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HYPERHOMOCYSTEINEMIA: AN UNKNOWN HIGH RISK FACTOR

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Abstract

The Homocysteine (Hcy) is a non-proteinogenic sulfur amino acid that results from methionine demethylation. Nutritional deficiency or genetic abnormalities of Hcy metabolism cofactors leads to hyperhomocysteinemia (hHcy) which is considered as an independent risk factor in the occurrence of several pathologies. The aim of this study is to determine the prevalence of hHcy in a general population in order to compare the relationship of Hcy betweenage, sex and its association with different pathologies in patients. The study involved a population of 140 patients, according to the age there are 131 adults and 9 children, and according to the gender there are 87 female patients and 53 male patients, recruited from severalservices. Then, the sampling was carried out at the medical biology department at the Ain Taya University Hospital (near Algiers). The patients' plasma Hcy level was measured using the principle of competitive immunodosage and enzyme dosage of Hcy. In Conclusion**,** our results state that the rate of total homocysteine (tHcy) increased with age, higher in men than in women and that this increase is due to metabolic disturbances associatedwith different pathological conditions. It can be said that an increase in tHcy in excess of 15 µmol/l is considered as an independent risk factor for various pathologies, such as venous thromboembolic diseases, stroke or renal failure.

Keywords: Hyperhomocysteinemia, Venous Thromboembolic Diseases, Stroke, Renal Failure.

GENERAL INTRODUCTION

Homocysteine (Hcy) has been the subject of much speculation since its discovery in 1932 by Vincent du Vigneaud **(Vigneaud, 1932; Ganguly and Alam, 2015).** This molecule is not found in the structure of proteins, but constitutes an important intermediate in the function of methyl donor of methionine (Met) and in the metabolism of the latter towards other sulfur-containing amino acids such as cysteine (Cys). **(Gaillard, and al., 2003).**

Nearly four decades later, McCully first associated Hcy with atherothrombosis; his theory was based on observations that children with very high plasma Hcy levels due to a metabolic abnormality had early atherothrombosis and died before the age of 20 **(McCully 1970)**. Then several reports described mental retardation and specific vascular

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lesions (including atherosclerosis, and thromboembolism) in patients with hyperhomocysteinemia (hHcy), leading to its association with these phenomena.

Hcy metabolism is a metabolic network centered on the folate and Met cycles in which single carbon (1-C) groups are transferred, supporting many physiological processes including nucleotide (NT) biosynthesis, amino acid (AA) homeostasis, epigenetic maintenance and redox defense.

Therefore, disruption of Hcy metabolism is associated with common pathologies, such as neurodegenerative diseases, cardiovascular diseases (CVD) and cancer. Any deficiency (genetic or acquired) relating to one of the key enzymes or one of their cofactors and co-substrates will induce a reduction in Hcy catabolism which will result in a more or less significant hHcy **(Caldeira-Araújo and al. 2019).**

It is still unclear whether Hcy is a marker or a causative agent of diseases. Growing research evidence suggests that Hcy is an important indicator for overall health. This opinion represents the current understanding of the molecular mechanism of Hcy and its link with hHcy-related pathologies in humans **(Škovierová and al. 2016).**

The aim of the present study consists of measuring the plasma total Hcy (tHcy) of different subjects in order to link the results obtained to appropriate diagnostics in order to better understand the real effect of this molecule, because these tests are applied on subjects suffering from venous thromboembolism (VTE), patients with stroke, as well as other complications such as kidney failure (KF).

The samples are in the form of frozen blood plasma, which will be analyzed using an immuno-enzymatic technique carried out by the IMMULITE 2000 machine (SIEMENS) and an enzymatic technique carried out by the COBAS INTEGRA 400 plus machine (ROCHE).

Bibliographic Synthesis

Homocystéine (Hcy):

Definition:

Homocysteine “2-amino-4-sulfanyl-butanoic acid” is a non-proteinogenic amino acid, comprising a reducing thiol group whose molecular formula is as follows:

C 4 H 9 N O 2 S. It is an essential intermediate in the intracellular metabolism of Met in mammals. Its molecular mass is approximately 135.19 g/mol.

