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Homocysteine and hematological indices in hemodialysis patients at Al-Shifa hospital, Gaza Strip

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BY

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of university of other institute, except where due acknowledgement has been made in the text.

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I

DEDICATION

I dedicate this work to:

My beloved parents who have always supporting me

My brothers and sisters, especially Khattab J. Abu taha,

without their patience, understanding, support and

most of all love, this work would not have been possible.

My brother and friend Mohammed T. al hoore who

supported me and was beside me step by step

throughout the study.

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Homocysteine and hematological indices in hemodialysis patients at Al-Shifa hospital, Gaza Strip

Abstract

Background: Renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8%. Although hyperhomocysteinemia has been strongly linked to end stage renal disease, biochemical test is restricted to monitoring kidney function. Therefore, introducing homocysteine as a biomarker of ESRD in Gaza hospitals is recommended.

Objective: To assess homocysteine and hematological indices in hemodialysis patients at Al-Shifa hospital, Gaza Strip.

Material and methods: This case-control study comprised 60 hemodialysis patients and 60 healthy controls. Questionnaire interview was applied. Serum homocysteine, urea and creatinine, white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrompin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) were determined. Data were analyzed using SPSS version 18.0.

Results: End stage renal disease was more prevalent among lower educated and unemployed individuals, families with low income as well as among individuals with family history of the disease. Clinical data showed that hypertension and diabetes are the most common self-reported disorders among the hemodialysis patients. Serum homocysteine was significantly higher in cases compared to controls (50.8±9.7 vs. 13.1±3.7 µmol/l, P=0.000). Serum urea and creatinine were also found to be significantly higher in cases

(169.6±42.4 and 9.96±2.40 mg/dl, respectively) compared to controls (27.4±7.1 and 0.77±0.14 mg/dl) with P=0.000. White blood cell count, MCHC and platelet count were significantly increased in cases compared to controls (7.18±1.37 x10^3 cell/μl, 33.8±1.2 mg/dl and 266.3±104.2 x10^9 L vs 5.95±1.37 x10^3 cell/μl, 28.4±2.0 mg/dl and 222.0±54.1 x10^9 L) with P=0.017, P=0.000 and 0.045, respectively. In contrast, RBC count, hemoglobin, hematocrit and MCH showed significant decreases in cases(3.12±0.54 x10^6 cell/μl, 8.9±1.5 gm/dl, 26.3±4.6% and 28.6±2.9 pg) compared to controls (4.03±0.37 x10^6 cell/μl, 12.8±1.6 gm/dl, 45.0±4.6**%** and 31.9±4.4 pg) with P<0.01. Prothrompin time and INR were significantly higher in cases compared to controls (16.2±2.6 sec and 1.23±0.17 vs 13.5±0.4 sec and 0.97±0.07, P=0.000), whereas APTT was decreased in cases (25.3±5.3 vs 32.6±2.1 sec, P=0.000). Homocysteine levels were higher among lower educated and unemployed individuals, families with low income as well as among individuals with family history of ESRD (P<0.01). Homocysteine was positively correlated with urea (r=0.827, P=0.000), creatinine (r=0.842, P=0.000), WBC count (r=0.338, P=0.008), MCHC (r=0.789, P=0.000) and platelet count (r=0.369, P=0.000) whereas negative correlations were found between homocysteine and RBC count (r=-0.648, P=0.000), hemoglobin (r=-0.733, P=0.000), hematocrit (r=-0.836, P=0.000) and MCH values (r=-0.402, P=0.001). In addition, homocysteine showed positive correlations with PT (r=0.564, P=0.000) and INR (r=0.657, P=0.000) and negative correlation with APTT (r=-0.690, P=0.000).

Conclusions: Serum homocysteine was significantly higher in hemodialysis patients compared to controls. Homocysteine was positively correlated with urea, creatinine, WBC count, MCHC, platelet count, PT and INR, and negatively correlated with RBC count, hemoglobin, hematocrit, MCH and APTT.

Keywords: Homocysteine, hematological indices, hemodialysis, Gaza Strip.

حالة الحمض الأميني الهوموسستين والمؤشرات الدموية عند مرضى الديال —

يعد الفشل الكلوي أحد الأمراض العشرة الأولى المؤدية إلى الوفاة في قطاع غزة بمعدل 2.8% : وعلى الرغم بأن ارتفاع مستوى الحامض الأميني الهوموسستين مرتبط بقوة بمرضى الديال الموي، إلى أن الفحوصات البيوكيميائية مقتصرة لرصد وظائف الكلى فقط، لذلك يوصى بإدخال فحص الهوموسستين كعلامة بيولوجية للمرحلة الأخيرة لمرضى الكلى في مستشفيات غزة.

الهدف: تهدف الدراسة إلى قياس حالة الحمض الأميني الهوموسستين والمؤشرات الدموية عند مرضى الديال — .

منهج الدراسة (دراسة مشهدة)، المجموعة المرضية تحتوي على 60 : الديال الدموي، 60 شخصا من الأصحاء كعينات ضابطة، وقد تم الحصول على النتائج من خلال المقابلة الشخصية لمرضى الديال الدموي والأصحاء، وقد تم قياس مستويات الهوموسستين، اليوريا والكرياتينين، عدد خلايا الدم البيضاء، عدد خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت، هيموغلوبين الكرية الوسطي، تركيز هيموغلوبين الكرية الوسطي، عدد الصفائح، زمن البروثرومبين، الزمن الجزئي للثرومبو بلاستين المنشط و نسبة التطبيع الدولية.

تم تحليل البيانات والنتائج التي تم الحصول عليها باستخدام البرنامج الإحصائي SPSS-18.0.

أظهرت النتائج بأن المرحلة الأخيرة من مرض الكلية تعد أكثر انتشارا بين الأشخاص العاطلين عن : العمل والأقل تعلما، العائلات ذات الدخل المحدود بالإضافة إلى الأشخاص ذوي تاريخ عائلي للمرض. كما أظهرت البيانات السريرية بأن الضغط والسكر هما المرضان الأكثر شيوعا بين مرضى الديال الدموي، كما أن تركيز كل من الهوموسستين واليوريا والكرياتينين أعلى في الحالات المرضية بالمقارنة مع الأصحاء وهذه النتائج ذات دلالة إحصائية، ولوحظ من النتائج بأن عدد خلايا الدم البيضاء، تركيز هيموغلوبين الكرية الوسطي وعدد الصفائح الدموية في الحالات المرضية بالمقارنة مع الأصحاء، وهذه النتائج ذات دلالة إحصائية أيضا. وفي المقابل بينت النتائج بأن معدلات عدد خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت و هيموغلوبين الكرية الوسطي ترتفع عند مرضى الديال الدموي بالمقارنة مع الأصحاء. كما أشارت الذ ثرومبين و عدل التطبيع العالمي أعلى في الحالات المرضية بالمقانة مع الأصحاء وهذه النتائج ذات دلالة إحصائية، وعلى النقيض كان زمن الثرومبوبلاستين الجزئي المنشط أقل في الحالات المرضية

منه في الأصحاء وهذه النتائج ذات دلالة إحصائية. مستوى الهوموسستين كان أعلى بين كل من الأشخاص العاطلين عن العمل، الأقل تعلما، العائلت ذات الدخل المحدود بالإضافة إلى الأشخاص ذوي تاريخ عائلي للمرض، وهي نتائج ذات دلالة إحصائية. وأوضحت النتائج وجود علاقة إيجابية بين الحامض الأميني الهوموسستين وكل من اليوريا، الكرياتينين، عدد خلايا الدم البيضاء، تركيز متوسط الهيموجلوبين الكروي وعدد الصفائح الدموية. في حين وجدت علاقة عكسية بين مستويات الحامض الأميني الهوموسستين، مع كل من عدد خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت ومتوسط الهيموجلوبين الكروي. وبين النتائ علاقة إيجابية بين الهوموسستين وكل من زمن الروثرومبين ومعدل التطبيع العالمي، وعلاقة سلبية مع زمن الثرومبوبلاستين الجزئي المنشط.

: وجد ارتفاع ذو دلالة إحصائية في مستوى الحامض الأميني الهوموسستين عند مرضى الديال

كما وجدت علاقة إيجابية بين مستويات الهوموسستين وكل من اليوريا، الكرياتينين، عدد خلايا الدم البيضاء، تركيز متوسط الهيموجلوبين الكروي، الصفائح الدموية، البروثرومبين ومعدل التطبيع العالمي. وأشارت النتائج لوجود علاقة عكسية بين الهوموسستين وكل من عدد خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت، متوسط الهيموجلوبين الكروي وزمن الثرومبوبلاستين

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الكلمات المفتاحية: الهوموسستين، المؤشرات الدموية، الديال الدموي، قطاع غزة.

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List of abbreviations

Full name	abbreviation
Activated partial thromboplastin time	APTT
Acute kidney disease	AKD
Chronic kidney disease	CKD
Chronic kidney failure	CKF
Complete blood count	CBC
End stage renal disease	ESRD
European Union	EU
Glomerular filtration rate	GFR
International normalized ratio	INR
Mean corpuscular hemoglobin	MCH
Mean corpuscular hemoglobin concentration	MCHC
Mean corpuscular volume	MCV
Ministry of health	MOH
National Kidney Foundation	NKF
Per million population	p.m.p.
Prothrompin time	PT
Red blood cell	RBC
S-adenosyl methionine	SAM
Tetrahydrofolate	THF
White blood cell	WBC

Chapter 1

Introduction

1.1 Overview

The kidneys are responsible for filtering and excreting wastes from the blood. Without proper functioning, toxic waste products will accumulate and the patient will die. Therefore, the kidneys are vital to maintain life. Kidney diseases were classified into acute kidney disease (AKD) and chronic kidney disease (CKD). Acute kidney disease is a syndrome characterized by rapid decline (hours to days) in glomerular filtration rate (GFR), retention of nitrogenous waste products, and perturbation of extracellular fluid volume and electrolyte and acid-base homeostasis **(Dennis et al., 2005)**. Chronic kidney disease is a progressive loss in renal function over a period of months or years, and it may lead to one of its recognized complications such as cardiovascular disease, anemia or pericarditis **(Nurko, 2006 and Herzog et al., 2011)**. Chronic kidney disease (CKD) is a well-known risk factor for end-stage renal disease, ESRD **(Iseki et al., 2003).**

National Kidney Foundation **(NKF, 2002)** classified the severity of CKD into five stages according to the level of GFR, with stage 1 being the mildest and usually causing few symptoms (GFR   90 ml/min/1.73 m^2) and stage 5 being a severe illness with poor life expectancy if untreated (GRF <15 ml/min/1.73 m^2 or dialysis). Stage 5 CKD is also called established CKD and is synonymous with the now outdated terms ESRD or chronic kidney failure (CKF). In renal failure, filtrate formation decreases or stops completely. Because toxic wastes accumulate quickly in the blood when the kidney tubule cells are not working, hemodialysis by an artificial kidney is necessary to cleanse the blood while the kidneys are shut down **(Marieb, 2003).**

Hemodialysis is the most common treatment option for ESRD patients. This treatment involves blood being taken from the body and circulated through a machine with an artificial kidney called a dialyzer, which performs ultrafiltration and diffusion through a semipermeable membrane **(The Kidney Foundation of Canada, 2004).** Under such circumstances, kidney dialysis is typically administered using a fixed schedule of three times per week **(Abo Shamala, 2008)**. Among the common complications seen in persons with ESRD, are anemia mainly due to loss of erythropoietin production **(Nurko, 2006; Jonathan, 2010; Lovci et al., 2011 and Portolés et al., 2013)**, abnormalities in WBC, platelets functions **(Kaw and Malhotra, 2006 and Turkmen et al., 2010)**, and uremia **(Depner, 2005 and Locatelli and Canaud, 2012).**

The number of patients being treated for ESRD globally was estimated to be 2,786,000 at the end of 2011 and, with a 6-7% growth rate, continues to increase at a significantly higher rate than the world population. Of these 2,786,000 ESRD patients, approximately 2,164,000 were undergoing dialysis treatment (hemodialysis or peritoneal dialysis) and around 622,000 people were living with kidney transplants. In the USA, Japan and the European Union, dialysis patient population growth rates between 2010 and 2011 were in a range of 1– 4% and, as such, were significantly lower than growth rates in regions such as Asia, Latin America, the Middle East and Africa **(Fresenius Medical Care, 2011).** The Palestinian Health Annual Report (2010) showed that renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8% **(Ministry of Health, MOH, 2010).**

Homocysteine is a sulphur containing intermediary amino acid which is derived by the demethylation of methionine **(Shipchandler et al. 1995)**. The primary source of methionine is animal protein **(Hankey and Eikelboom, 1999)**. The normal range of homocysteine is 5 to15 µmol/L **(Ueland et al., 1993 and Graham et al., 1997)**. Elevated serum homocysteine beyond the normal range (>15 µmol/L) is traditionally referred to as

hyperhomocysteinemia. Recently, hyperhomocysteinemia has been linked to different stages of CKD including ESRD **(Van Guldener, 2006; Vieira et al., 2010 and Paterson, 2011).**

Although CKD is prevalent in the Gaza strip **(MOH, 2010)**, few recent studies targeted the disease. **Abo Shamala, (2008)** studied parathormone, calcium and phosphorus levels in hemodialysis patients at Al-Shifa hospital, Gaza-Palestine. **Abu Nada, (2012)** investigated homocysteine levels in chronic kidney disease patients in Gaza Governorate. **Muhaisen et al. (2012)** assessed risk factors of cardiovascular disease among children with chronic kidney disease in Gaza Strip. The present study will be the first to asses homocysteine level and hematological indices in hemodialysis patients from Gaza Governorate, Gaza Strip.

1.2 General objective

To asses homocysteine and hematological indices in hemodialysis patients at Al-shifa hospital, Gaza Strip.

1.3 Specific objectives

1. To determine homocysteine level in hemodialysis patients compared to healthy controls.
2. To determine urea and creatinine concentrations in hemodialysis patients and controls.
3. To measure complete blood count (CBC) in hemodialysis patients and controls.
4. To asses prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) in hemodialysis patients in comparison to controls.
5. To verify possible relations between homocystein with the studied parameters.

1.4 Significance

1. In the last decade the number of hemodialysis patients in the Gaza Strip has been doubled with mortality rate of 2.8% **(MOH, 2010 and Personal Communication, 2013).**

2. In Gaza Strip only one study have been carried out on hemodialysis patients without speculation of the status of homocysteine and hematological parameters in the disease **(Abo Shamala, 2008).** Therefore, this will be the first study to investigate homocysteine and hematological indices in hemodialysis patients in Gaza Governorate.

3. Assessments of homocysteine and hematological indices in hemodialysis patients could be useful in the intervention and management strategies of renal disease.

Chapter 2

Literature Review

2.1 The kidneys

2.1.1 Location and structure

The kidneys are small, dark red organs lie against the dorsal body wall beneath the parietal peritoneum in superior lumbar region where they receive some protection from the lower part of the rib cage (Figure 2.1). An adult kidney (about 12 cm long, 6 cm wide, and 3 cm thick) has a medial indentation (the hilus) in which there is two renal arteries, renal vein, and ureter **(Marieb, 2003).** The kidney has three regions, outer granulated layer called renal cortex, renal medulla that consists of cone shaped tissue masses called medullary pyramids, and renal pelvis which is a central space or cavity that is continuous with the ureter **(Mader, 2004).**
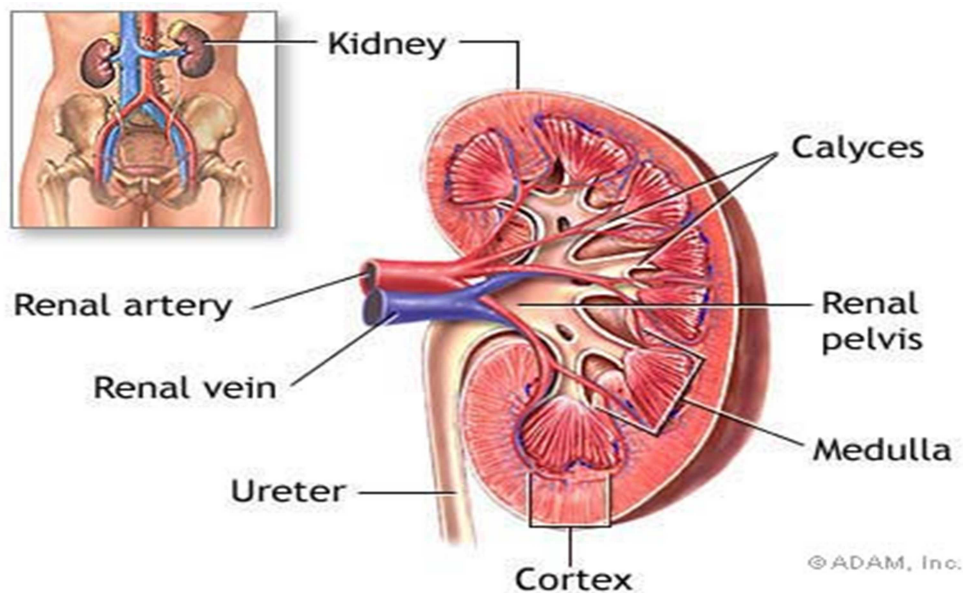
Figure 2.1. Location and structure of the kidney (Marieb, 2003)

Each kidney contains approximately one million tiny structures called nephrons (Figure 2.2). Nephrons are responsible for the processes of filtration, reabsorption, and secretion that go on in the kidney to form the urine product **(Marieb, 2003).** The nephron consists of two main structures, a glomerulus, which is a knot of capillaries, and a renal tubule. The closed end of the renal tubule is enlarged and cup-shaped and completely surrounds the glomerulus. This portion of the renal tubule is called Bowman's capsule. In order from Bowman's capsule they are the proximal convoluted tubule, loop of Henle, and the distal convoluted tubule. Most of the nephron is located in the cortex, only portion of the loops of Henle dip into the medulla. Urine from many nephrons is collected in the collecting ducts, which deliver the final urine product into the calyces and pelvis of the kidney **(Thibodeau and Patton, 1999; Mader, 2004 and Guyton and Hall, 2011).**
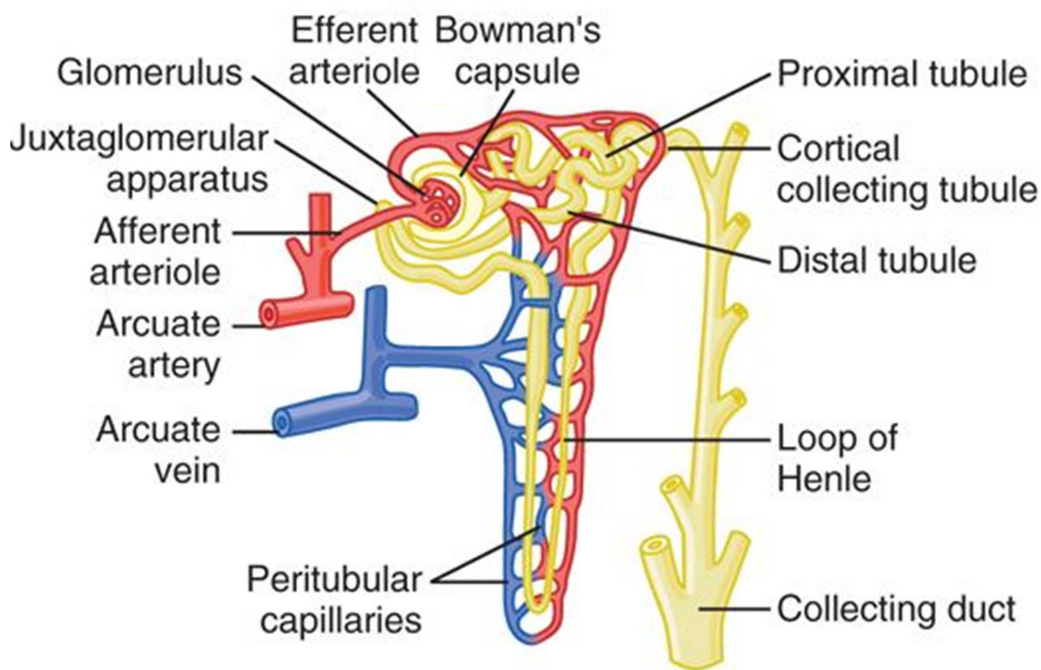
Figure 2.2. Structure of the nephron (Guyton and Hall, 2011)

2.1.2 Functions of the Kidney

The kidneys excrete natural waste products, including urea and creatinine, as well as foreign substances like alcohol and drugs, from the body. The kidneys also regulate the water and electrolyte (dissolved salts) balance and the acid-base balance **(Faratro et al., 2004).** The kidneys also produce and secrete important hormones, including renin, erythropoietin, and vitamin D. Renin is involved in regulating blood pressure, erythropoietin is used to stimulate the bone marrow to produce red blood cells, and vitamin D is needed to absorb the calcium from food in the intestine **(Faratro et al., 2004 and Barrett et al., 2010).**

2.2 Kidney disease

Renal disease may be acute or chronic. Acute renal failure occurs when the kidney fails suddenly, but this may be a temporary problem, and after a short period of treatment the patient may recover. Chronic renal failure results from an abnormal loss of renal function over months to years. Chronic renal disease is rarely reversible and leads to progressive decline in renal function **(Faratro et al., 2004).** Reduction in renal mass leads to hypertrophy of the remaining nephrons with hyperfiltration, and the GFR in these nephrons are transiently at supranormal levels, that may worsen renal function **(Lawrence et al., 2003).**

2.2.1 Chronic kidney disease

Levey et al. (2005) defined CKD as kidney damage or GFR<60 ml/min/1.73 m^2 for 3 months or more, irrespective of cause. Kidney damage in many kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio >30 mg/g in two of three spot urine specimens. Chronic kidney disease has been classified into various stages for the purpose of prevention, early identification of renal damage and institution of preventive measures for progression of the primary damage and appropriate guidelines for instituting management for prevention of complications in severe CKD **(Vijayakumar et al., 2007).** National kidney foundation classified

CKD into 5 stages according to the level of GFR (Table 2.1). For stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio **(National Kidney Foundation, NKF, 2002).**

Table 2.1 Classification of the stages of chronic kidney disease (CKD).

