lipoprotein lipase, the insulin dependent enzyme responsible for removing VLDL from the plasma. Therefore, VLDL stays in plasma for a longer period and ultimately leads to low density accumulation LDL lipoprotein (Toescu *et al*., 2002).

In Figure (3-19) show there is a positive correlation between homocysteine and very low density lipoprotein in premature group

with correlation coefficient (r= 0.40) and positive correlation in preeclampsia group with correlation coefficient (r= 0.11).

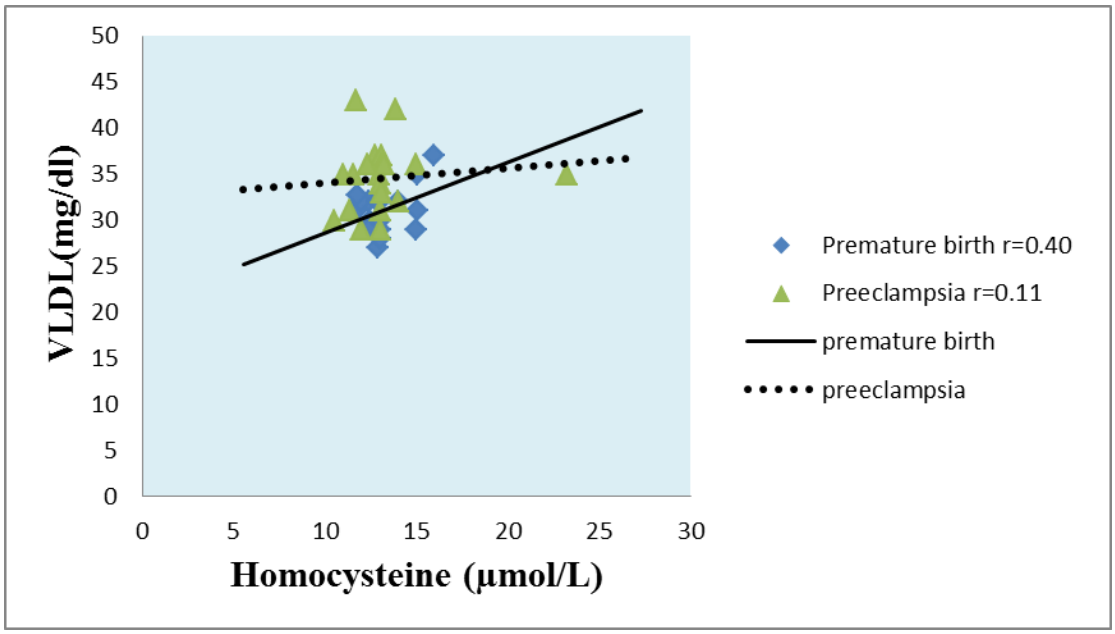


Figure (3-19): Correlation between serum Hcy and very low density lipoprotein VLDL in preeclampsia and premature birth groups

Conclusions

Among the most important conclusions of the study :

Through our current study, the high levels of homocysteine in the groups under study (preeclampsia and the risk of premature birth) may be an important cause of the mentioned pregnancy problems and its effect on important indicators in the blood of pregnant women, including :

1. Increased homocysteine in the groups of preeclampsia and risk of premature birth Compared to the healthy and non-pregnant groups.
2. Significant increase in the oxidative stress index of malondialdehyde in the groups of preeclampsia and risk of premature birth relative to the control groups .
3. Significant decrease in the values of the trace elements copper, selenium and magnesium When compared to control groups .
4. Increased ferritin levels in women at risk of premature birth and women with preeclampsia relative to the control groups .
5. The antioxidants albumin and ceruloplasmin decreased in the preeclampsia group and the premature birth risk group relative to the control groups .
6. A decrease in the values of vitamins B12 and C was observed in the groups of preeclampsia and the risk of premature birth Compared to the healthy and non-pregnant groups .
7. There was a significant increase in LDL, VLDL, TG, and TC while a decrease in HDL values was observed Compared to the healthy and non-pregnant groups .
8. We observed a positive correlation between homocysteine with ferritin and malondialdehyde and both LDL, VLDL, TG and TC .
9. We also noticed a negative relationship between homocysteine with Se, Cu, Mg and vitamins B12, C and the antioxidants Alb, Cp and HDL .