Its chemical properties showed similarity with cysteine (Cys), hence its name homocysteine **(Ganguly and Alam, 2015).**

Trans-methyation biosynthesis of Met, an essential amino acid, represents the only means by which Hcy is produced in humans **(Škovierová and al. 2016).**

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Hyperhomocysteinemia (hHcy):

Definition:

Hyperhomocysteinemia (hHcy) is defined as a condition characterized by an abnormally high level (greater than 15 µmol/L) of Hcy in the blood **(Guo and al. 2009).** That is, hHcy results from increased levels of intracellular Hcy that is readily released into the extracellular medium (plasma or body fluid) **(Ji and Kaplowitz, 2004).**

Total plasma homocysteine (tHcy) is defined as the pool of free Hcy, Hcy bound to the protein, and Hcy linked to another Hcy molecule or to Cys by a disulfide bond. tHcy is used as a predictive risk factor for cardiovascular disorders, stroke progression, screening for inborn errors of Met metabolism and as an additional test for Vit B12 deficiency **(Škovierová and al. 2016).**

The Biological Reference Interval:

The methods used to determine the Hcy level in different biological samples can be classified into chromatographic methods, enzymatic assays and combined assays (enzymatic reaction followed by immunoassay) **(Škovierová and al. 2016).**

The concentration of tHcy in the plasma of healthy (fasting) humans is low and ranges between [5.0 and 15.0] µmol/L using HPLC, or between [5 .0 and 12.0] µmol/L with immunoassay methods **(Ganguly and Alam, 2015).**

Based on the work carried out, the range of tHcy concentration in the plasma of healthy adults is 5 to 15 µmol/L. The desired upper limit for Hcy concentration should be 10 µmol/L **(Ji and Kaplowitz, 2004).**

The causes of hHcy:

Genetic abnormalities, age, sex, and various nutritional and hormonal determinants contribute to hHcy. However, genetic and nutritional disorders are the main factors **(Ji and Kaplowitz, 2004).**

- The remethylation cycle has three limiting factors: MTHFR, Vit B9 and Vit B12.
- The trans-sulfuration reaction depends on CBS and pyridoxine (Vit B6).

Insufficiency of any of these vitamins or a polymorphism in the genes of any of these enzymes will reduce the rate of Hcy metabolism and cause its accumulation in the blood, i.e. 'hHcy **(Bhargava, 2018).**

Genetic Anomalies:

- MTHFR gene:

It is present on the short arm of chromosome 1 at position 36.3 **(Goyette and al., 1998).** MTHFR is encoded by a 20,328 bp gene, composed of 11 exons. There are 18 known mutations in this gene, but the most common are C677T and A1298C. The minor allele

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frequency for MTHFR C677T and A1298C is 0.15 and 0.44, respectively, indicating greater dominance of the latter polymorphism **(Kumar and al. 2005).**

- CBS gene:

It is present on the long arm of chromosome 21 at position 22.3 **(Munke and al. 1988).** It is 23,678 bp located on chromosome 21q22.3. More than 150 homocystinuria-causing mutations have been identified in this gene, but the most common are T833C, G919A, and G1330A **(Bhargava, 2018).**

The Toxicity of Hcy:

Over the years, different hypotheses focusing on Hcy toxicity have been developed. However, despite the efforts made, none have been able to clearly explain the biotoxicity of Hcy. The three main pathways of Hcy biotoxicity have been reviewed in the literature:

- Homocysteylylation;
- Induction of oxidative stress;
- Excitotoxicity**. (Škovierová and al.2016).**

Homocysteylylation:

Hcy toxicity is proposed as a consequence of the covalent binding of this compound to proteins, followed by the modification of their functions. The process is called “Homocystéylylation”, which is considered a post-translational modification of proteins **(Škovierová and al. 2016).**

“S” homocysteylylation occurs when Hcy binds through its free thiol group to another free thiol group, derived from a Cys residue in a protein molecule and makes the disulfide bond. These modifications have a strong influence on the thiolo-dependent redox status of proteins.