CKD Stage	Description	GFR (ml/min/1.73 m^2)
1	Kidney damage with normal or increased	90
2	Kidney damage with mild reduction of GFR	60-89
3	Moderate reduction of GFR	30-59
4	Severe reduction of GFR	15-29
5	Kidney failure (ESRD)	<15 or dialysis

GFR: Glomerular filtration rate. Adopted from National Kidney Foundation **(NKF, 2002).**

2.3 End stage renal disease

End stage renal disease, a continuum of CKD, is defined as irreversible kidney failure treated with dialysis or transplantation **(Anderson et al., 2009). Smeltzer and Bare (2004)** defined ESRD as the final stage of chronic renal failure were in there is a progressive, irreversible deterioration in renal function in which the body's ability to maintain metabolic and fluid and electrolyte balance fails, resulting in uremia. The National Kidney Foundation (2002) rated ESRD as the last and the fifth stage among CKD, which is based on the presence of kidney damage and level of kidney function whereas GFR <15 ml/min/1.75m². When the kidneys cease functioning, as in end stage renal disease, medications, diet, and fluid restriction will help to optimize the patient's health and wellbeing, but dialysis or kidney transplantation also need to occur **(Krueger, 2007 and Ganesh and Lee, 2011).**

2.3.1 Prevalence of end stage renal disease

End stage renal disease prevalence is highest in Taiwan with around 2,850 patients per million population (p.m.p.), closely followed by Japan with around 2,490 p.m.p. and then the USA with around 1,970 p.m.p. It averages about 1,040 p.m.p. in the 27 countries that make up the European Union (EU). In Asia, Latin America, the Middle East and Africa, dialysis patient population growth rates between 2010 and 2011 were higher than growth rates in regions such as the USA, Japan and the European Union **(Fresenius Medical Care, 2011).** The Palestinian Health Annual Report (2010) showed that renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8% **(MOH, 2010).** The number of the hemodialysis patients in Gaza strip, in year 2003 was 204 patients. In the year 2008, this figure was increased to reach about 361 patients, of them about 180 patients were in the hemodialysis unit at Al-Shifa hospital, which had 32 hemodialysis machines **(Abo Shamala, 2008).** Nowadays, there are 420 patients maintained on regular hemodialysis in Gaza strip, of them about 230 patients were in the hemodialysis unit at Al-Shifa hospital, which had 33 hemodialysis machines **(Personal Communication with Renal Unit at Al-Shifa Hospital, 2013).**

2.3.2 Causes of end stage renal disease.

End stage renal disease has many causes that vary from one patient to another. The most common causes include **(Sandra, 2005; Hyman, 2006; Soyibo and Barton, 2007; Hartmann et al., 2009 and Herzog, 2011):**
- Uncontrolled hypertension.
- Glomerulonephritis.
- Atherosclerosis.
- Obstruction of the urinary tract by stones or cancer.
- Diabetes mellitus.
- Obesity.
- Polycystic kidney disease.

- Medications such as the use of some analgesics regularly over long durations of time.

2.3.3 Treatment of end stage renal disease

The most important treatment alternatives for ESRD include hemodialysis, peritoneal dialysis and kidney transplantation. The populations of ESRD patients, dialysis patients and patients living with a transplanted kidney have increased steadily over the past years, whereby consistently more than three-quarters of all ESRD patients were treated by dialysis **(Fresenius Medical Care, 2011).**

2.3.3.1 Hemodialysis

Hemodialysis is a method that is used to achieve the extracorporeal removal of waste products such as creatinine and urea and free water from the blood by an artificial kidney machine when the kidneys are in a state of renal failure. The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which unwanted substances in the blood pass by diffusion **(Guyton and Hall, 2011)**. Hemodialysis is found in two variants: conventional hemodialysis, where patients receive hemodialysis in a clinic three times a week for 4 hours/session, and nocturnal hemodialysis, where patients are trained to do their own hemodialysis while they sleep, 5–6 nights/week **(Crawford and Lerma, 2008).** Hemodialysis is a relatively safe procedure, but there are several complications that can occur including hypotension, cardiac arrhythmias, muscle cramps, anaphylaxis, and restless leg syndrome **(Crawford and Lerma, 2008).** However, with proper monitoring and prompt treatment, many of these complications can be avoided. Of note, better glycemic control (HbA1c < 7.5 %) has been shown to predict better survival of diabetic ESRD patients starting hemodialysis treatment **(Morioka et al., 2001).**

2.3.3.2 Peritoneal dialysis

Compared to hemodialysis, peritoneal dialysis offers lower risk of death across all subgroups for the first 1–2 years of dialysis and is now recommended for use as the initial modality of dialysis in the majority of ESRD patients due to the lower prevalence of infections and better preservation of residual renal function **(Chung et al., 2009).** The two common choices for peritoneal dialysis are continuous ambulatory peritoneal dialysis and automated continuous cycling peritoneal dialysis, both of which function by infusing peritoneal dialysis fluid in the peritoneal cavity and draining it 4–6 hours later with the number of exchanges varying according to patient size, peritoneal membrane permeability, and residual kidney function **(Crawford and Lerma, 2008).**

2.3.3.3 Kidney transplantation

Is the surgical procedure of placing a fully functioning kidney into a person with ESRD. This procedure is usually an elective one, performed in patients who have undergone careful preoperative assessment and preparation. The transplanted kidney may originate from a deceased donor or from a related or unrelated person **(Cueto-Manzano AM, 2007).** Several recent studies have demonstrated significantly improved patient and allograft survival as well as lower rates of delayed graft function or acute rejection episodes in those with preemptive transplants versus those who were on dialysis for a period of time before transplantation **(Gill et al., 2004 and Baura, 2012).**

2.4 Hematological complications of ESRD

End stage renal disease is associated with a variety of hemopoietic changes. Anemia is common among ESRD patients, with more than 95% of individuals on dialysis receiving some form of anemia treatment **(Zadeh and Aronoff, 2009 and Anees et al., 2010)**. The main contributing factor to anemia in renal failure is loss of erythropoietin production **(Nurko, 2006).** Hemolysis may result from a number of biochemical and toxic insult during the dialysis

procedure. The life span of red blood cells is reduced by approximately one third in hemodialysis patients **(Marticorena et al., 2004).** Other factors include suppression of bone marrow erythropoiesis, hematuria, and gastrointestinal blood loss **(Suresh et al., 2012).** Abnormalities in white blood cell and platelet functions lead to increased susceptibility to infection and easy bruising. Patients with ESRD develop increased bleeding tendency, which is characterized by defective interaction of platelets with damaged sub endothelium due to impaired platelet functions **(Mohsin et al., 2010).** In this context, conventional hemostasis parameters PT, APTT and INR were disturbed and thrombotic complications and bleeding abnormalities are common among patients undergoing hemodialysis **(Holley, 1999; Rios et al., 2010 and Alghythan and Alsaeed, 2012).**

2.5 Homocysteine

2.5.1 Definition and structure

Homocysteine is an amino acid with the formula HSCH2CH2CH(NH2)CO2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. Homocysteine exists at neutral pH values as a zwitterion: Betatine form of (S)-Homocysteine and (R)-Homocysteine (Figure 2.3).
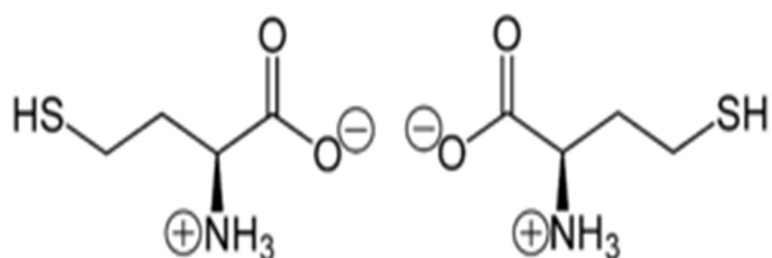
Figure 2.3. Structure of homocysteine. Betatine form of (S)-Homocysteine (left) and (R)-Homocysteine (right) (Champe et al., 2008).

2.5.2 Biosynthesis of homocysteine

Homocysteine is not obtained from the diet **(Selhub, 1999).** Instead, it is biosynthesized from methionine via a multi-step process (Figure 2.4). First, methionine receives an adenosine group from ATP, a reaction catalyzed by Sadenosyl- methionine synthetase, to give S-adenosyl methionine (SAM). SAM then transfers the methyl group to an acceptor molecule, (i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-homocysteine. L-homocysteine has two primary fates: conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine **(Champe et al., 2008).**
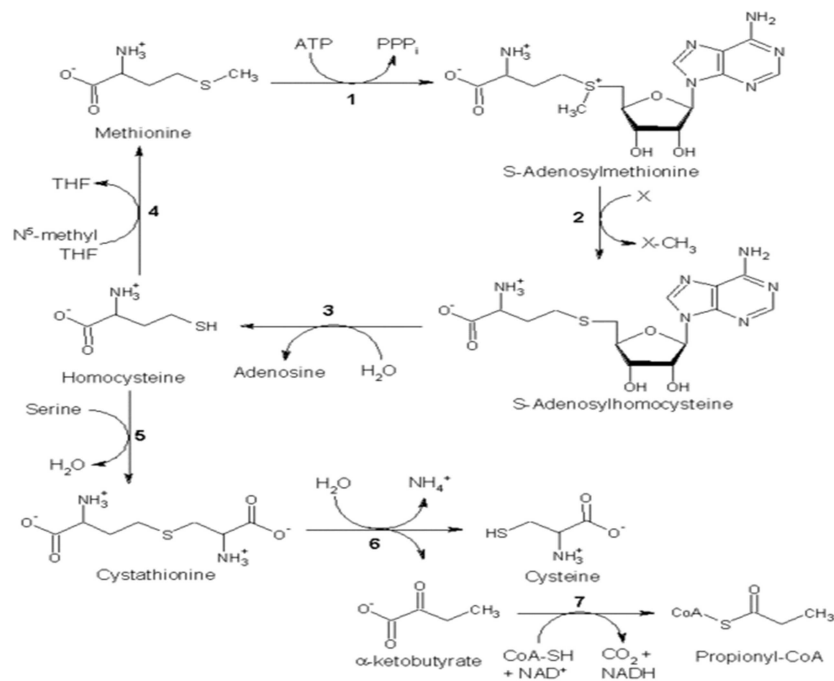
Figure 2.4. Biosynthesis of homocysteine. Sited from Champe et al. (2008).

2.5.3 Homocysteine species

Several homocysteine species have been identified in human plasma including albumin-(protein)-bound, free circulating disulfides and sulfhydryl forms **(Maron & Loscalzo, 2007)**. However, total homocysteine plasma concentrations are commonly reported in the literature as current analytical methodology involves the reduction of homocysteine disulfide bonds, quantifying all forms as free total homocysteine **(Perla-Kajan, et al., 2007 and Maron and Loscalzo, 2009)**.

2.5.4 Metabolism of homocysteine

The metabolism of homocysteine can be divided into three distinct pathways (Figure 2.5): the remethylation of homocysteine to methionine by the vitamin B12 dependent methionine synthase; the transsulfuration pathway, converting homocysteine to cystathionine and then cysteine via vitamin B6 dependent cystathionine -synthase enzyme; in the liver and kidneys, homocysteine can be remethylated back to methionine by betaine-homocysteine methyltransferase **(Maron & Loscalzo, 2009).**
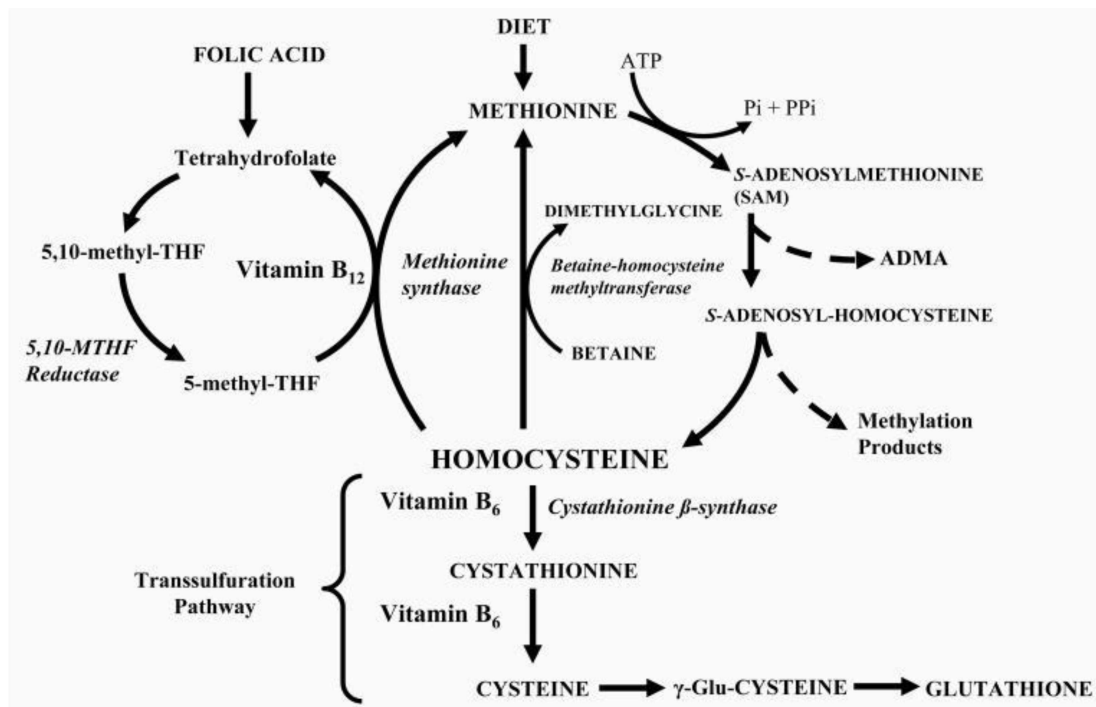
Figure 2.5. Homocysteine metabolic cycle. Sited from Maron & Loscalzo, (2009).

2.5.5 Homocysteine values

The American Heart Association released an advisory statement classifying total homocysteine plasma concentrations as follows: 5-15 µmol/L homocysteine as normal, 16-30 µmol/L homocysteine as moderate, 31-100 µmol/L homocysteine as intermediately elevated and total homocysteine levels above 100 µmol/L as severely elevated concentrations **(Malinow et al., 1999).**

2.5.6 Hyperhomocycteinemia

Hyperhomocysteinemia, is defined as total homocyteine concentrations elevated above 15µmol/L. Plasma homocysteine concentration exhibits a strong relationship with (indices of) renal function. Hyperhomocysteinemia has been implicated in patients with CKD **(van Guldener, 2006 and Vieira et al., 2010).** There are several indications that whole body homocysteine metabolism is altered in renal insufficiency **(van Guldener, 2005).** Stable

isotope studies in dialysis patients have shown a decreased homocysteine clearance by transsulfuration and decreased homocysteine remethylation and methionine transmethylation **(van Guldener et al., 1999 and Stam et al., 2005)**. Several, but not all, prospective studies have linked hyperhomocysteinemia to adverse cardiovascular outcomes in renal failure patients. Hyperhomocysteinemia has been also associated with the pathogenesis of cardiovascular disease **(Al-obaidi et al., 2001; Lubos et al., 2007 and coldea et al., 2011)**. Deficiencies of the vitamins folic acid (B9), pyridoxine (B6), or B12 (cyanocobalamin) can lead to hyperhomocysteinemia **(Brosnan, 2004 and Abraham et al., 2010)**. Hyperhomocysteinemia also occur in the rare hereditary disease homocystinuria and in the methylenetetrahydrofolate reductase polymorphism genetic traits **(Qi et al., 2003)**. The latter is quite common (about 10% of the world population) and it is linked to an increased incidence of thrombosis and cardiovascular disease, which occurs more often in people with above minimal levels of homocysteine (about 6μmol/L).

2.6 Related studies

Bostom et al., (1995) determined fasting total plasma homocysteine in 24 ESRD patients on dialysis, and 24 age, gender, and race matched controls with normal renal function. Mean plasma homocysteine was markedly higher in the ESRD patients versus controls (22.7 vs. 9.5 pmol/l). End stage renal disease patients were 33 times more likely than controls to have hyperhomocysteinemia (>15.8 pmol/l) (95% confidence interval, 5.7-189.6). Hematological evaluation of hemodialysis patients showed that sustained increases in hematocrit (Hct) levels are associated with improved patient survival **(Ma et al., 1999).**

Chou et al. (2000) found that the mean concentrations of plasma total homocysteine in Taiwanese hemodialysis patients (21.3±4.3 μM) were statistically higher than in age-matched normal subjects (11.0±2.9 μM).