Recommendations & Future Studies

- 1.Make homocysteine level test as a biomarker with pregnancy complications .
- 2.Comparative study between high of homocysteine level with other parameters such as Zinc and vitamins D and E .
- 3. Conducting the same study on other pregnancy problems such as gestational diabetes.
- 4. Measuring sex hormones in conjunction with a similar study, and the parathyroid hormone.

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الخلاصة

الهوموسستين هو حمض أميني يحتوي على سلفهيدريل يتشكل عندما يتم إزالة الميثيونين وهو ضروري لعملية التمثيل الغذائي داخل الأوعية الدموية. يُعتقد أن الهوموسيستين عامل خطر محتمل لتطور عمليات أمراض الأوعية الدموية لتصلب الشرايين التي تؤدي إلى أمراض القلب والأوعية الدموية (CVD) والموت بسكتة دماغية .

الهدف من الدراسة التحقيق في العلاقة بين ارتفاع مستويات الهوموسيستين ومستويات العناصر النزرة (السيلينيوم والنحاس والمغنيسيوم والفيريتين) ومضادات الأكسدة (الألبومين والسيرولوبلازمين) والفيتامينات (B12, C) والدهون في مصل النساء الحوامل مع تسمم الحمل وخطر الولادة المبكرة. اشتملت الدراسة على (130) امرأة ، اربع مجاميع، مجموعة من (30) امرأة تتمتع بحمل صحي ، (30) امرأة غير حامل, (35) امرأة تعانين من تسمم الحمل و (35) امرأة معرضة لخطر الولادة المبكرة.

النتائج اظهرت زيادة معنوية في مستوى الهوموسيستين في تسمم الحمل والولادة المبكرة مقارنة بالمجموعات السليمة (غير الحوامل ، الحمل الصحي). أظهرت النتائج ايضا وجود انخفاض كبير في المغنيسيوم ، النحاس و Se في جميع مجموعات المرضى في تسمم الحمل و الولادة المبكر, بينما تظهر النتائج وجود زيادة ملحوظة في الفيريتين في جميع مجموعات المرضى في تسمم الحمل والولادة المبكرة ، حيث وجد أن هناك علاقة سلبية بين الهوموسيستين وكل من السيلينيوم والمغنيسيوم والنحاس ، ووجود ارتباط إيجابي بين الهوموسيستين والفيريتين. كما أظهرت النتائج انخفاضًا معنويًا في مستوى مضادات الاكسدة الالبومين والسيرولوبلازمين، كما أظهرت النتائج انخفاضًا في الفيتامينات B12 و C في تسمم الحمل ومجموعات المخاض قبل الأوان . أظهرت الدهون زيادة كبيرة في كل من LDL و TG و TC و VLDL وانخفاض في HDL في مجموعات الدراسة. كانت هناك زيادة معنوية في مالونديالديهيد في تسمم الحمل والولادة المبكرة.

خلصت دراستنا إلى أن مستوى الهوموسيستين في الدم كان أعلى في مجاميع مقدمات الارتعاج والولادة قبل الأوان مقارنة بمجموعات الضبط ، وخلصت النتائج أيضًا إلى أن الهوموسيستين مرتبط بمستوى أعلى من الفيريتين ، MDA ، الدهون، ومرتبط بمستوى منخفض من السيلينيوم ، Mg، Cu،HDL, ألبومين ، سيرولوبلازمين ، فيتامين ب 12 ، فيتامين C.

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دراسة مستويات الهوموسستين وبعض المعايير الكيموحيوية لدى النساء الحوامل في محافظة ذي قار

الرسالة

مقدمة الى مجلس كلية العلوم / جامعة ذي قار

كجزء من متطلبات نيل شهادة الماجستير في الكيمياء الحياتية

من قبل

رؤى حسن ناصر

بكالوريوس علوم كيمياء (جامعة ذي قار) 2008

بأشراف

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