“N” homocysteylylation occurs when Hcy interacts through its amine group with the ε-amino group of a lysine (Lys) residue in a protein and alters the structure and function of the modified protein [L' homocysteylylation in N results from the high reactivity of Hcy-thiolactone (Hcy-TL), the synthesis of which is catalyzed by methionyl-tRNA synthetase (EC.6.1.1.10) in the presence of ATP].

In vivo, Hcy-TL targets and modifies blood albumin, hemoglobin, immunoglobulins, LDL, HDL, transferrin, antitrypsin and fibrinogen. Furthermore, it has been proven that proteins modified by N-homocysteylylation can act as neoantigens, triggering the activation of the inflammatory response which is a key component of atherogenic, atherothrombosis and etiology of stroke **(Škovierová and al., 2016).**

Induction of Oxidative Stress

One of the proposed mechanisms for the deleterious effects of Hcy is its ability to generate reactive oxygen species (ROS), thereby producing oxidative stress which can potentially lead to cardiovascular disease **(Davy and Davy, 2006)**. Different studies have

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shown that redox reactions may be key factors in the development of atherosclerosis, vascular hypertrophy and thrombosis in animals with hHcy **(Škovierová and al. 2016).**

Oxidative stress will appear when there is a profound imbalance between antioxidants and pro-oxidants in favor of the latter. This situation can result from a dysfunction of the mitochondrial chain (ischemia-reperfusion, aging), activation of enzymatic systems (NADPH oxidase, glucose oxidase), release of free iron from chelating proteins (ferritin) or an oxidation of certain molecules (glucose, hemoglobin catecholamines, etc.). Finally, a poor diet low in antioxidants will also contribute to the appearance of oxidative stress **(Pincemail and al. 2002).**

Oxidative stress is generated during the oxidation of the free thiol group of Hcy which binds via a disulfide to plasma proteins, mainly albumin, to other plasma thiols of low molecular mass or to a second molecule of 'Hcy. Hcy increased the production of ROS, they can form hydroxyl radicals capable of stripping electrons from other molecules and inducing the subsequent oxidation of lipids, proteins, carbohydrates and nucleic acids which can lead to a endothelial dysfunction or vessel wall damage, followed by platelet activation and thrombus formation **(Škovierová and al. 2016).**

Excitotoxicity: (Hcy as a neurotoxin)

The toxic effect of Hcy on brain tissue is influenced by the absence of two of the main metabolic pathways for Hcy elimination:

- The remethylation pathway (Hcy to Met) by betaine;
- The trans-sulfuration pathway (Hcy to Cys).

In addition, Hcy acts as an agonist of glutamate receptors:

Overstimulation of these receptors results in increased cytoplasmic calcium levels, increased free radical production, and activation of caspases leading to apoptosis.

Furthermore, hHcy can often lead to intracellular Ca2+ mobilization and endoplasmic reticulum stress, followed by the development of apoptotic events, extracellular matrix (ECM) remodeling in the brain parenchyma, and endothelial dysfunction. In humans, increased Ca2+ levels damage mitochondria, reducing mitochondrial membrane potential and ATP production is suppressed. Furthermore, the consequent leakage of cytochrome c, mitochondria and ROS activates the caspase 3 pathways, which leads to DNA fragmentation, characteristic of apoptosis **(Škovierová and al. 2016).**

The Involvement of Hcy in Diseases

Hcy can be involved in CVD and several other pathologies; it can also damage several organs and tissues **(Fig.1).**