Nasri and Baradaran (2006) demonstrated a significant inverse correlation of serum homocysteine with white blood cell count (r=-0.34, P=0.036) and a significant positive correlation of serum homocysteine with body mass index (r=0.35, P=0.039) in 25 hemodialysis patients. In addition, **Nasri (2006)** showed a significant positive correlation of platelet count with serum homocysteine in 39 hemodialysis patients (r=0.35, P=0.044)**.**

Cabarkapa et al. (2007) examined 105 subjects: 73 patients in different stage of chronic renal failure, 31 of them on hemodialysis, and 32 subjects as a control group. The results showed high prevalence of hyperhomocysteinemia in chronic renal failure patients, especially with reduction of glomerular filtration rate over 50% of the surface normalized value. **Khanam et al. (2007)** found that the mean hemoglobin concentration, hematocrit and total count of red blood cells were significantly lower in three stages of chronic renal failure (CRF) patients (n=65) with anemia compared to those of healthy subjects (n=25). All the hematological parameters showed positive correlation with creatinine clearance in all three stages of CRF.

Ali et al. (2008) demonstrated an increase of hemoglobin, hematocrit, red cell count, PT and APTT in Sudanese patients after dialysis session. In addition, **Shojaei et al. (2009)** found that homocysteine level in Iranian patients on maintenance hemodialysis was 33% higher on average than the reference value.

The relationship of hemoglobin and homocysteine levels and mortality of patients on hemodialysis was assessed **(Anees et al., 2010)**. Fifty patients on hemodialysis and 20 healthy individuals were enrolled in the study. Forty-three patients (86%) were anemic (hemoglobin < 11 g/dL). Serum ferritin was high (> 500 ng/mL) in 33 patients (66%). Mortality was 28% in one year (33% in anemic patients versus no death among patients with a hemoglobin level greater than 11 g/dL). The relative risk of mortality was increased by 1.58 with every one g/dL decrease in hemoglobin level. All of the patients had a high

homocysteine level, and a significant difference was observed between the homocysteine levels of the patients on hemodialysis and the control group (P<0.001). In addition, **De Almeida et al. (2011)** found that the total prevalence of hyperhomocysteinemia in 70 renal failure patients on dialysis treatment was 85.7%. Homocystien was positively correlated with albumin and creatinine.

Suresh et al. (2012) assesed the hematological changes in 50 patients suffering from chronic renal failure compared to 50 age and sex matched controls. In chronic renal failure patients, RBC count, hemoglobin concentration, hematocrit and platelet count where significantly (P<0.05) reduced, whereas total leukocyte count was reduced but not statistically significant. The concentration of serum creatinine shows negative correlation with all the haematological parameters. And the degree of changes depends on the severity of renal failure. In addition, **Alghythan and Alsaeed, (2012)** observed that most of hematological parameters elevated after hemodialysis. More significantly, PT, APTT and fibrinogen were found to increase post hemodialysis while there was a concurrent decrease of platelet counts. In addition, Baseline data on co-morbidities and anemia management in 4591 European hemodialysis patients on hemodialysis >180 days were collected **(Locatelli et al., 2012)**. Mean hemoglobin (Hb) concentration was 11.0 g/dl; 53% patients had hemoglobin concentration 11 g/dl. Higher hemoglobin concentrations were associated with decreased relative risk for mortality and hospitalization. Patients with hemoglobin <10 g/dl were 29% more likely to be hospitalized than patients with hemoglobin 11–12 g/dl (P<0.001).

Chapter 3

Materials and Methods

3.1 Study design

The present study is a case-control design.

3.2 Study population

The study population comprised hemodialysis patients diagnosed as end stage renal disease on hemodialysis and healthy controls.

3.3 Setting of the study

The hemodialysis unit at Al-Shifa hospital in the Gaza strip.

3.4 Sample size

A total of 60 patients (34 males and 26 females) maintained on hemodialysis were included in the present study. Sixty healthy individuals (34 males and 26 females) were served as controls. Controls and patients were age and sex matched.

3.5 Exclusion criteria

- Pregnant women.
- Patients with hepatitis.

3.6 Ethical considerations

The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information obtained through the questionnaire and blood analysis.

3.7 Data collection

3.7.1 Body mass index

The body weight of each individual dressed in light clothing without shoes was weighed using a carefully calibrated electrical balance (Seca model 762, Germany). The height of each was measured using vertical measuring rod. Body mass index was calculated as the ratio of body weight in Kg/height in meter square. Individuals with BMI=18.5-24.9 were considered to have normal weight, individuals with BMI=25.0-29.9 were classified overweight, individuals with BMI 30.0 were considered obese **(World Health Organization, WHO, 2000).**

3.7.2 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designated for matching the study need (Annex 1). All interviews were conducted face to face by the researcher himself. During the survey the interviewer explained any of the questions that were not clear. Most questions were one of two types: the yes/no question, which offers a dichotomous choice; and the multiple choice questions, which offer several fixed alternatives **(Backestrom and Hursh-Cesar, 1981).** The validity of the questionnaire was tested by six specialists in the fields of nephrology, public health and hematology. The questionnaire included personal information (age, gender, marital status and education), socioeconomic characters (employment, family income/month, family history of ESRD and smoking) and clinical data (hemodialysis duration, hemodialysis frequency and other chronic disease). A pilot study was done prior to beginning real data collection to know the length and clarity of the

questionnaire and to evaluate the outcome. Ten cases were interviewed. At the end of the pilot study, a comprehensive revision to questionnaire was made and modified as necessary. The pilot subjects were not included in the study.

3.7.3 Blood sampling and processing

Blood samples were collected by the researcher himself from all the subjects who agreed to participate in the study (before hemodialysis sessions for the patients). Nine ml blood were obtained from each subject and divided into EDTA tube (2 ml) for CBC analysis, Sodium citrate 3.2% tube for PT and APTT determination in plasma (3 ml) and vacutainer plain tube (4 ml) that was left for a while to allow blood to clot. Then, serum samples were obtained by centrifugation at 3000 rpm for 15 minutes. Serum homocysteine, creatinine and urea were determined.

3.8 Hematological analyses

3.8.1 Complete blood count

Blood samples were processed by an automatic counter for hemoglobin concentration and other whole blood component concentrations (cell dyn 1800, USA).

3.8.2 Determination of prothrombin time (PT)

Principle

Single-stage prothrombin time measures the clotting time of test plasma after the addition of the thromboplastin reagent containing calcium chloride. The reagent supplies a source of "tissue thromboplastin," which activates factor VII, and is therefore sensitive to all stage II and III factors. Deficiencies in stage I factors (VIII, IX, XI, and XII) are not detected by the test.

International sensitivity index (ISI)

The International Committee for Standardization in Hematology and the International Committee on Thrombosis and Hemostasis have agreed on recommendations for the reporting of prothrombin time results based upon an International Sensitivity Index (ISI) for thromboplastin reagents and an International Normalized Ratio (INR). Thromboplastin reagents are assigned an ISI value by calibrating them against an international reference preparation, (IRP, 67/40) which by definition has an ISI = 1.0. The ISI value assigned to commercial thromboplastin reagents, therefore, defines a comparative slope, or relative sensitivity, in comparison to the reference thromboplastin. The lower the ISI value, the more "sensitive" is the reagent. By knowing the ISI of a particular thromboplastin reagent, the ratio can be calculated which would have been found if the IRP 67/40 had been used as the reagent.

This is termed the international normalized ratio (INR), and is determined by:

$$\text{INR} = \text{RISI} = \text{Ratio ISI} = \left(\frac{\text{Patients PT (s)}}{\text{Normal PT (s)}} \right) ISI$$

For hemostat thromboplastin-si an ISI value was assigned in relation to the WHO standardized thromboplastin.

Preparation

Anticoagulant

3.2% buffered sodium citrate was used.

Procedures

1. Fifty µl plasma into cuvette was pipetted.

2. Plasma was pre-warmed for 3 minutes.

3. Cuvette was transferred to measuring position.

4. 100 µl pre-warmed hemostat thromboplastin-si was added (measurement starts automatically).

5. The result is displayed in seconds and INR.

Normal value

The expected range is between 11 and 14 s.

3.8.3 Determination of activated partial thromboplastin time (APTT)

Principle

The APTT test measures the clotting time of test plasma after the addition of APTT reagent, then allowing an "activation time," followed by the addition of calcium chloride. Deficiencies of approximately 40% and lower of factors VIII, IX, XI and XII will result in prolonged APTT. Heparin, in the presence of adequate amounts of AT-III will also result in prolonged APTT.

Reagent

Hemostat APTT-EL

Calcium chloride

Preparation

Anticoagulant

3.2% buffered sodium citrate was used.

Procedures

1. Fifty µl plasma was pipetted into cuvette.

2. Plasma was pre-warmed for 1-2 minutes

3. Fifty µl APTT reagent was added to plasma.

4. Incubate for 3-5 minutes (for consistent results, test all plasmas with the same time)

5. Cuvette was transferred to measuring position.

6. Fifty µl pre-warmed calcium chloride was added (the measurement starts automatically).

7. The result was displayed in seconds and ratio.

3.9 Biochemical analyses

3.9.1 Determination of serum homocysteine

Serum homocysteine was determined by enzymatic colorimetric method for the quantitative determination of homocysteine **(Refsum, 2002)**, using Globe diagnostics kit, Italy.

Principle

The Globe Diagnostics Enzymatic Test for the quantitative homocysteine determination is based on a series of enzymatic reactions causing a decrease in absorbance value due to NADH oxidation to NAD+. HCY concentration in the sample is directly proportional to the quantity of NADH converted to NAD+ (A 340nm). The enzymatic reactions are the following:

$$\text{T(HCY)} \xrightarrow{\text{s-Methyltransferase}} \text{(HCY)free}$$

$$\text{(HCY)free + SAM*} \xrightarrow{\text{SAM Hydrolase + Adenosine deaminase}} \text{Methionine + SAH**}$$

$$\text{SAH + NADH} \xrightarrow{\text{Glutamatedehydrogenase}} \text{Inosine + NAD+}$$
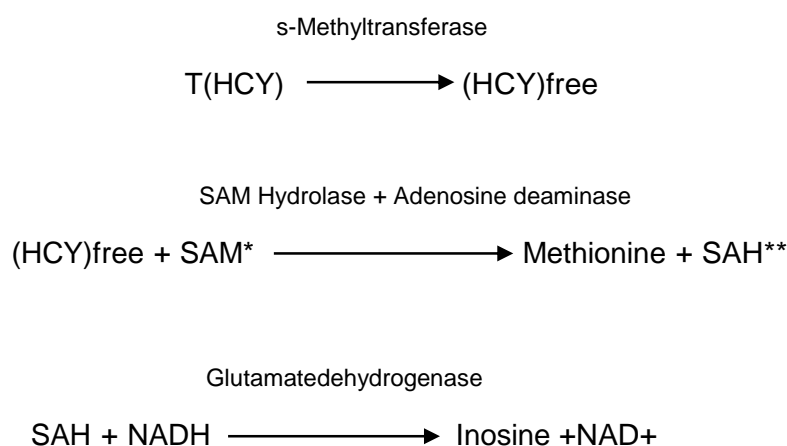
* SAM = S-Adenosyl-methionine

** SAH = S-Adenosyl-homocysteine

Reagents

Reagent	Concentration
Reagent A:	
S-adenosylmethionine	0.1 mmol/l
NADH*	0.2 mmol/l
TCEP	0.5 mmol/l
2-oxoglutarate	5.0 mmol/l
Reagent B:	
Glutamate dehydrogenase	10 KU/l
SAH hydrolase	3.0 KU/l
Adenosyne deaminase	5.0 KU/l
HCY** methyltransferase	5.0 KU/l

*Nicotinamide Adenine Dinucleotide (NADH)

**Homocysteine (HCY)

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent 1 (µl)	180
Reagent 2 (µl)	47
Serum (µl)	9
Incubation period (s)	25 cycles (5 minutes)
Reaction type	Fixed time
Wavelength (nm)	340
Reaction	Descending

Calculation of results

The △A (A2 - A1) calculated for blank and each calibrator against its concentration (concentrations are reported on the calibrator vial label). Results was found by comparing the sample △A against the plotted curve. A curve fitting system software was suggested to achieve more precise results.

Reference value

National Institute of Standards and Technology (NIST) standardized study shows 15 µmol/l as the cut-off value for normal level of homocysteine for adults.

Standarization	NIST
Adult normal cut-off	15 µmol/l

3.9.2 Determination of serum urea

Principle

Serum urea was determined by using "Urease-GLDH": enzymatic UV test, according to Thomas method (Thomas, 1998) using DiaSys reagent kits.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + 2HCO$$

$$\text{2-Oxoglutarate} + NH_4 + NADH \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

27
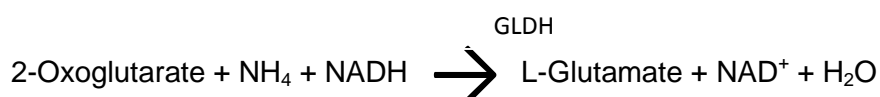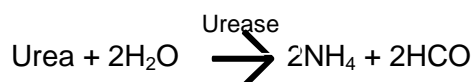
Reagents

Concentrations are those in the final test mixture.

Reagent	Concentration
R1: TRIS	120 mmol/l
2- Oxoglutarate	7 mmol/l
ADP	0.6 mmol/l
Urease	0.6 ku/l
GLDH	1 ku/l
R2: NADH	0.25 mmol/l
Standard	50 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 340 nm

Optical path: 1cm

Temperature: 37 ⁰C

Measurement: against distilled water.

- Ten µl of standard (sample or control) was added to 1 ml of working reagent and mixed well.
- The mixture was incubated for 30 sec then absorbance (A1) was recorded.
- After exactly further 60 sec the absorbance (A2) was measured.

Calculation

A = (A1 – A2) sample or standard

$$\text{Urea (mg/dl)} = \frac{\text{A sample X concentration of standard}}{\text{standard}} \quad \text{A}$$

Reference value

(Palestinian Clinical Laboratory Test Guide, PCLTG, 2005)

Child	5 - 30 mg/dl
Adult	13 - 43 mg/dl

3.9.3 Determination of serum creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method **(Newman and Price, 1999)** using DiaSys reagent kits.

Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid   creatinine picrate complex

Reagents

Concentrations are those in the final test mixture.

Reagent	Concentration
R1: Sodium hydroxide (pH approx. 13)	0.16 mol/l
R2: Picric acid (pH approx. 1.2)	4.0 mmol/l
Standard	2.0 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 490 nm

Optical path: 1cm

Temperature: 37 °C

Measurement: against distilled water.

- Fifty µl of standard (sample or control) was added to 1 ml of working reagent, add and mixed well.
- The Mixture was incubated for 60 sec then absorbance (A1) was recorded.
- After exactly further 120 sec the absorbance (A2) was measured.

Calculation

A = (A1 − A2) sample or standard

$$\text{Creatinine (mg/dl)} = \frac{\text{A sample} \times \text{concentration of standard}}{\text{A standard}}$$

Reference value (in serum) **(PCLTG, 2005).**

Infant	0.2 – 0.4 mg/dl
Child	0.3 - 0.7 mg/dl
Adolescent	0.5 - 1.0 mg/dl
Adult: Male	0.6 - 1.2 mg/dl
Female	0.5 -1.1 mg/dl

3.10 Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0) statistical package.

• Simple distribution of the study variables and the cross tabulation were applied.

• Chi-square (t^2) was used to identify the significance of the relations, associations, and interactions among various variables. Yates's continuity correction test, t^2(corrected), was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small.

• The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between cases and controls homocysteine levels.

• The one-way ANOVA test was used for analysis of variance.

• Pearson's correlation test was applied.

• The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).

• Ranges as minimum and maximum values were used.

• The percentage difference was calculated according to the formula:

Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

Percent difference = (| (V1 - V2) | / ((V1 + V2)/2)) X 100.

• SPSS version 18.0 was used for plotting of correlation graphs plotting between homocysteine and the other studied parameters.

Chapter 4

Results

4.1 Personal profile of the study population

Personal profile of the study population is summarized in Table 4.1. Age classification showed that 19 (31.7%) controls and 18 (30.0%) cases were ≤45 years old. Age group 46-56 years comprised 18 (30.0%) controls and 16 (26.7%) cases. Controls and cases aged >56 years were 23 (38.3%) and 26 (43.3%), respectively. Chi-Square test showed that the difference between controls and cases in term of age distribution was not significant (P=0.845). The mean ages of controls and cases were 53.4±8.7and 53.9±10.1 years with ranges of 38-68 and 39-70 years, respectively. The independent sample t-test also showed no significant difference between mean ages of controls and cases (P=0.669). Each control and case group comprised 34 (56.7%) males and 26 (43.3%) females. Five (8.3%) and 55 (91.7%) of controls were single and married, respectively compared to 10 (16.7%) and 50 (83.3%) of cases (P=0.168). Analysis of the educational status of the study population showed that 32 (53.3%) controls and 18 (30.0%) cases had a university degree, 20 (33.3%) and 14 (23.3%) finished secondary school, 6 (10.0%) and 6 (10.0%) finished preparatory school, one (1.7%) and 16 (26.7%) passed primary school, and one (1.7%) and 6 (10%) were illiterate. The difference between the various educational levels of controls and cases was significant (P=0.001), with ESRD was more prevalent among persons with lower educational levels.

Table 4.1 Personal profile of the study population

Personal character	Controls (n=60)		Cases (n=60)		test		p-value
	No.	%	No.	%			
Age (Year)							
45	19	31.7	18	30.0	t^2	0.328	0.845
46-56	18	30.0	16	26.7			
>56	23	38.3	26	43.3			
Mean±SD	53.4±8.7		53.9±10.1		t	0.529	0.669
Range *(min-max)*	*38-68*		*39-70*				
Gender							
Male	34	56.7	34	56.7	t^2	0	1.000
female	26	43.3	26	43.3			
Marital status							
Single	5	8.3	10	16.7	t^2	1.905	0.168
Married	55	91.7	50	83.3			
Education							
university	32	53.3	18	30.0	t^2	18.014	0.001*
Secondary school	20	33.3	14	23.3			
Preparatory	6	10.0	6	10.0			
Primary	1	1.7	16	26.7			
illiterate	1	1.7	6	10.0			

*P-value of χ^2 (corrected) test.
P>0.05:not significant, P<0.05:significant.

4.2 Socioeconomic data of the study population

Table 4.2 provides socioeconomic data of the study population. The employed controls and cases were 37 (61.7%) and 12 (20.0%) whereas 23 (38.3%) controls and 48 (80.0%) cases were unemployed. The difference between the two groups was significant (P=0.000), with increasing ESRD among unemployed individuals. Regarding family income/month, significant difference was also recorded between controls and cases (P=0.000) with ESRD more frequent among family with less income. In addition, family history of ESRD revealed that 2 (3.3%) controls and 16 (26.7%) cases reported that they had family history of ESRD whereas 58 (96.7%) controls and 44 (73.3%) cases did not (P=0.000), indicating that family history is

associated with ESRD. Twenty (33.3%) controls were smokers compared to 15 (25.0%) cases (P=0.315).

Table 4.2 Socioeconomic characters of the study population

Family character	Controls (n=60)		Cases (n=60)		t^2	p-value
	No.	%	No.	%		
Employment						
Yes	37	61.7	12	20.0	21.558	0.000
No	23	38.3	48	80.0		
Family income/month (NIS)[**]						
<1000	5	8.3	26	43.3	24.704	0.000
1000-2000	18	30.0	20	33.3		
>2000	37	61.7	14	23.3		
Family history						
Yes	2	3.3	16	26.7	11.046	0.000*
No	58	96.7	44	73.3		
Smoking						
Yes	20	33.3	15	25.0	1.008	0.315
No	40	66.7	45	75.0		

*P-value of χ^2 (corrected) test.
[**] NIS: New Israeli Shekels.
P>0.05:not significant, P<0.05:significant.

4.3 Clinical data of the hemodialysis patients

Clinical data of the hemodialysis patients are presented in Table 4.3. The mean duration of hemodialysis among patients was 3.2±2.9 year and the mean frequency of hemodialysis per week was 2.6±0.6 sessions. The most common self-reported disorders among the hemodialysis patients were hypertension 49 (81.7%) and diabetes 32 (53.3%).

Table 4.3. Clinical data of the hemodialysis patients

Clinical data	mean±SD
Hemodialysis duration (Year)	3.2±2.9
(min-max)	*(0.5-10.0)*
Hemodialysis frequency/week	2.6±0.6
(min-max)	*(1-3)*
Other disorders	**No. (%)**
Yes	53 (88.3)
No	7 (11.7)
If Yes	**No. (%)**
Diabetes	32 (53.3)
Hypertension	49 (81.7)
CVD	6 (10.0)
Asthma	2 (3.3)

4.4 Anthropometric measurements of the study population

Anthropometric measurements of the study population are illustrated in Table 4.4. The mean weight of cases was 81.1±18.8 Kg compared to 81.9±11.1 Kg of controls. The weight difference between cases and controls was not significant (P=0.835). However, there was a significant difference in the mean height of cases compared to controls (1.64±0.07 vs. 1.71±0.08 m, P=0.001). Therefore, the difference in the body mass index (BMI) between cases and controls was not significant (30.1±6.9 vs. 27.9±2.7 Kg/m^2, P=0.109).

Table 4.4. Anthropometric measurements of the study population.

Anthropometric measurement	Control (n=60) mean± SD	Case (n=60) mean±SD	% difference	t	P-value
Weight (Kg)*	81.9±11.1	81.1±18.8	1.0	0.209	0.835
(min-max)	*(60-100)*	*(56-130)*			
Height (m)*	1.71±0.08	1.64±0.07	4.2	3.500	0.001
(min-max)	*(1.56-1.89)*	*(1.5-1.75)*			
BMI (Kg/m^2)*	27.9±2.7	30.1±6.9	7.6	1.625	0.109
	(23.4-34.6)	*(21.4-46.7)*			

*Kg: kilogram, ** m: meter. ***BMI: Body mass index (Kg/m^2): People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight, people with BMI 30.0 were considered obese **(WHO, 2000)**. All values are expressed as mean ±SD. P>0.05: not significant, P<0.05:significant.

4.5 Serum homocysteine levels of the study population

Table 4.5 shows the average serum homocysteine levels of the study population. There was a significant elevation in the mean level of homocysteine in cases compared to controls (50.8±9.7 vs. 13.1±3.7 μmol/l, % difference=118.0, P=0.000).

Table 4.5 Serum homocysteine levels of the study population

Parameter	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
Homocysteine (μmol/l)	13.1±3.7	50.8±9.7	118.0	19.802	0.000
Range *(min-max)*	*(7-19)*	*(37-75)*			

P<0.05: significant.

4.6 Serum urea and creatinine concentrations of the study population

Serum urea and creatinine concentrations of the study population are illustrated in Table 4.6. The average concentrations of urea and creatinine were found to be significantly higher in cases (169.6±42.4 and 9.96±2.40 mg/dl) compared to controls (27.4±7.1 and 0.77±0.14 mg/dl) with % differences of 144.4 and 171.4, P=0.000.

Table 4.6 Serum urea and creatinine of the study population

Parameter (mg/dl)	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
Urea	27.4±7.1	169.6±42.4	144.4	18.114	0.000
(min-max)	*(15-40)*	*(88-256)*			
Creatinine	0.77±0.14	9.96±2.40	171.4	20.970	0.000
(min-max)	*(0.5-1.0)*	*(5.9-15.0)*			

P<0.05: significant.

4.7 Hematological parameters of the study population

4.7.1 White blood cell count of the study population

Table 4.7 gives white blood cell count of the study population. There was a significant increase in the mean level of WBCs count in cases compared to controls (7.18±2.37 vs. 5.95±1.37 x10^3 cell/μl, % difference=18.7, P=0.017).

Table 4.7. White blood cell (WBC) count of the study population

Parameter	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
WBC count (x10^3 cell/~l)	5.95±1.37	7.18±2.37	18.7	2.468	0.017
(min-max)	*(3.9-9.0)*	*(2.0-14.1)*			

P<0.05: significant.

4.7.2 Red blood cell/mass indices of the study population

Red blood cell mass of the study population including red blood cell (RBC) count, hemoglobin and hematocrit of the study population are illustrated in Table 4.8. The means of RBC count, hemoglobin and hematocrit were found to be lower in cases (3.12±0.54 x10^6 cell/ml, 8.9±1.5 gm/dl and 26.3±4.6 %) compared to controls (4.03±0.37 x10^6 cell/ml, 12.8±1.6 gm/dl and 45.0±4.6 %) with % differences of 25.4, 36.0 and 52.4%, respectively, P=0.000.

Table 4.8. RBC count, hemoglobin (Hb), and hematocrit (Hct) of the study population

Parameter	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
RBC count (x10^6 cell/~l)	4.03±0.37	3.12±0.54	25.4	7.689	0.000
(min-max)	(3.2-5.0)	(2.3-4.7)			
Hb (gm/dl)	12.8±1.6	8.9±1.5	36.0	9.559	0.000
(min-max)	(10.2-15.0)	(6.8-12.7)			
Hct (%)	45.0±4.6	26.3±4.6	52.4	15.743	0.000
(min-max)	(38.0-53.0)	(20.4-37.0)			

P<0.05: significant.

4.7.3 Red blood cell indices of the study population

Table 4.9 shows RBC indices of the study population. The average level of mean corpuscular volume (MCV) exhibited a non-significant decrease in cases compared to controls (83.0±6.6 vs. 85.5±5.0 (fl), % difference=3.0, p=0.105). However, mean corpuscular hemoglobin (MCH) displayed significant decrease in cases (28.6±2.9 vs. 31.9±4.4 (pg), % difference=11.0, p=0.001). In contrast, mean corpuscular hemoglobin concentration (MCHC) was significantly increase in cases compared to controls (33.8±1.2 vs. 28.4±2.0 % and p=0.000).

Table 4.9. mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of the study population.

Parameter	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
MCV (fl)	85.5±5.0	83.0±6.6	3.0	1.646	0.105
(min-max)	(70-92)	(68-98)			
MCH (pg)	31.9±4.4	28.6±2.9	11.0	3.333	0.001
(min-max)	(26.1-46.9)	(22.1-33.4)			
MCHC (mg/dl)	28.4±2.0	33.8±1.2	17.4	13.005	0.000
(min-max)	(24.5-31.7)	(30.3-36.0)			

P>0.05: not significant.

4.7.4 Blood platelet count of the study population

Table 4.10 shows blood platelet count of the study population. There was a significant difference in the mean blood platelet count between cases and controls (266.3±111.9 vs. 222.0±54.1 x10^9 L, % difference=18.1, p=0.045).

Table 4.10. Platelets count of the study population

Parameter	Control (n=60) mean±SD	Case (n=60) mean±SD	% difference	t	P-value
PLT (x10^9 L) *(min-max)*	222.0±54.1 *(146.0-340.0)*	266.3±111.9 *(118.0-618.0)*	18.1	2.052	0.045

P>0.05: not significant.

4.7.5 Hemostasis parameters of the study population

Hemostasis parameters of the study population including prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) are presented in Table 4.11. The mean PT and INR were significantly increase in cases compared to controls (16.2±2.6 sec and 1.23±0.17 vs. 13.5±0.4 sec and 0.97±0.07, % difference=18.2 and 23.6, P=0.000). In contrast, the mean APTT was significantly decreased in cases compared to controls (25.3±5.3 vs. 32.6±2.1 sec, % difference=25.2, p=0.000).

Table 4.11. Hemostasis parameters prothrombin time (PT) and activated partial thromboplastin time (APTT) and international normalized ratio (INR) of the study population.

Parameter	Control (n=30) mean±SD	Case (n=30) mean±SD	% difference	t	P-value
PT (sec) *(min-max)*	13.5±0.4 *(13.0-14.1)*	16.2±2.6 *(13.5-26.8)*	18.2	5.733	0.000
APTT (sec) *(min-max)*	*32.6±2.1* *(30.0-37.0)*	*25.3±5.3* *(17.0-40.0)*	25.2	6.930	0.000
INR *(min-max)*	0.97±0.07 *(0.9-1.1)*	1.23±0.17 *(1.0-1.9)*	23.6	7.745	0.000

P<0.05: significant.

4.8 Hemocysteine relations

4.8.1 Homosycteine levels in relation to socio-demographic data of the study population

Table 4.12 provides the relationship of homocysteine with socio-demographic data of the study population. Results showed that the lower the educational level, the higher the level of homocysteine (Figure 4.1). Using ANOVA test, this inverse relationship was found to be significant (F=7.848, P=0.000). The t-test showed that unemployed individuals had significant increase in homocysteine level compared to employed ones (Figure 4.2, t=4.111, P=0.000). Regarding family income, homocysteine level was increased with decreased family income/month (Figure 4.3). This negative relation was significant (F=9.862, P=0.000). In addition homocysteine level was significantly higher in individuals with family history ESRD (Figure 4.4, t=3.033, P=0.004).

Table 4.12. Homosycteine levels in relation to sociodemographic data of the study population

| Sociodemographic character	homosyctien level (µmol/l) Mean±SD	Statistical test		p-value
Education				
University	24.0±16.7	F	7.848	0.000
Secondary school	27.7±15.8			
Preparatory school	32.5±26.0			
Primary school	55.8±12.7			
Illiterate	60.3±9.0			
employment				
Yes	20.6±14.9	t	4.111	0.000
No	40.1±20.0			
Family income/month (NIS)[**]				
<1000	48.7±17.6	F	9.862	0.000
1000-2000	31.0±16.2			
>2000	23.0±19.1			
Family history				
Yes	51.0±10.6	t	3.033	0.004
No	29.0±20.0			

P>0.05:not significant.
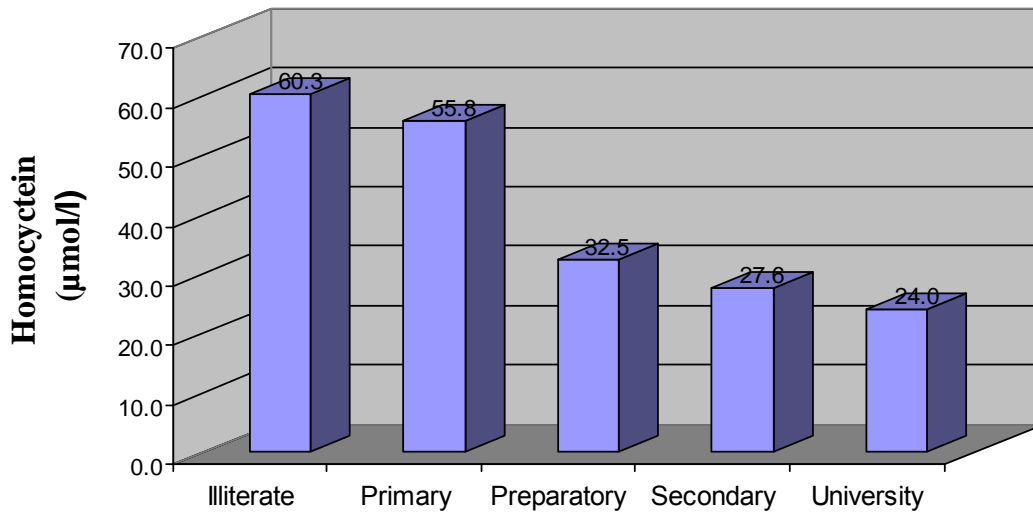
Figure 4.1. Homocysteine level in various to educational levels
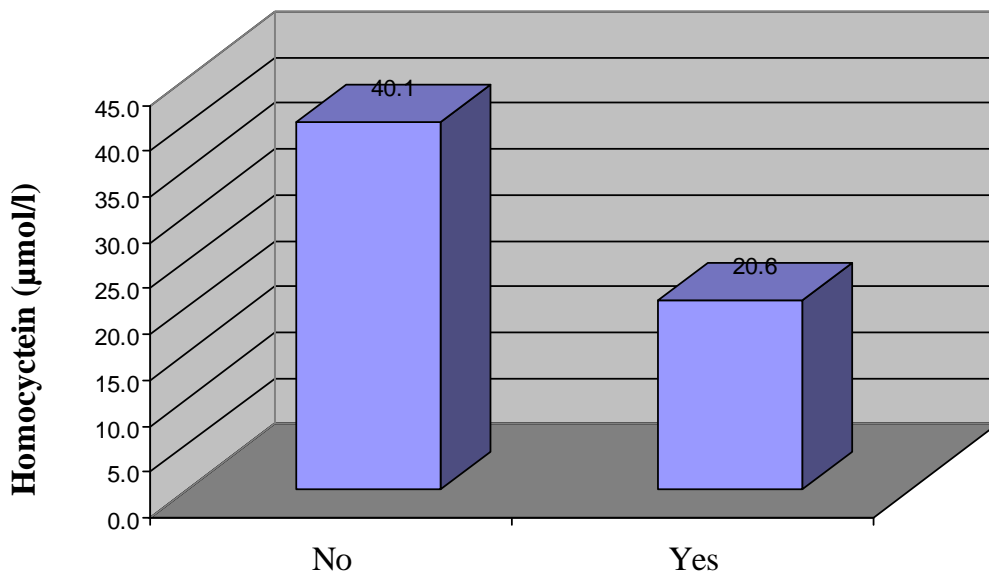
Figure 4.2. Homocysteine level in relation to employment
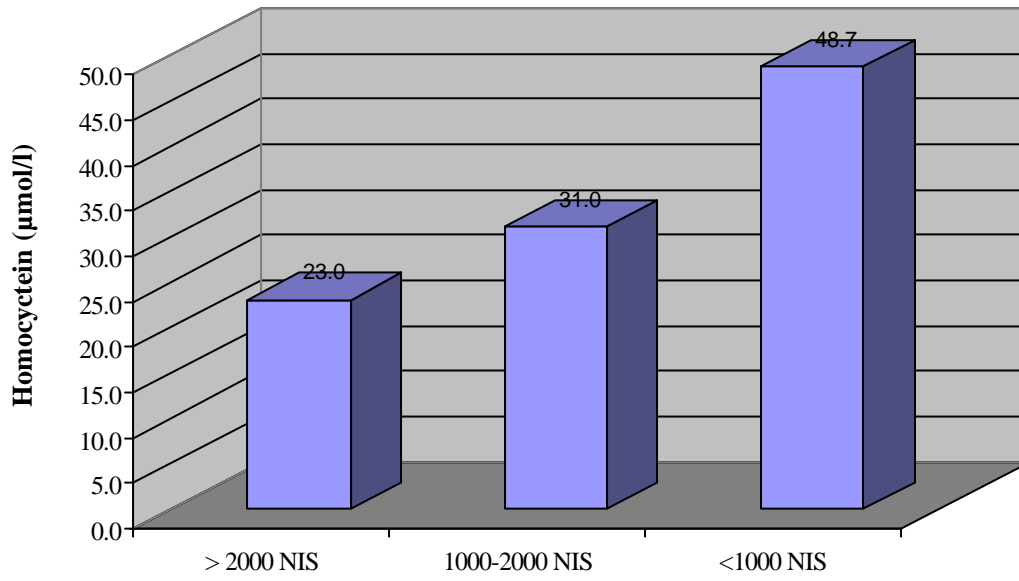
Figure 4.3. Homocysteine level in relation to Family income/month (NIS)
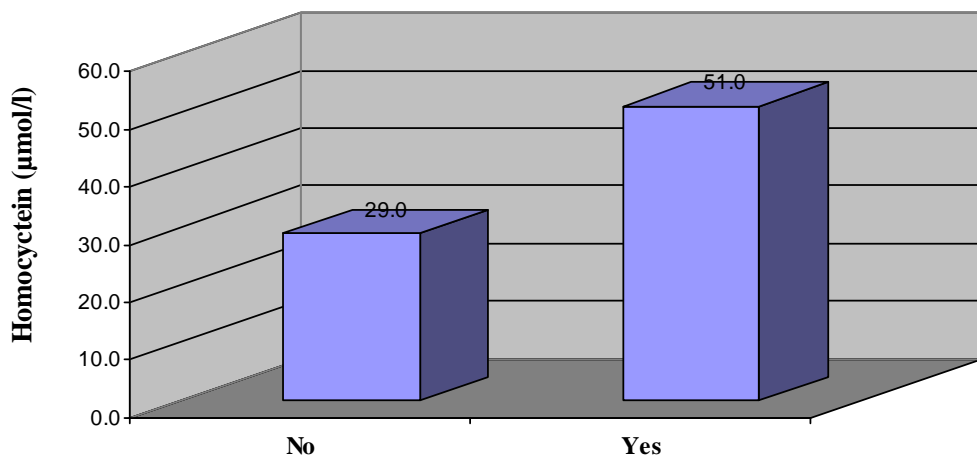
Figure 4.4. Homocysteine level in relation to Family history

4.8.2 Homosyctien level in relation to BMI of the study population

Homocysteine level in relation to BMI of the study population is presented in Table 4.13 and Figure 4.5. The Pearson correlation test showed that the higher the BMI, the higher the level of homocysteine. This positive correlation was not statistically significant (r=0.243, P= 0.062).

Table 4.13. Homocysteine level in relation to BMI

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
BMI (Kg/m^2)	0.243	0.062

* People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight, people with BMI 30.0 were considered obese **(WHO, 2000)**. P>0.05: not significant.
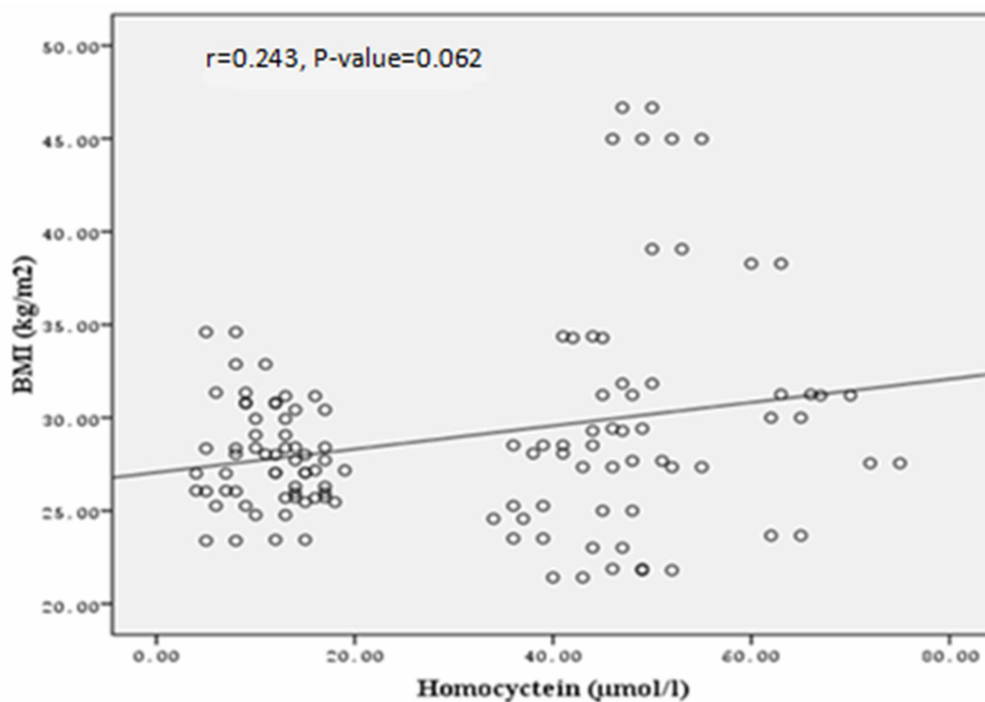
Figure 4.5. Correlation between homocysteine level and BMI of the study Population

4.8.3 Homosycteine level in relation to urea and creatinine of the study population

Table 4.14. shows the correlation between homocysteine levels with urea and creatinine of the study population. The Pearson correlation test showed that elevation in homocysteine level was accompanied with elevations in both urea and creatinine. Such positive correlations were significant (Figure 4.6. r=0.827, P=0.000 for urea and Figure 4.7. r=0.842, P=0.000 for creatinine).

Table 4.14. Homocysteine level in relation to urea and creatinine of the study population

Parameter (mg/dl)	Homocysteine level (µmol/l)	
	Pearson correlation (r)	P-value
Urea	0.827	0.000
Creatinine	0.842	0.000

P<0.05: significant.

Figure 4.6. Correlation between homocysteine level with urea of the study population
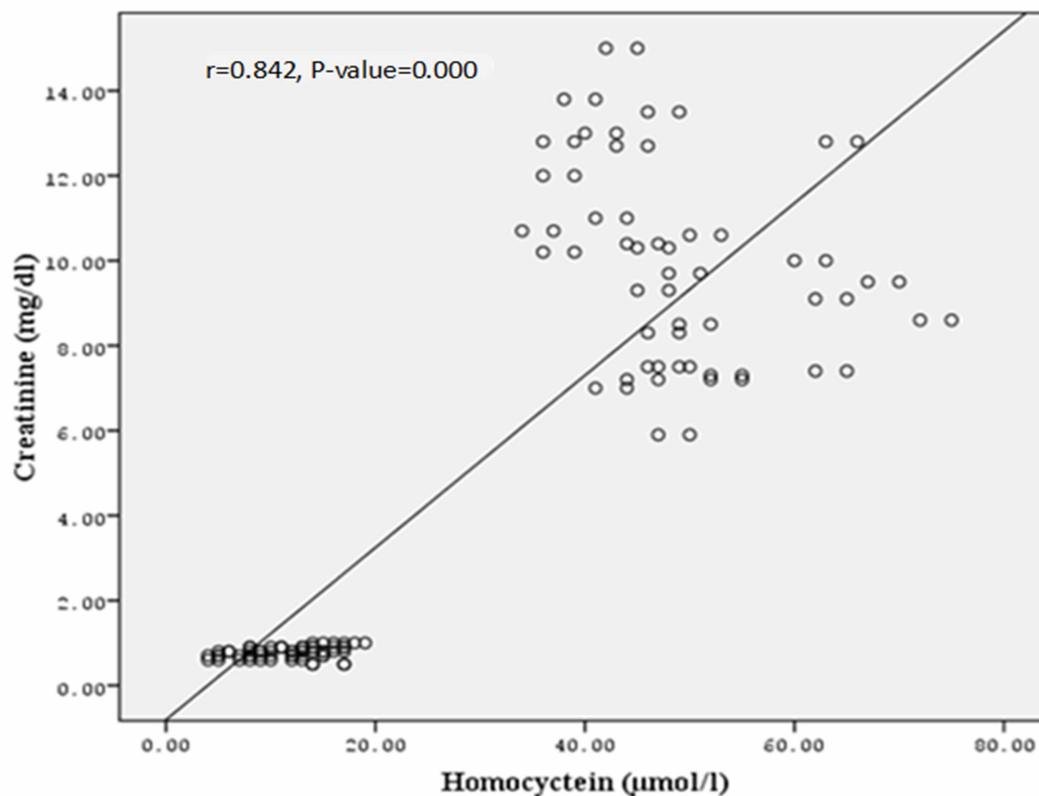
Figure 4.7. Correlation between homocysteine level with creatinine of the study population.

4.8.4 Homosycteine level in relation to WBC count of the study population

Homosycteine level in relation to WBC count of the study population is illustrated in Table 4.15 and fugure 4.8. The Pearson correlation test showed that the higher the homosycteine, the higher the WBC count. This positive correlation was statistically significant (r=0.338, P= 0.008).

Table 4.15. Homocysteine level in relation to WBC count of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
WBCs (x10^3 cell/~l)	0.338	0.008

P<0.05: significant.

Figure 4.8. Correlation between homocysteine level with WBC count of the study population

4.8.5 Homosycteine level in relation to RBC mass of the study population

Table 4.16 shows the correlation between homocysteine level with RBC count, hemoglobin and hematocrite of the study population. The Pearson correlation test showed that increase in homocysteine level was accompanied with decrease in RBC count, hemoglobin and hematocrite. Such inverse correlations were significant (Figure 4.9, r=-0.648, P=0.000; Figure 4.10, r=-0.733, P=0.000 and Figure 4.11, r=-0.836, P= 0.000, respectively).

Table 4.16. Homocysteine levels in relation to RBC count, HB, and HT of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
RBCs (x10⁶ cell/~l)	-0.648	0.000
Hb (gm/dl)	-0.733	0.000
Hct (%)	-0.836	0.000

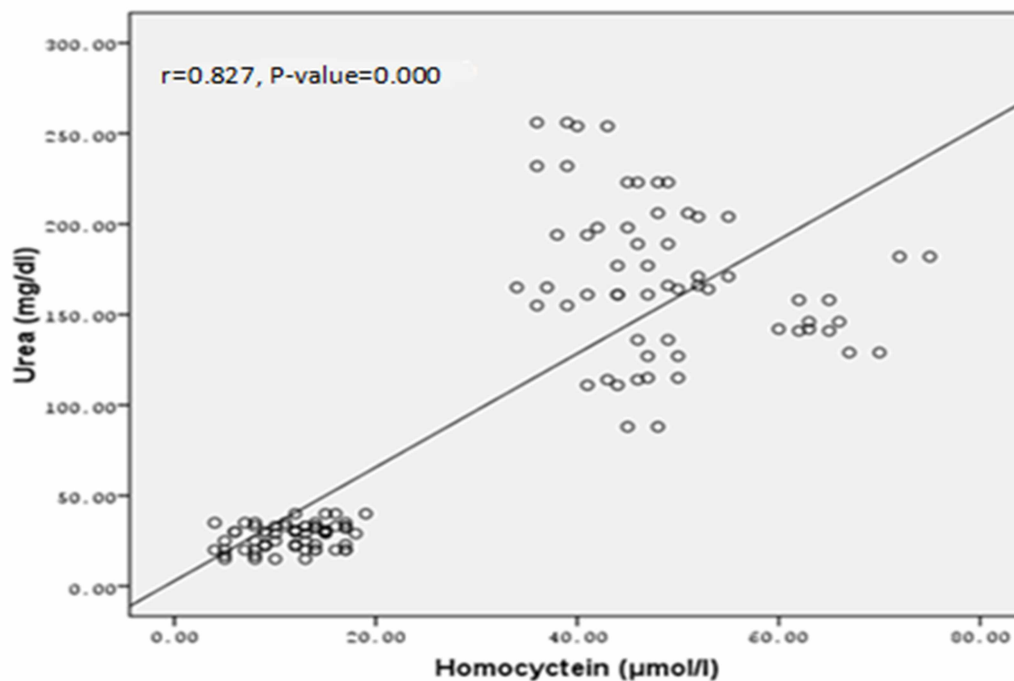All values are expressed as mean ±SD. P<0.05: significant.

Figure 4.9. Correlation between homocysteine level with RBC count of the study population
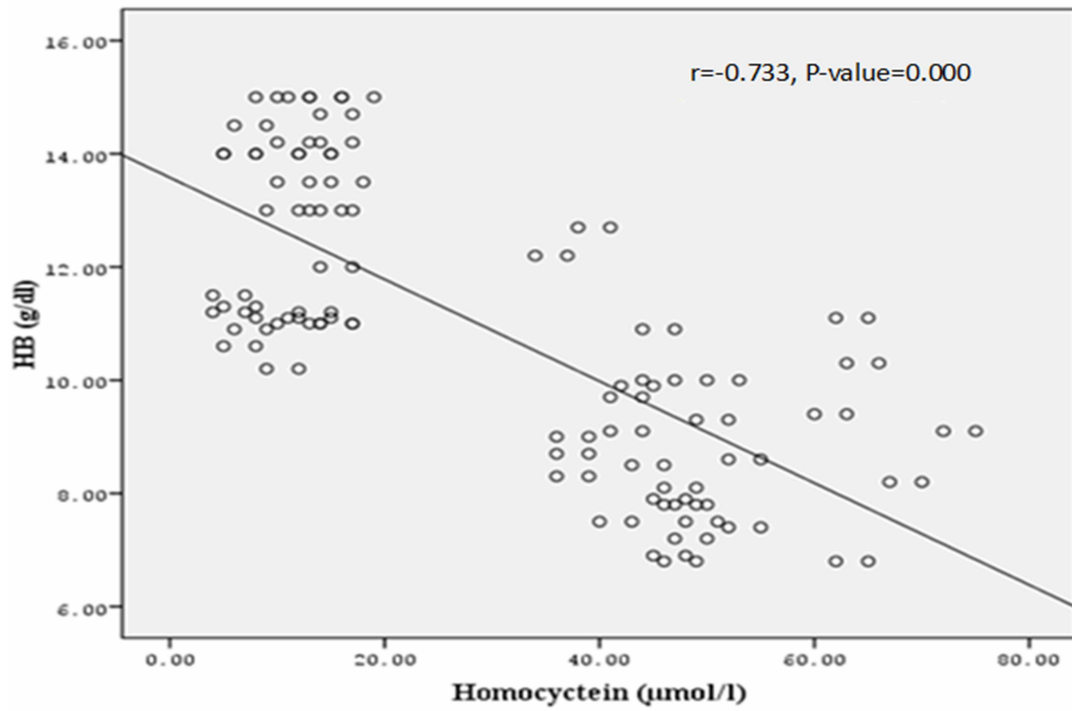
Figure 4.10. Correlation between homocysteine level with hemoglobin (HB) content of the study population
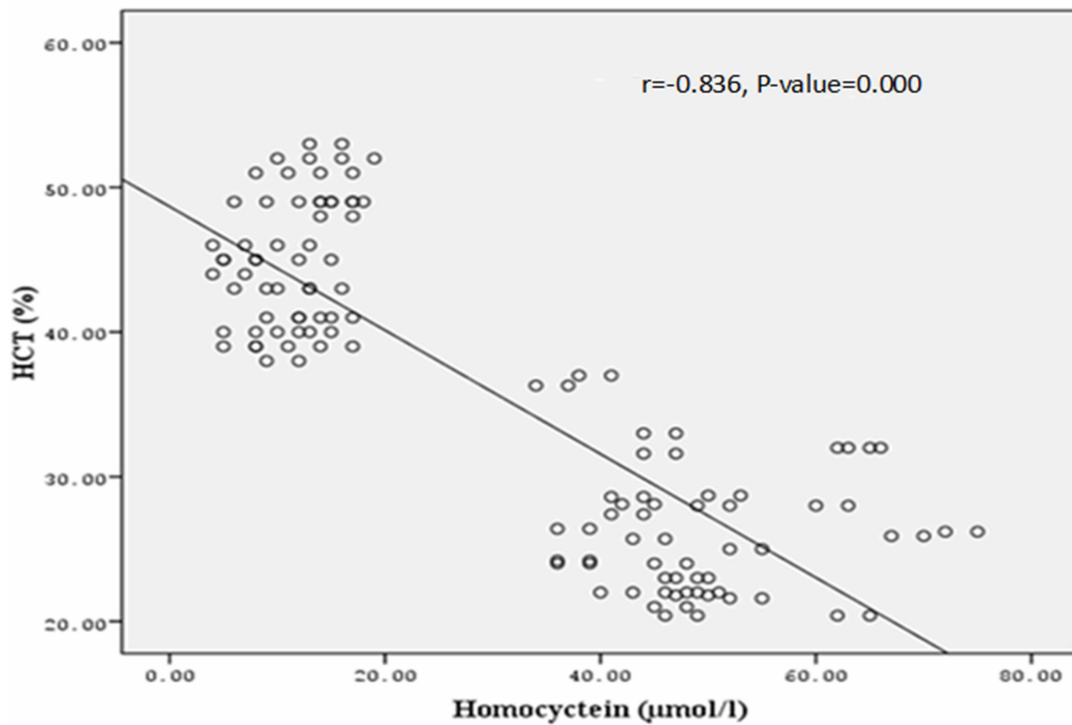
Figure 4.11. Correlation between homocysteine level with hematocrit (HTC) of the study population.

4.8.6 Homosycteine level in relation to RBC indices of the study population

Homosycteine level in relation to MCV, MCH and MCHC of the study population is illustrated in Table 4.17, The Pearson correlation test showed negative significant correlations of homocysteine level with MCV and MCH levels (Figure 4.12, r=-0.267, P=0.039; and Figure 4.13, r=-0.402 P=0.001, respectively). On the other hand, there was a positive significant correlation between homocysteine level and MCHC (Figure 4.14, r=0.789, P=0.000).

Table 4.17. Homocysteine level in relation to MCV, MCH, and MCHC of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
MCV (fl)	-0.267	0.039
MCH (pg)	-0.402	0.001
MCHC (mg/dl)	0.789	0.000

All values are expressed as mean ±SD. P<0.05: significant.

Figure 4.12. Correlation between homocysteine level with MCV level of the study population

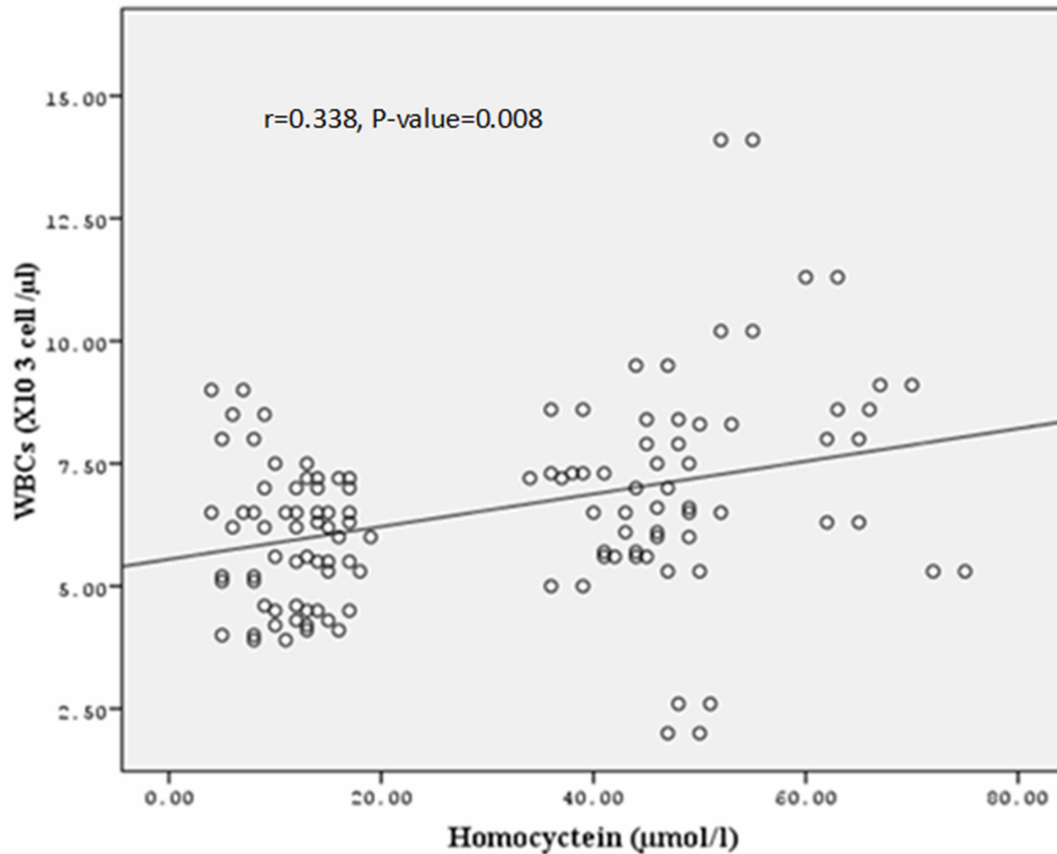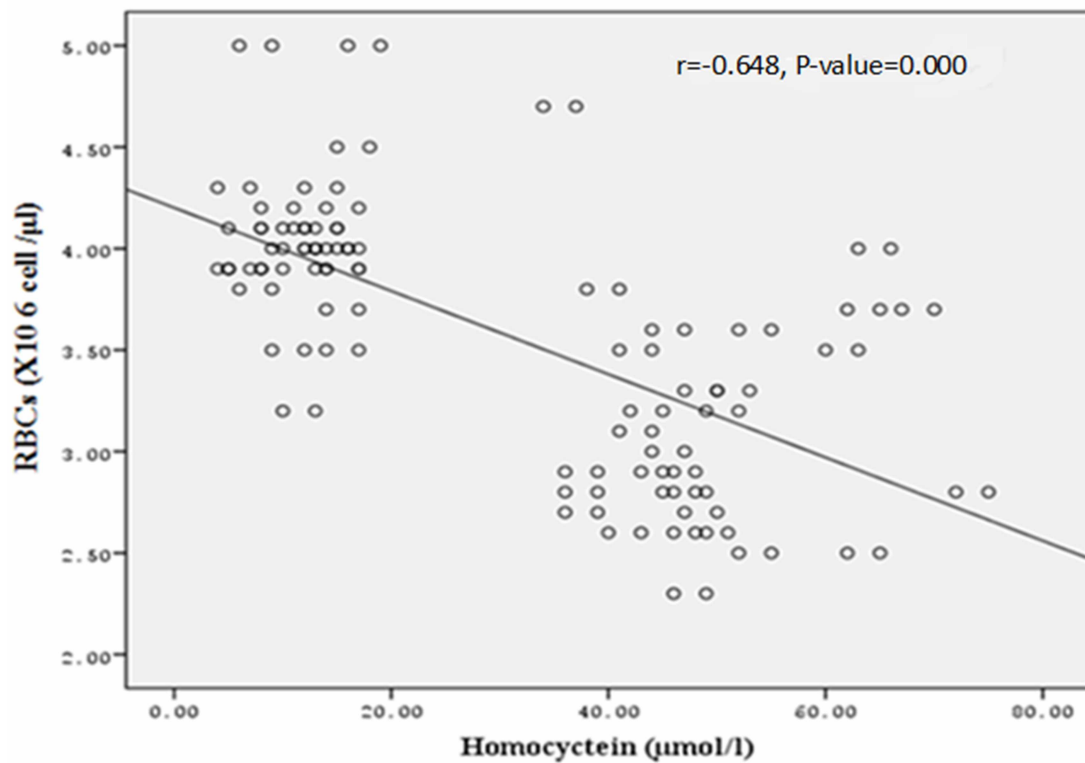Figure 4.13. Correlation between homocysteine level with MCH of the study population.

Figure 4.14. Correlation between homocysteine level with MCHC of the study population

4.8.7 Homosycteine level in relation to blood platelet count of the study population

Table 4.18 and Figure 4.15 points out the correlation between the homocysteine level and platelet count of the study population. Pearson corrlation test showed a positive significant correlation between homocysteine level and platelet count (r=0.369, P= 0.000).

Table 4.18. Homocysteine level in relation to platelet count of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
platelet count (x10^9 L)	0.369	0.000

All values are expressed as mean ±SD. P<0.05: significant
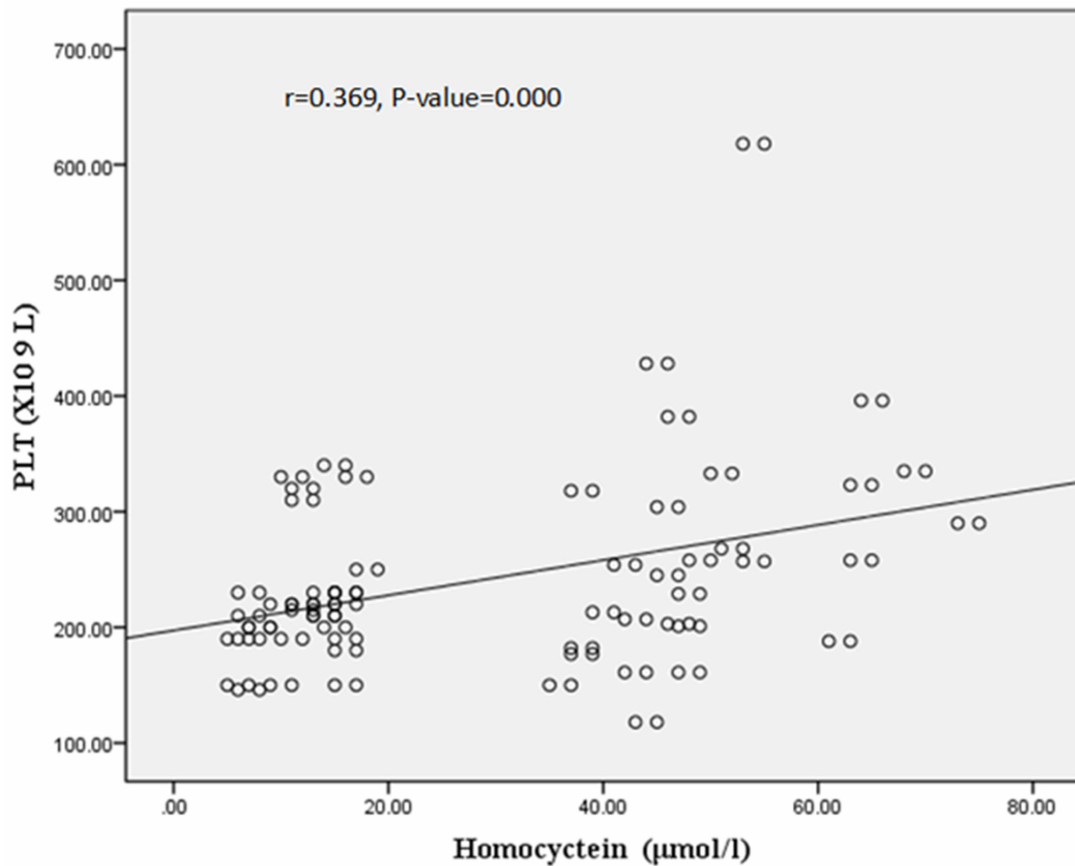
Figure 4.15. Correlation between homocysteine level with platelet (PLT) count of the study population

4.8.8 Homosycteine level in relation to hemostasis parameters of the study population

Homosycteine level in relation to PT, APTT and INR of the study population is illustrated in Table 4.19. Homosycteine level exhibited positive significant correlations with PT, INR (Figure 4.16, r=0.564, P=0.000 and Figure 4.17, r=0.657, P=0.000). On the other hand, an inverse significant correlation was recorded between homocysteine level and APTT (Figure 4.18, r=-0.690, P=0.000).

Table 4.19 Homocysteine level in relation to PT, APTT and INR of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
PT (sec)	0.564	0.000
APTT (sec)	-0.690	0.000
INR	0.657	0.000

All values are expressed as mean ±SD. P<0.05: significant.
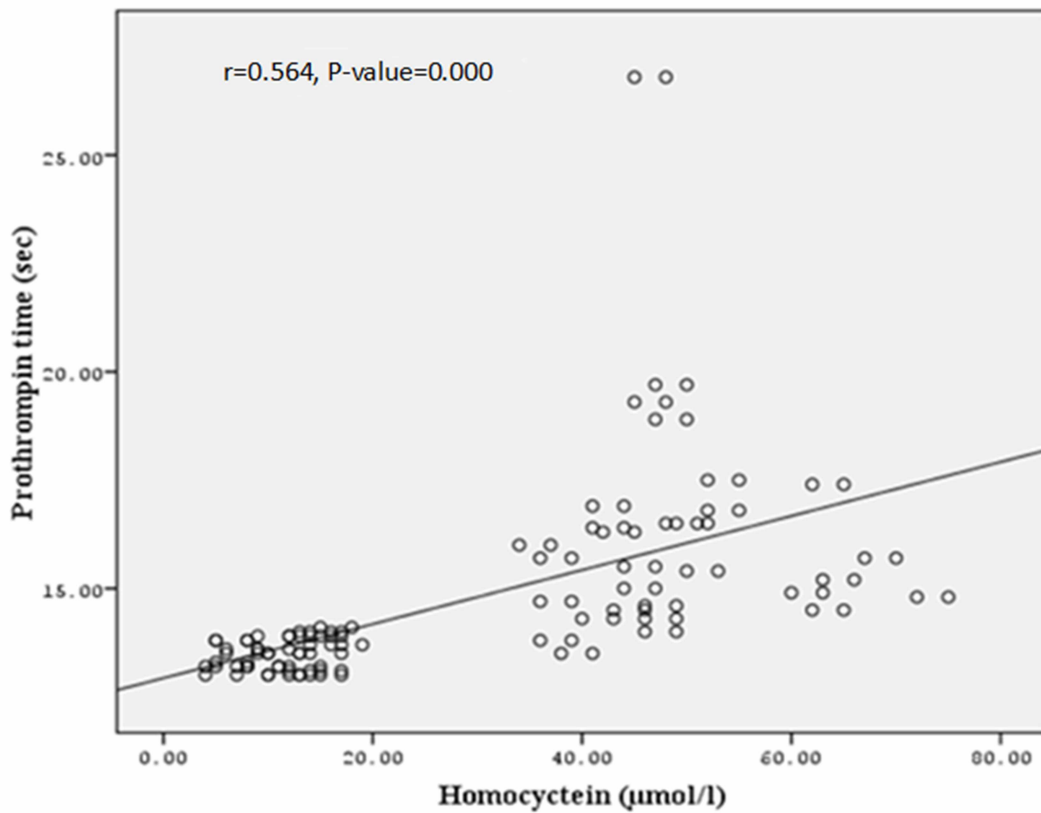
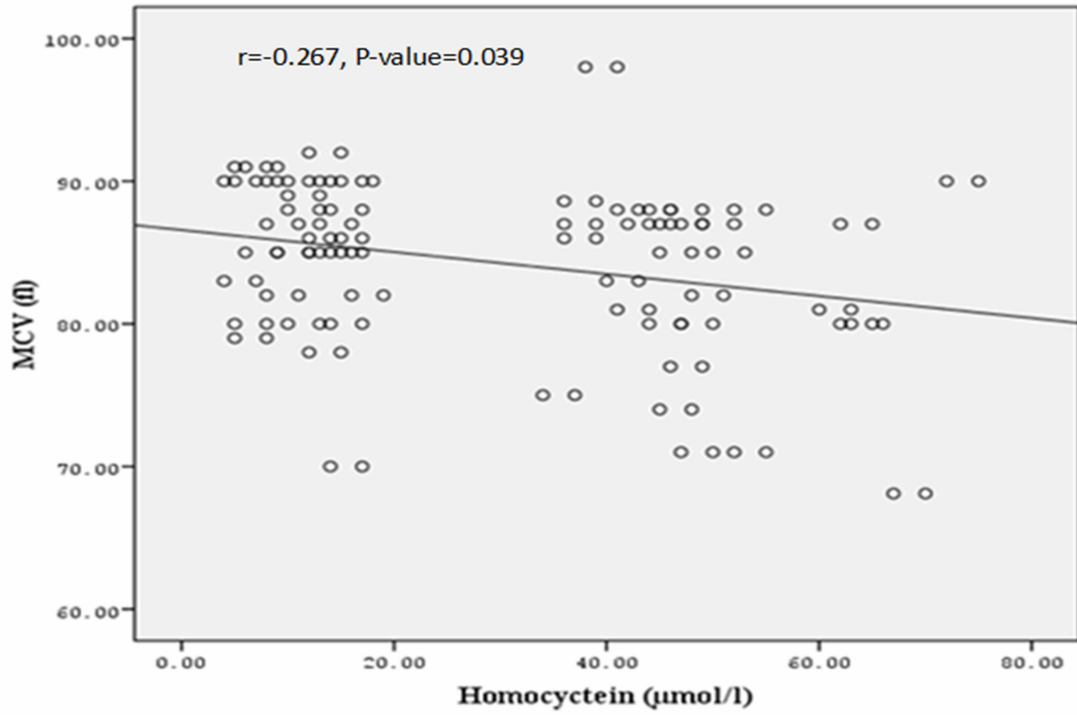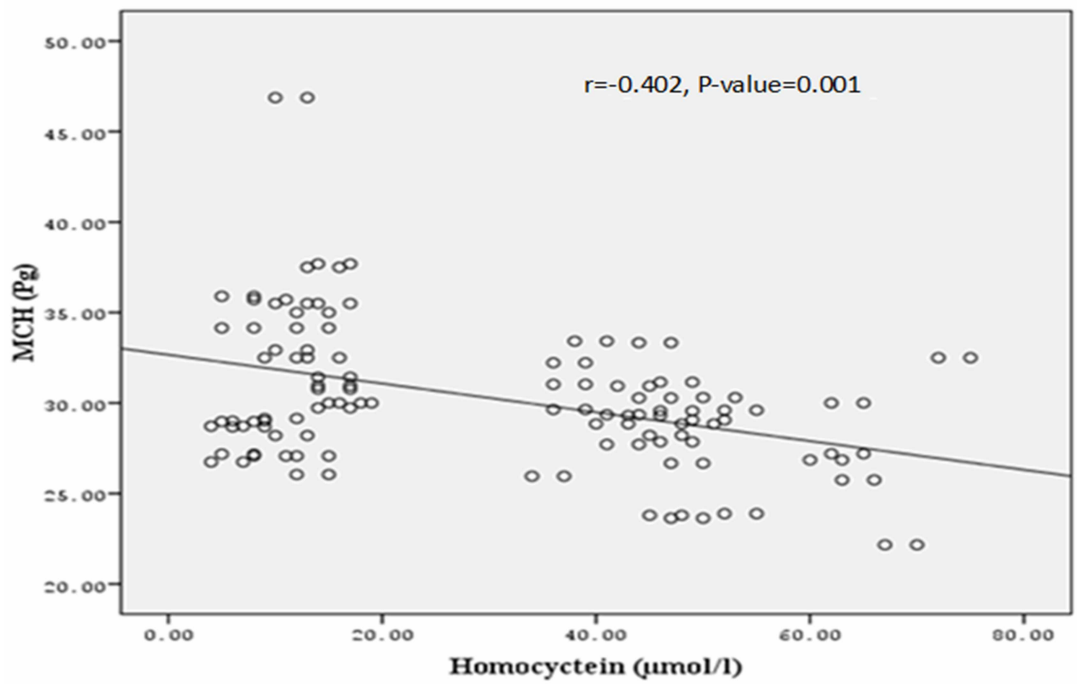Figure 4.16. Correlation between homocysteine level with PT of the study population
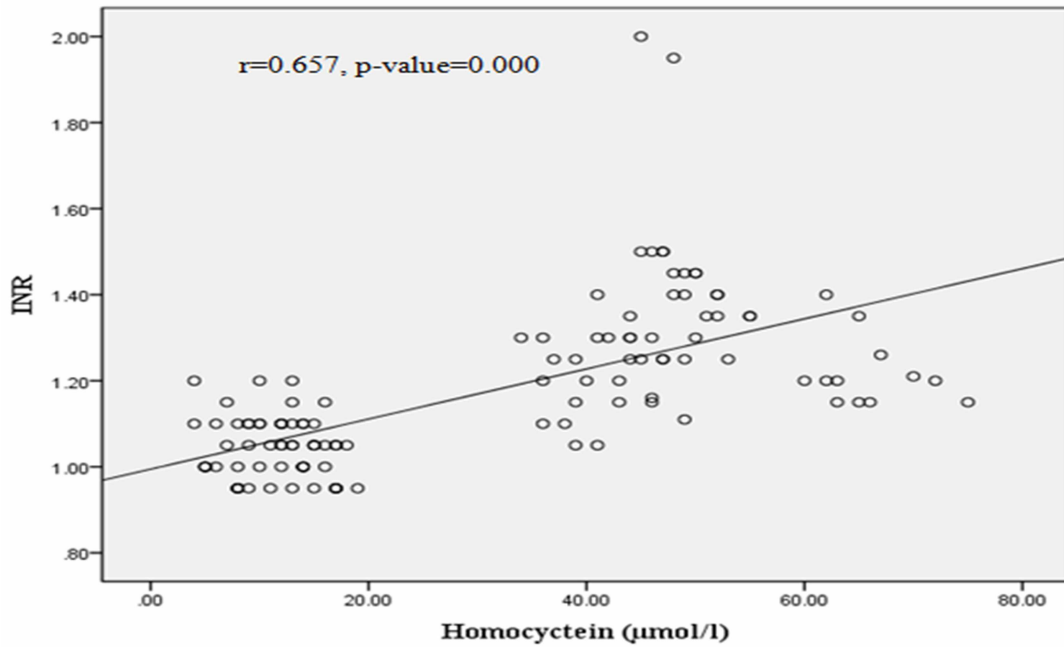
Figure 4.17. Correlation between homocysteine level with INR of the study population

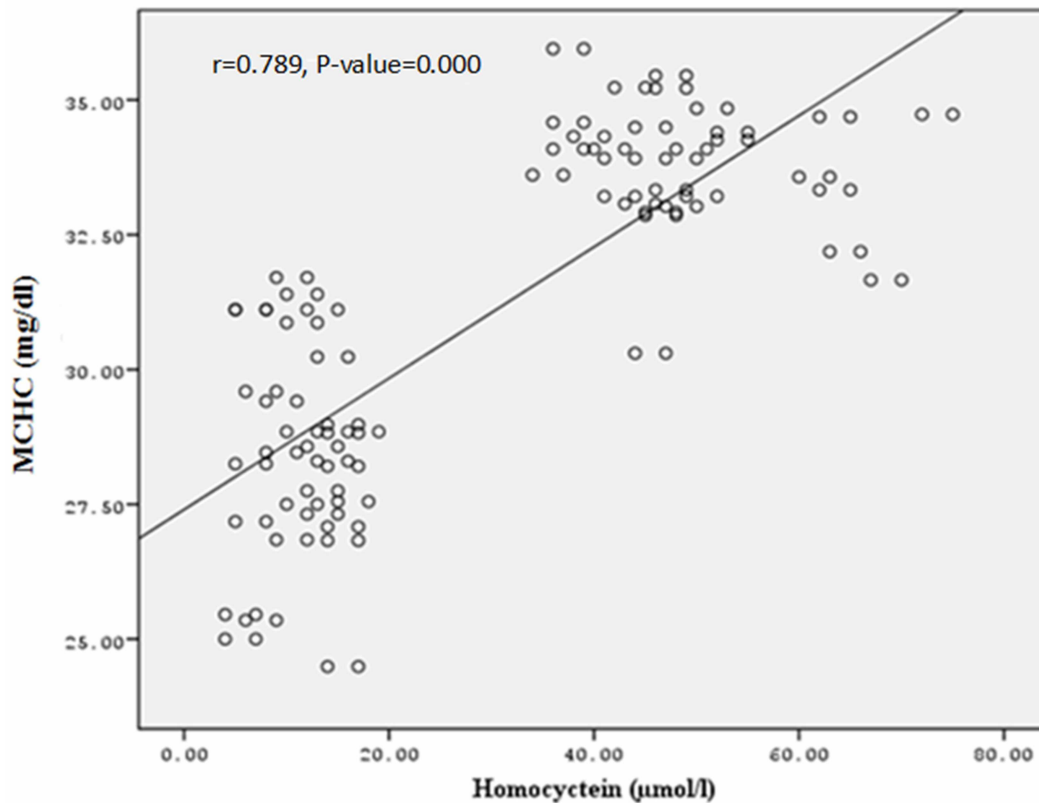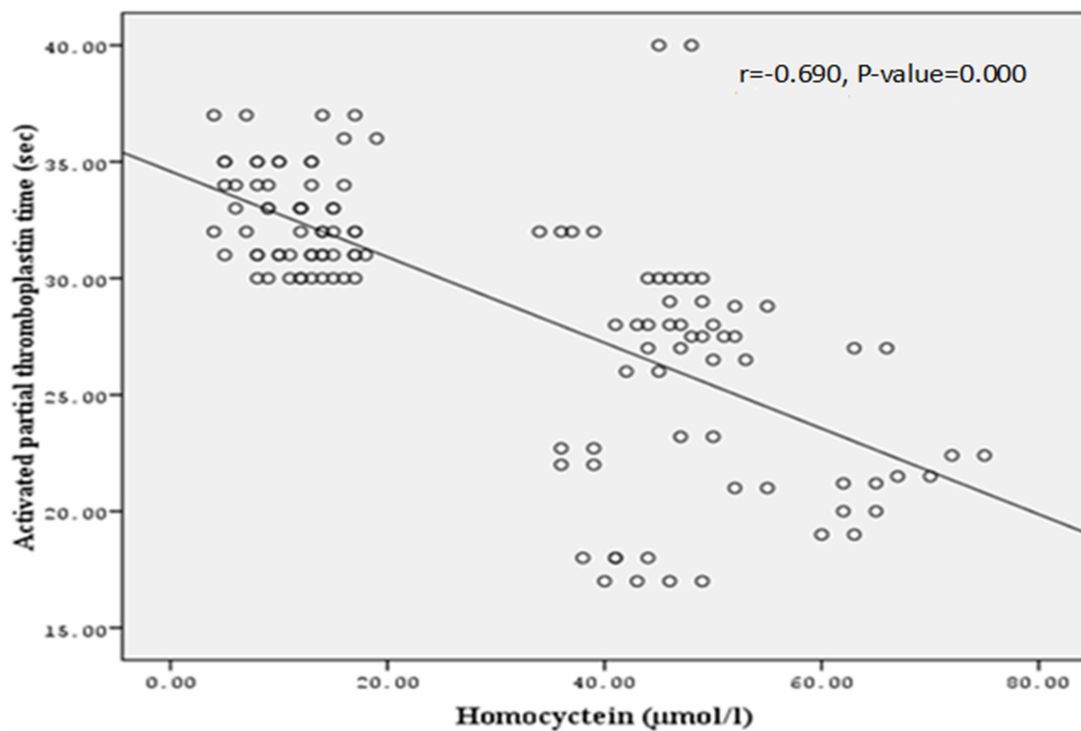Figure 4.18. Correlation between homocysteine level with APTT of the study population.

Chapter 5

Discussion

Although the number of hemodialysis patients in the Gaza Strip has been doubled in the last decade with mortality rate of 2.8%, the published data on the ESRD are few and restricted to annual reports produced by the Palestinian Ministry of Health. The available biochemical and hematological tests of ESRD were limited to routine traditional kidney function including urea and creatinine tests and CBC analysis. To our knowledge only one study assessed parathormone, calcium and phosphorus levels in hemodialysis patients in Gaza strip **(Abo Shamala, 2008).** This necessitated further assessment of other biochemical and hematological features such as homocystine which was recently linked to ESRD **(Nasri and Baradaran, 2005 and Anees et al., 2010)** as well as hemostasis paramaters (**Alghythan and Alsaeed, 2012)**. Introduction of homocystine test in the Gaza hospital and clinics may be useful to get a clearer picture on the patient condition, and to help in the disease management.

5.1 Socio-demographic characters of the study population

The present study is a case control investigation included 60 hemodialysis patients (34 males and 26 females) with mean age 53.9±10.1 years and 60 controls (34 males and 26 females) with mean age 53.4±8.7 years. End stage renal disease was more prevalent among low educated individuals. This finding is in agreement with the study of **Khattak et al., (2012)** who concluded that higher education level is associated with improved survival of patients on dialysis. The result that ESRD is more frequent among unemployed may coincide with the idea that dialysis treatment also causes a significant change

in daily living, disruption in work schedule and shift in social role which in turn imposes financial, housing, marital and employment problems **(Chau et al., 2003).** In addition, **White et al. (2008)** showed that shorter duration of education and being unemployed increased the incidence of kidney disease. Regarding family income, ESRD was more prevalent among families with less income/month. This result is in agreement with that obtained by **NKF (2002)** and **Mahmoodreza et al., (2009)** who pointed out that the dialysis patients' families had lower socioeconomic level in comparison with the healthy population. Finally the previous socio-demographic factors may be secondary to other disorders like hypertension and diabetes disorders reported in hemodialysis patients. Furthermore, ESRD was more frequent in patients with family history of the disease, i.e. ESRD is associated with family history. Similar results were previously documented **(McClellan et al., 2007; Tsai et al., 2010 and Kong et al., 2013).** In the context of socio-demographic aspects, **Sajadi et al., (2010)** reported that fatigue in chronic renal failure patients treated with hemodialysis increased with decrease in education level and income and increase in age, dialysis history and risk of chronic renal failure. When related to socio-demographic factors, homocysteine levels were found to be higher among the lower educated and unemployed individuals, among families with low income as well as among individuals with family history of ESRD, indicating that homocysteine level could be a predictor of ESRD in terms of education, employment, family income and family history. Low socioeconomic status was found to be associated with an increased risk of ESRD where homocysteine levels are elevated **(Khurelbaatar and Huang 2006; Shishehbor et al., 2008 and Abo Nada, 2012).**

5.2 Clinical data of the hemodialysis patients

Clinical data of the hemodialysis patients showed that the mean duration of hemodialysis among patients was 3.2±2.9 year and the mean frequency of hemodialysis per week was 2.6±0.6 sessions. The mean duration and frequency of hemodialysis registered in the present study are in agreement

with that previously obtained in hemodialysis patients in Gaza Strip by **abo Shamala (2008).** The most common self-reported disorders among the hemodialysis patients were hypertension and diabetes. This finding coincides with the fact that high blood pressure and diabetes play a key role in the progression of renal failure **(Oishi, 2000; Whitworth, 2005; Ganesh and Lee, 2011 and Etemadi et al., 2012).**

5.3 Serum homocysteine level of the study population

As indicated in the present study, there is a significant elevation in the mean level of homocysteine in hemodialysis patients compared to controls. This means that high homocysteine levels are linked to ESRD. Such finding is in agreement with that demonstrated by **Friedman et al. (2003); Takenaka et al. (2005); Sjöberg et. (2006); Anees, (2010); Koning and Hu, (2010) and Chao Wu et al. (2012).** In addition, **Friedman et al., (2001)** reported that hyperhomocysteinemia is very common in patients with chronic renal insufficiency and is nearly ubiquitous in patients with end-stage renal disease; who have up to a 30 times higher risk of cardiovascular related death than the general population. Hyperhomocysteinemia recorded in the present study in hemodialysis patients may be explained on the basis of: 1) homocysteine disposal in the kidneys themselves is almost ceased and 2) extrarenal homocysteine metabolism is impaired **(van Guldener, 2006)**. The first hypotheses is supported by our findings that 1) urea and creatinine was markedly elevated in hemodialysis patients compared to controls and 2) there was a strong positive correlation between urea and creatinine and homocysteine **(Guerra-Shinohara et al., 2007; Lu et al., 2011 and Hassan et al., 2012).**

5.4 Serum urea and creatinine concentrations of the study population

The average concentrations of serum urea and creatinine were found to be markedly elevated in cases compared to controls. It is known that urea and

creatinine are markers of the kidney function **(Depra Manzella, 2008)**. Consequently, the present result is logic as the kidney function is almost ceased in hemodialysis patients. Such findings are in agreement with that previously reported in ESRD patients **(Goldwasser et al., 1997; Iseki et al., 2004; Depner, 2005; Li et al., 2009; Bmbch et al., 2010; Bhavsar et al., 2011 and Vartia, 2013).** Person's correlation test showed significant positive correlations of homocysteine with urea and creatinine. This implies that hyperhomocysteinemia is linked to kidney function and that homocysteine could be a predictive biomarker of ESRD. The recorded positive correlation of homocysteine with urea and creatinine was previously reported **(Hoffer et al., 2000; Guerra-Shinohara et al., 2007; De Almeida et al., 2011 and Abu Nada, 2012).**

5.5 Hematological profile of the study population

White blood cell count and platelet count were significantly increased in hemodialysis patients compared to controls. Leukocytosis recorded in the present study is in concurrent with that obtained by **Reddan et al. (2003); Nasri, (2007); Wei Hsu et al. (2010) and Molnar et al. (2011).** It is known that hemodialysis patients suffer inflammation which is associated with increase in WBCs **(Nasri and Bradran, 2006 and Nasri, 2006; Afshar., 2009 and Molnar et al., 2011).** In addition, **Wei Hsu et al. (2010)** pointed out that WBC count was closely associated with index of inflammation. When related to homosycteine level, results revealed that the higher the homosycteine, the higher the WBC count. This positive correlation between homosycteine level and WBC count was reported by **Ventura et al. (2004); Nasri and Baradaran, (2005) and Guerra-Shinohara et al. (2007).**

Regarding blood plateletes, there was a significant increase in the mean platelet count in hemodialysis patients compared to controls. Such finding is in agreement with that demonstrated by **Nasri. (2006); Molnar et al. (2011) and Alghythan and Alsaeed. (2012).** A significant positive correlation of platelet count with serum homocysteine was found. Similar result was obtained by

nasri (2006) who reported that in hemodialysis patients high homocystiene levels make the platelets more likely to clump and cause clots and contributes to the possibility of thrombotic events among these patients.

Red blood cell count, hemoglobin, hematocrit and MCH values were significantly lower, whereas MCHC was significantly higher in hemodialysis patients compared to controls. This indicates that hemodialysis patients are more likely to be anemic. Such results are inagreement with that reported in earlier studies **(Besarab et al., 1998; Afshar et al., 2009; Anees et al., 2010 Mohsin et al., 2010; Suresh et al., 2012 and Poudel et al., 2013).** Anemia in hemodialysis patients may be due to many factors including blood loss, shortened red cell life span, vitamin deficiencies, the "uremic milieu," renal erythropoietin deficiency due to kidney failure, iron deficiency, and inflammation **(Nurko, 2006; Locatelli et al., 2007 and Anees et al., 2010).** In addition, **Shittu et al. (2013)** reported that hematological parameters are commonly affected in ESRD. Of all the parameters, red cell indices are the ones commonly and severely affected. This is because as high as 90% of erythropoietin is produced in the juxta glomerular apparatus of the kidney while 10% are produced in the liver and other organs. The severity of effect depends on the stage of renal failure. Person's correlation test showed negative significant correlations of homocysteine with RBC count, hemoglobin, hematocrit and MCH values wherase MCHC exhibited a positive significant correlation with homocysteine. Such correlations were previously obtained by **Bachmann et al. (1995); Nasri and Baradaran, (2005); Anees et al. (2010) and Poudel et al. (2013),** and reinforced the idea that homocysteine is a suitable biomarker of ESRD, where most patients suffered hematological disorders.

5.6 Hemostasis parameters of the study population

The mean PT and INR were significantly increased in hemodialysis patients compared to controls. In contrast, the mean APTT was significantly decreased in hemodialysis patients. Such results are in agreement with that found by **Brophy et al. (2006); Ali et al. (2008) and Park et al. (2010).** This means that hemostasis process is impaired in hemodialysis patients. **Jalal et al. (2010)** reported that disorders of hemostasis are associated with chronic kidney disease. The hemostasis abnormalities in ESRD involved intrinsic and extrinsic pathways in which there are a defect of coagulation factors and platelets dysfunction **(smits 2000; Boccardo et al., 2004; Małyszko et al., 2005; Kaw and Malhotra, 2006; Rios et al., 2010 and Mannucci and Tripodi, 2012).** Person's correlation test showed positive significant correlations of homocysteine with PT and INR, whereas negative significant correlation was recorded between homocysteine and APTT. Similar results were reported by other authors **(Shemin et al., 1999; O'shea et al., 2003; Nasri, 2006. and Özkan and Ulusoy, 2013)**. Possible interactions of homocysteine with endothelial cells, blood platelets, plasmatic fibrinogen and plasminogen, as the important major components of hemostasis were discussed **(Karolczak and Olas, 2009).**

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. End stage renal disease was more prevalent among lower educated and unemployed individuals, families with low income as well as among individuals with family history of the disease.

2. Clinical data showed that hypertension and diabetes are the most common self-reported disorders among the hemodialysis patients.

3. The mean levels of homocysteine were significantly higher in hemodialysis patients compared to controls.

4. The average levels of urea and creatinine were significantly higher in cases compared to controls.

5. White blood cell count, MCHC and platelete count were significantly increased in cases compared to controls, whereas RBC count, hemoglobin, hematocrit and MCH showed a significant decrease in cases.

6. Prothrompin time and INR were significantly increased whereas APTT was decreased in cases compared to controls.

7. Homocysteine levels were higher among lower educated and unemployed individuals, families with low income as well as among individuals with family history of ESRD.

8. Homocysteine levels were positively correlated with urea, creatinine, WBC count, MCHC and platelet count, whereas negative correlations were found between homocysteine and RBC count, hemoglobin, hematocrit and MCH values.

9. Homocysteine levels showed positive correlations with PT and INR and negative correlation with APTT.

6.2 Recommendations

1. Introducing of homocysteine as a prognostic test for ESRD patients in Gaza hospitals and clinics is highly recommended.

2. Frequent monitoring of homocysteine levels particularly in hypertensive and diabetic individuals as well as individuals with family history of ESRD.

3. Launching of health education programs on risk factors contributing to ESRD particularly in hypertensive and diabetic individuals.

4. Further research on the relation of homocysteine with clotting factors and the role of homocysteine in fibrinolysis is needed.

Chapter 7

References

Abu Nada A. (2012): Homocysteine levels in chronic kidney disease patients in Gaza Governorate, Gaza Strip. [Master Thesis] The Islamic University of Gaza, Gaza, Palestine p:85.

Abo Shamala H. (2008): Parathormone, calcium and phosphorus levels in hemodialysis Patients at Al-Shifa Hospital, Gaza-Palestine. [Master Thesis] P:84.

Abraham M., Joell Y.N. and Leslle C. (2010): The Homocysteine hypothesis, still relevant to the prevention and treatment of cardiovascular disease? Cleveland Clinic Journal of Medicine 77 (12): 911-918.

Afshar R., Sanavi S., Salimi J. and Ahmadzadeh M. (2009): Hematological profile of chronic kidney disease patients (Ckd) in Iran, in predialysis stages and after initiation of hemodialysis. Saudi J Kidney disease transplant 20(1): 368-371.

Alghythan K and Alsaeed H. (2012): Hematological changes before and after hemodialysis. Academic Journals 7 (4): 490-497.

Ali M., Babiker M., Merghani L., Ali F. and Abdulmajeed M. (2008): Hematological changes post-hemo and peritoneal dialysis among renal failure

patients in sudan. Saudi journal of kidney diseases and transplantation 19(2): 274–279.

Al-Obaidi M.K., Stubbs P.J., Amersey R and Noble M.I.M. (2001): Acute and convalescent changes in plasma homocysteine concentrations in acute coronary syndromes. Heart 85:380–384.

Anderson S., Halter JB., Hazzard WR., Himmelfarb J., Horne FM and Kaysen GA. (2009): Prediction, progression, and outcomes of chronic kidney disease in older adults. Journal of the American Society of Nephrology 20:1199–1209.

Anees M., Mumtaz A., Ibrahim M., Shaheen SM and Asghar A. (2010): Effect of anemia and hyperhomocysteinemia on mortality of patients on hemodialysis. Iranian Journal of Kidney Diseases 4 (1) 60-65.

Backestrom C. and Hursh-Cesar G. (1981): Survey research, 2nd ed. london: Macmillan 128-140.

Bachmann J., Tepel M ., Raidt H., Riezler R., Graefe U., Longer K. and Zidek W. (1995): Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. Journal of the American Society of Nephrology.; 6:121-125).

Barrett E, Barman M, Boitano S and Brooks L. (2010): Ganong's Review of Medical Physiology, 23rd ed. McGraw Hill, Boston.

Baura G. (2012): Hemodialysis delivery system. Medical device technologies p: 193-216.

Besarab A., Bolton K., K. Browne J., Egrie J., Nissenson A., Okamoto D., Schwab S. and Goodkin D. (1998): The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. The New England Journal of Medicine 339 (9): 590-584.

Bhavsar N., Lawrence J., Appel L., Kusek J., Contreras G., Bakris G., Coresh J. and Astor B.(2011): Comparison of measured GFR, serum creatinine, cystatin C, and beta-trace protein to predict ESRD in african americans with hypertensive CKD. Am J Kidney Dis.;58 (6):886-893.

Bmbch C., Howie A., Nightingale P., Mbchb S, Adu D, MD,1 FMedSci C and Mbchb P. (2010): Prediction of ESRD in Pauci-immune necrotizing glomerulonephritis: Quantitative histomorphometric assessment and serum creatinine. American Journal of Kidney Diseases, 55 (2): 250-258.

Bostom A., Shemin D., Lapane K, Millerd J., Sutherland P, Nadeaud M, Seyoumd E, Hartmand W, Priord R, Wilson P and Selhubd J. (1995): Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: a case-control study. Atherosclerosis 114 : 93-103.

Boccardo P., Remuzzi G. and Galbusera M. (2004): Platelet dysfunction in renal failure. Seminars in thrombosis and hemostasis 30 (5): 579-589.

Brosnan J. T. (2004): Homocysteine and cardiovascular disease, interactions between nutrition, genetics and lifestyle. Canadian Journal of Applied Physiology, 29 (6): 773-780.

Brophy D., Martin E., Gehr T., Best A. Paul K and Carr J R M. (2006): Thrombin generation time is a novel parameter for monitoring enoxaparin therapy in patients with end-stage renal disease. Journal of thrombosis and hemostasis, (4): 372‑376.

Cabarkapa V., Stosi Z., Zeravica R. and Ilinci B. (2007): The importance of homocysteinemia measurement in chronic renal failure. Medici ski Preged Journal: 60 (2): 81-83.

Champe P.C. and Harvey R.A. (2008): Biochemistry. Lippincott's Illustrated Reviews 4th ed. Lippincott Williams and Wilkins.

Chao Wu C., Zheng C., Lin Y., Lo L., Liao M and Cheng Lu K. (2012): Role of homocysteine in end-stage renal disease. Clinical Biochemistry 45: 1286–1294.

Chau KF., Chak WL., Wong Mk., Choi KS., Wong KM., Chan YH., Wong HS., Cheung CY. and Li CS. (2003): Rehabilitation of Patients with End-stage Renal Disease. Medical Section, Vol. 8 No. 2. 3-13.

Chou S.T, Ko L.E., Lim P.S., Huang J.L. and Yang C.S. (2000): Effect of age and sex on plasma total homocysteine in Taiwanese. Chinese Journal of Physiology 43(4): 159-164.

Chung SH., Noh H., Ha H and Lee HB. (2009): Optimal use of peritoneal dialysis in patients with diabetes. PDI 29 (Suppl 2):S132–134.

Coldea L., Teodora Mocan., Marc Gatfosse., Silvia Lupu and Dan L. (2011): Plasma Homocysteine and the severity of heart failure in patients with previous myocardial infarction. Cardiology Journal 18 (1) 55-62.

Crawford W and Lerma V. (2008): Treatment options for end stage renal disease. Prim Care 35:407–432.

Cueto-Manzano M and Rojas-Campos E. (2007): Status of renal replacement therapy and peritoneal dialysis in mexico. Peritoneal Dialysis International, Vol. 27, pp. 142–148.

De Almeida C., Guerra D., Vannucchi M., Geleilete T., Vannucchi H. and Chiarello P. (2011): What Is the Meaning of Homocysteine in Patients on Dialysis?. Journal of Renal Nutrition 21 (5): 394–400.

Dennis L., Anthony S. and Dan L. (2005): Style Manual Used: American Psychological Association, 5 th edition. p:148.

Depner T. (2005): Hemodialysis adequacy: Basic essentials and practical points for the nephrologist in training. A.Hemodialysis International vol. 9 issue 3. P: 241-254.

Depra Manzella N. (2008): Kidney disease in diabetes, http://diabetes.about.com/od/preventingcomplications/p/kidneydisease.htm 21 March. 2013.

Etemadi J., Zolfaghari H., Firoozi R., Ardalan M.R., Toufan M., Shoja M.M. and Ghabili K. (2012): Unexplained pulmonary hypertension in peritoneal dialysis and hemodialysis patients. Elsevier España, Rev Port Pneumol.;18 (1):10—14.

Faratro, R., D'Gama, C., Fung, S., and Tantalo R. (2004): Nocturnal home hemodialysis clinic: Home hemodialysis manual, Toronto, Ontario.

Fresenius Medical Care report. (2011): Global View of Hemodialysis Patients. End stage renal disease patients in 2011. 61346 Bad Homburg v. d. H. Germany.

Friedman N., Bostom G., Selhub J., Levey A.S.and Rosenberg I.H. (2001): The kidney and homocysteine metabolism. Journal of American Society of Nephrology 12(10): 2181-2189.

Friedman A., Bostom A., Laliberty P., Selhub J. and Shemin D. (2003): The Effect of N-Acetylcysteine on Plasma Total Homocysteine Levels in Hemodialysis: A Randomized, Controlled Study. American Journal of Kidney Diseases, 41 (2): 442-446.

Ganesh A and Lee K. (2011): Management of chronic kidney disease and End-stage renal disease in diabetes. Faculty of medicine, university of Calgary, UBCMJ 3(1) 13-15.

Gill JD., Tonelli M., Johnson N., Pereira BJ. (2004): Why do preemptive kidney transplant recipients have an allograft survival advantage? Transplantation (78):873–879.

Goldwasser P., Aboul-Magd A. and Maru M. (1997): Race and creatinine excretion in chronic renal insufficiency. American Journal of Kidney Diseases, 30(1): 16-22.

Graham, I.M., Daly L.E., Refsum H.M., Robinson K., Brattstrom L.E., Ueland P.M., Palma R.J., Boers G.H., Sheahan R.G. and Israelsson B. (1997): Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 277: 1775-178.

Guyton A.C. and Hall J.E. (2011): Textbook of Medical Physiology, 12th Edition, Saunders, Philadelphia, USA.

Guerra-Shinohara E., Morita O., Pagliusi R., Avila V., Allen R. and Stabler S. (2007): Elevated serum S-adenosylhomocysteine in cobalamin-deficient megaloblastic anemia. Metabolism Clinical and Experimental (56): 339– 347.

Hankey G.J. and Eikelboom J.W. (1999): Homocysteine and vascular disease. US National Library of Medicine National Institutes of Health, Page :407-413.

Hartmann A., Aasebø W., Jenssen T. (2009): Predictors of anemia in patients on hemodialysis. Hemodialysis International 13:335–339.

Hassan S., Kristal B., Khazim K., Hassan F., Hassan D. and Hassan K. (2012): Do we have a biocompatible peritoneal dialysis fluid? Open Journal of Nephrology (2): 29-34.

Herzog C., Asinger R., Berger A., Charytan D., Dı´ez J., Hart R., Eckardt K., Kasiske B., McCullough P., Passman R., DeLoach S., Pun P. and Ritz E.

(2011): Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). International Society of Nephrology.10.1038/ki .223.

Hoffer L., Bank L., Hongsprabhas P., Shrier I., Saboohi F., Davidman M., . Bercovitch D. and Barr P. (2000): A tale of two homocysteinesmand two hemodialysis units. Metabolism, 149 (2): 215-219.

Holley JL. (1999): Acute complications during hemodialysis. Up to Date Inc.

Hyman C. (2006): Obesity is a risk factor for kidney failure. Journal of the American Medical Association (JAMA) 27(5) :67 – 71.

Iseki K., Kinjo K., Iseki C. and Takishita S. (2004): Relationship between predicted creatinine clearance and proteinuria and the risk of developing ESRD in okinawa, Japan. American Journal of Kidney Diseases, 44(5): 806-814.

Iseki k., Ikemiya Y., Iseki C. and Takishita S. (2003): Proteinuria and the risk of developing end-stage renal disease, Kidney Int. vol. 63 issue 4, p: 1468-1474.

Jalal D., Chonchol. M. and Targher G. (2010): Disorders of hemostasis associated with chronic kidney disease. Seminars in thrombosis and hemostasis 36 (1): 34-40.

Jonathan J. (2010): Anemia and chronic kidney disease: What's the connection? Journal of Family Practice, Vol. 59 Issue 1, p:14.

Karolczak K and Olas B. (2009): Mechanism of action of homocysteine and its thiolactone in hemostasis system. Physiol. Res. (58): 623-633.

Kaw D. and Malhotra D. (2006): Platelet dysfunction and end-stage renal disease. ision of Nephrology, Department of Medicine, Medical University of Ohio, Toledo, Ohio, Vol. 19, No. 4 P: 317–322.

Khanam S., Begum N., Begum S. and Hoque EAM. (2007): Changes in hematological indices in different stages of chronic renal failure. J Bangladesh Soc Physiol (2): 38-41.

Khattak M., Sandhu GS., Desilva R. and Goldfarb-rumyantzev AS. (2012): Association of education level with dialysis outcome. International Society for Hemodialysis, (16): 82–88.

Kong X., Liu L., Zuo L., Yuan P., Li Z., Li W., Cai M., Chen X., Jiang A., Long G., Xu J., Lin H., Wang S., Huang W., Wang Y., Guo Y., Cao P., Wu H., Jia Q., Zhang L., Wang M. and Wang H. (2013): Association between family members of dialysis patients and chronic kidney disease: a multicenter study in China. BMC Nephrology 14:19.

Khurelbaatar M and Huang S. (2006): Homocysteine, dietary pattern and cardiovascular disease among middle aged women in mongol. International Health Program, Institute of Public Health School of Medicine, National Yang Ming University. Master Thesis.

Koning L and Hu F. (2010): Homocysteine lowering in end stage renal disease : Is there any cardiovascular benefit?. American Heart Association (121):1379-138.

Krueger L. (2007): Wisconsin hmong experiences with hemodialysis. M.S. Thesis, University of Wisconsin-Stout.

Lawrence M., Maxine A., Stephen J. (2003): Current medical diagnosis and treatment,42 nd ed. Lange Medical Books :867-902.

Levey S., Eckardt U., Tsukamoto Y., Levin A., Coresh J., Rossert J., De

Zeeuw D., Hostetter T.H., Lameire N. and Eknoyan G. (2005): Definition and

classification of chronic kidney disease: a position statement from Kidney

Disease: Improving Global Outcomes （KDIGO）. Kidney International 67:

2089-2100.

Li H., Zhang X ., Xu G., Wang X. and Zhang C. (2009): Determination of reference intervals for creatinine and evaluation of creatinine-based estimating equation for Chinese patients with chronic kidney disease. Clinica Chimica Acta (403): 87–91.

Lovci V., Vuji J., Basi -Juki N., Kurtovi I., Jankovi RI., Lovci P. and Dzapo M. (2011): Treatment of renal anemia in hemodialysis patients in General Hospital Bjelovar from 2007 to 2010. Vol. 65 Suppl. 3 p: 49-53.

Locatelli F and Canaud B. (2012): Dialysis adequacy today: a European perspective. Nephrology Dialysis Transplantation 27:8, p: 3043-3048.

Locatelli F., Pozzoni P. and Del Vecchio L. (2007): Recombinant Human Epoietin beta in the Treatment of Renal Anaemia. Ther Clin Risk Manag. 3(3): 433-9.

Lubos E., Lasclazo J., and Handy D. (2007): Homocysteine and glutathione peroxidase-1. Antioxid. Redox. Signal , 1923-1940.

Ma Z., Ebben J., Xia H., Collins AJ. (1999): Hematocrit level associated mortality in hemodialysis patients. J Am Soc Nephrol 10:610–619, 1999.

Mader S. (2004): Mader understanding human anatomy & physiology,5th ed. 323-340.

Mahmoodreza K., Mohammad E., Delaram K., Yalda H. and Sofla Raheleh AA. (2009): The Effects of Family Income and Educational Status on Children's Pattern of End-Stage Renal Disease in Mashhad. Medical Science Journal Islamic Azad University-Mashhad Branch, 4(4): 243-250.

Marieb E. (2003): Essentials of human anatomy and physiology,7th ed.USA p:479-501.

McClellan W., Speckman R., McClure L., Howard V., Campbell R.,Cushman M., Audhya P., Howard G. and Warnock DG. (2007): Prevalence and characteristics of a family history of end-stage renal disease among adults in

the united states population: reasons for geographic and racial differences in stroke (REGARDS) renal cohort study. J Am Soc Nephrol (18): 1344–1352.

Ministry of Health (MOH). (2010): Health annual report, Health Status in Palestine 2009,. Ministry of Health Nablus – Palestine.

Muhaisen M., Sharif F. and Yassin M. (2012): Risk factors of cardiovascular disease among children with chronic kidney disease in Gaza strip. Journal of Cardiovascular 3(2):91-8.

Małyszko J., Małyszko JS., My liwiec M. and Buczko W. (2005): Hemostasis in chronic renal failure. Annales academiae medicae bialostocensis (50): 126-131.

Mannucci P and Tripodi A. (2012): Hemostatic defects in liver and renal dysfunction. American society of hematology P:168-173.

Morioka T., Emoto M., Tabata T., Shoji T, Tahara H., Kishimoto H. (2001): Glycemic control is a predictor of survival for diabetic patients on hemodialysis. Diabetes Care 24(5):909–913.

Marticorena R., Ly J., Donnelly S. (2004): Red blood cell survival in chronic renal failure. Am J Kidney Dis 44:715–719.

Malinow M., Bostom A., and Krauss R. (1999): Homocyst(e)ine, Diet, and Cardiovascular Diseases. A statement for healthcare professionals from the nutrition committee, American Heart Association. Circulation , 178-182.

Maron B. and Loscalzo J. (2009): The treatment of hyperhomocysteinemia. Annu. Rev. Med., 39-54.

Maron B. and Loscalzo J. (2007): Should hyperhomocysteinemia be treated in patients with atherosclerotic disease? Curr. Atheroscler. , 375-383.

Mohsin S, Aslam M, Anees M., Hussain S, Ahmed T and Qamar U. (2010): Platelets dysfunction in patients of end stage renal disease. Biomedica 26 (14): 157 – 161.

Molnar M., Streja E, Kovesdy C., Budoff M., Nissenson A, Krishnan M. and Anker s., Norris k., Fonarow G. and Kalantar-Zadeh K. (2011): High platelet count as a link between renal cachexia and cardiovascular mortality in end-stage renal disease patients. Am J Clin Nutr (94): 945–54.

Nasri H and Bradran A. (2006): Association between white blood cell count and levels of serum homocysteine in end-stage renal failure patients treating with hemodialysis. Department of Biochemistry, Center of Research and Reference Laboratory of Iran, Hospital Bu Ali, Tehran J Ayub Med Coll Abbottabad ;18 (1).

Nasri H and Baradaran A. (2005): Association of serum homocysteine with anemia in maintenance hemodialysis patients. Pakistan Journal of Nutrition 4 (6): 414-417.

Nasri H. (2006): Influence of serum homocysteine on platelet count in stable hemodialysis patients. Pak J Physiol 2(2).

Nasri H. (2007): Association of white blood cell count with left ventricular hypertrophy and ejection fraction in stable hemodialysis patients. Saudi J Kidney disease transplant 18 (1): 31-63.

National Kidney Foundation (NKF). (2002): K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. American Journal of Kidney Disease 39 (2 Suppl 1): S1-266.

Newman D.J. and Price C.P. (1999): Renal function and nitrogen metabolites.

In: Burtis C.A, Ashwood E.R, (editors) Text book of clinical chemistry. 3rd edition, Philadelphia: W.B Standers Company. pp 1204-1207.

Nurko S. (2006): Anemia in chronic kidney disease Causes, diagnosis, treatment. Cleveland clinic journal of medicine Vol. 73, No. 3, p:289.

Oishi K., Nagake Y. and Yamasaki H. (2000): The significance of serum homocysteine levels in diabetic patients on haemodialysis. Nephrol Dial Transplant 15:851–5.

O'shea, S.I., Lawson, J.H., Reddan, D., Murphy, M. & Ortel, T.L. (2003): Hypercoagulable states and antithrombotic strategies in recurrent vascular access site thrombosis. J Vasc Surg, 38(3): 541-548.

Özkan G and Ulusoyin S. (2013): Hemodialysis. Chapter 4, Bleeding Diathesis in Hemodialysis Patients.

Palestinian Clinical Laboratory Test Guide, PCLTG, 2005. Ministry of health.

Park Y., Waldrum M. and Marques M. (2010): Platelet Count and Prothrombin Time Help Distinguish Thrombotic Thrombocytopenic Purpura–Hemolytic Uremic Syndrome From Disseminated Intravascular Coagulation in Adults. American society for clinical pathology (133): 460-465.

Paterson J. (2011): Prevalence of hyperhomocysteinemia in patients with predialysis chronic kidney disease after folic acid food fortification of the canadian food supply. Department of Nutritional Sciences University of Toronto p:133.

Perla-Kajan, J., Twardowski, T., & Jakubowski, H, (2007): Mechanisms of homocysteine toxicity in humans amino acids. The Journal of The Federation of American Societies For Experimmental Biology: p 561-572.

Personal Communication. (2013): Ministry of Health, Hemodialysis Unit at Al-Shifa Hospital, Gaza Strip.

Poudel B., Kumar Yadav B., Jha B., Raut K. and Pandeya D. (2013): Prevalence and association of anemia with CKD: A hospistal based crosssectional study from Nepal. Biomedical Research; 24 (1): 99-103.

Portolés J., Gorriz JL., Rubio E., de Alvaro F., García F., Alvarez-Chivas V., Aranda P. and Martinez-Castelao A. (2013): The development of anemia is associated to poor prognosis in NKF/KDOQI stage 3 chronic kidney disease. BMC Nephrol, 7; 14(1):2.

Qi Z., Hoffman G., Kurtycz, D. and Yu J. (2003): Prevalence of the C677T substitution of the methylenetetrahydrofolate reductase (MTHFR) gene in

Wisconsin. Genetic In Medicine :Official Journal of The American College of Medical Genetics; 5(6):458-467.

Reddan D., Klassen P., Szczech L., Coladonato J., O'Shea S., Owen Jr W. and Lowrie E. (2003): White blood cells as a novel mortality predictor in hemodialysis patients. Nephrol Dial Transplant (18): 1167–1173.

Refsum H. (2002): Clinical Laboratory News May. 2-14.

Rios DRA., Carvalho MG., Lwaleed BA., Silva ACS., Borges KBG., Duss LMS. (2010): Hemostatic changes in patients with end stage renal disease undergoing hemodialysis. Elsevier 411: 135–139.

Sajadi A., Farmahini Farahani B., Esmaeilpoor Zanjani S., Durmanesh B. and Zare M. (2010): Factors affecting fatigue in chronic renal failure patients treated with Hemodialysis. Iranian Journal of Critical Care Nursing Spring, (3) (1):33-38.

Sandra W (2005): Protecting renal function in people with diabetes. The British of journal primary care nursing 1(4): 18.

Selhub, J, (1999). "Homocysteine metabolism". Annual Review of Nutrition 19: 217–246.

Shemin D., Lapane K., Bausserman L., Kanaan E., Kahn S., Dworkin L. and Bostom A. (1999): Plasma total homocysteine and hemodialysis access thrombosis: A prospective study. J Am Soc Nephrol (10): 1095–1099.

Shipchandler M.T., Moore E.G. (1995): Rapid, fully automated measurement of plasm homocysteine with the Abbott IMx analyzer. Clin Chem. 41:991.

Shishehbor M., Oliveira L., Lauer M., Sprecher D., Wolski K., Cho L., Hoogwerf B. and Hazen S. (2008): Emerging cardiovascular risk factors that account for a significant portion of attributable mortality risk in chronic kidney disease. Am J Cardiol 101 (12): 1741–1746.

Shojaei M., Djalali M., Siassi F., Khatami M., Boroumand M. and Eshragian M. (2009): Serum levels of lipoprotein(a) and homocysteine in patients on hemodialysis who take hydroxymethylglutaryl- coA reductase inhibitors, vitamin B6, and folic acid. Iranian Journal of Kidney Diseases 3:141-144.

Sjöberg B., Anderstam B., Suliman M. and Alvestrand A. (2006): Plasma reduced homocysteine and other aminothiol concentrations in patientsWith CKD. American journal of kidney diseases, 47 (1): 60-71.

Shittu A., Chijioke A., Biliaminu S., Makusidi A., Sanni M., Abdul-Rahman M. and Abdul-Azeez I. (2013) : Haematological profile of patients with chronic kidney disease in Nigeria. Journal of Nephrology and Renal Transplantation (JNRT) 5(1): 2 –10.

Smeltzer C. and Bare B. (2004): Brunner & Suddarth's textbook of medical-surgical nursing. (10th ed.) Philadelphia: USA: Lippincott Williams & Wilkins.

Smits J., Linden J., Blankestijn P. and Rabelink T. (2000): Coagulation and hemodialysis thrombosis. Nephrology dialysis transplantation (15): 1755-1760.

Soyibo K and Barton N. (2007): Caribbean renal registry data. West Indian Medical Journal 5(3):45-51.

Stam F., Van Guldener C., M ter Wee P., Jakobs C., De Meer K. and Stehouwer C. (2005): Effect of folic acid on methionine and homocysteine metabolism in end-stage renal disease. Kidney International (67): 259–264.

Suresh M., reddy M N., Singh SM., Hari Krishna BandiHK., keerthi SG., Chandrasekhar M. (2012): Hematological changes in chronic renal failure. International Journal of Scientific and Research Publications 2(9).

Takenaka T., Itaya Y. and Suzuki H. (2005): Young hemodialysis patients are exposed to hyperhomocysteinemia. Journal of renal nutrition, 15(4): 435-440.

The Kidney Foundation of Canada. (2004): Hemodialysis. Retrieved November 8, 2006.

Webpage: http://www.kidney.ca/page.asp?intNodeID=22130.

Thibodeau G and Patton K. (1999): Anatomy and physiology, urinary system, chapter 28, fourth edition, Mosby, 823-825.

Turkmen K., Guney I., Yerlikaya FH. and Tonbul HZ. (2010): The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail; 34(2):155-9.

Tsai JC., Chen SC., Hwang SJ., Chang JM., Lin MY. and Chen HC. (2010): Prevalence and risk factors for CKD in spouses and relatives of hemodialysis patients. Am J Kidney Dis, 55 (5): 856–866.

Ueland Per m., Mansoor Azam Anhe B. Guttormsen Fredrik Mãoeller, Pal Aãoekrust, Helga Refsãoem and Asbj0rn M. Svarda. (1993): Colloquium: Homocyst(e)ine, vitamins and arterial occlusive diseases. The journal of nutrition.

Van Guldener C. (2006): Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? Nephrol Dial Transplant 21: 1161–1166.

Van Guldener C., Frank S., and Coen D. A. S. (2005): Hyperhomocysteinaemia in chronic kidney disease: focus on transmethylation Clin Chem Lab Med 43(10):1026–1031.

Van Guldener C., Wim K.R.B., Denise A., Cornelis J., Dirk-J., Donker., Ab J.M., Coen D.A and Kees D.e. (1999): Homocysteine and methionine metabolism in ESRD: A stable isotope study Kidney International 56, 1064–1071.

Vartia A. (2013): Urea concentration and hemodialysis dose. Hindawi publishing corporation. Article ID 341026.

Ventura P., Panini R., Tremosini S., Salvioli G. (2004): A role for homocysteine increase in haemolysis of megaloblastic anaemias due to vitamin B12 and folate deficiency: results from an in vitro experience. Biochimica et Biophysica Acta 1739: 33– 42.

Vieira C., Baptista A., Malho A., Pinho A., Silva A.P., Bernardo I.C. and Neves P.L. (2010): Homocysteine is a risk factor in predialysis patients when associate with malnutrition and inflammation. International Journal of Nephrology: p1-4.

Vijayakumar M., Nammalwar B.and Prahlad N. (2007): Prevention of chronic kidney disease in children. Indian journal of nephrology, 17 (2): 47-52.

Wei Hsu C.,Lin J., Lin-Tan D.,Yen T. and Chen K. (2010): White blood cell count predicts all-cause, cardiovascular disease-cause and infection-cause one-year mortality of maintenance hemodialysis patients. Therapeutic Apheresis and Dialysis 14 (6):552–559.

White S.L., McGeechan K. and Jones M. (2008): Socioeconomic disadvantage and kidney disease in the United States, Australia, and Thailand. Am. J. Public. Health, (98): 1306–13.

Whitworth A. (2005): Progression of renal failure – The role of hypertension. Annals academy of medicine Vol. 34 No. 1.

Wolfe RA., Hulbert-Shearon TE, Ashby VB., Mahadevan S., and Port FK. (2005): Improvements in dialysis patient mortality are associatedWith improvements in urea reduction ratio and hematocrit, 1999 to 2002. American Journal of Kidney Diseases, 45(1): 127–135.

World Health Organization (2000): Obesity: Preventing and managing the global epidemic. Report of WHO, consultation on obesity. Technical report series,894.

Zadeh K. And Aronoff R. (2009): Hemoglobin variability in anemia of chronic kidney disease. Journal of the American Society of Nephrology 20(3):479-87.

الجامعةالإسلامية –
كلية العلوم

Questionnaire (HD patients)

A. Sociodemographic data:

1. Patient name:---
2. Patient No : --
3. Age (Year):---
4. Gender: Male   Female
5. Marital status: Single   Married   Widowed   Divorced
6. Education: University   Secondary school   Preparatory school
    Primary school   Illiterate
7. Employment: Yes   No
8. Family history: Yes   No
9. Income/month:<1000   1000-2000   > 2000 NIS
10. Smoking : Yes   No

B. Medical information:

1. Date of diagnosis:---
2. When did you start hemodialysis? --
3. How many times you receive hemodialysis per week? ---------------------
4. Do you complain any other disease?   Yes   No
    If yes mention it: --

Questionnaire (Healthy control)

A. Sociodemographic data:

1. Patient name:--
2. Patient No : ---
3. Residence: Gaza North   Gaza   Mid Zone   Gaza South
4. Age (Year): --
5. Gender: Male   Female
6. Marital status: Single   Married   Widowed   Divorced
7. Education: University   Secondary school   Preparatory school Primary school   Illiterate
8. Employment: Yes   No
9. Family history: Yes   No
10. Income/month:<1000 1000-2000 > 2000 NISincome:
11. Smoking: Yes   No