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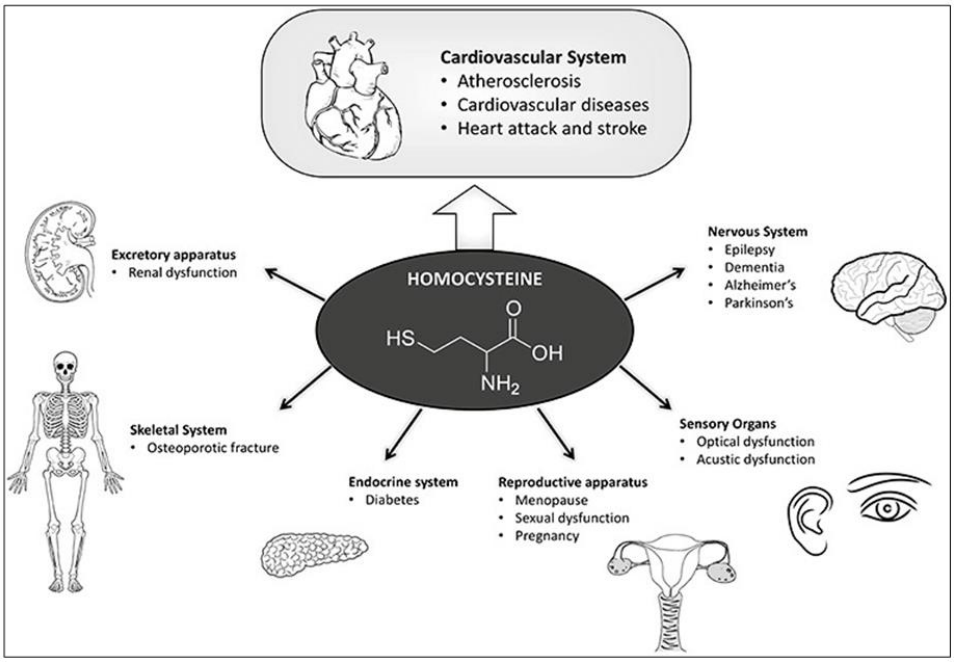

Figure 1: Pathologies in which hHcy has a Role in their Appearance and Development (Tinelli and al. 2019).

Venous Thromboembolic Diseases (VTE):

A large number of studies have shown that hHcy is associated with a risk of venous and arterial thrombosis **(Cattaneo, 2001**). Hcy increases the risk of venous thrombosis **(Guadagnino and al., 1999),** by interfering with the coagulation cascade by promoting the coagulant pathway, by increasing the expression of tissue factor (factor III), factors V and XII, the plasminogen activator inhibitor 1 (PAI-1) gene and the production of thromboxane AII by platelets, thus leading to the formation of thrombin (Rigaud, 1999).The alteration of the metabolism of lipoproteins (mainly LDL) under the action of the thiol groups of Hcy potentiates the atherogenic risk **(McCully and al. 1990).**

Kidney Failure (KF):

The kidney plays a major role in Hcy metabolism (a locus of transsulfidation and remethylation cycles) **(Soria and al. 1990).** It also plays another essential role in the clearance of Hcy, the quantity of Hcy filtered daily is 0.5 MU **(El Mohamed Cherifi and al. 2007).**

Hcy is linked to glomerular filtration rate (GFR), so when kidney function decreases, Hcy values increase (DGF falls below 75 ml/min) **(Tinelli and al. 2019).**

It also appears that renal failure contributes to the inactivation of crucial enzymes in Hcy metabolism, mainly trans-sulfuration and remethylation enzymes, as well as a reduction in sulfate excretion (Cys, Hcy and cysteine-sulfinic acid) could increase tHcy levels **(Kim and al. 2018).**

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The mechanisms behind the accumulation of Hcy during renal failure are not yet known; they may be a vitamin deficiency, metabolism abnormalities or renal elimination of Hcy **(Friedman and al. 2001).**

Stroke:

In people who have a stroke, the blood supply to the brain is interrupted, leading to neuronal cell death and loss of function in specific regions of the brain, which is attributed to a lack of oxygen and nutrients. **(Sacco and al. 1997).**

The main cause of ischemic stroke is arterial athero-thromboembolism. A high Hcy level is both atherogenic and prothrombotic **(Gariballa, 2008).** hHcy is considered a marker of the acute phase of ischemic events, based on an observation which shows an increase in blood Hcy levels in stroke patients **(Tinelli and al. 2019).**

MATERIAL AND METHODS

This work was carried out at the central medical biology laboratory within the Ain Taya hospital establishment near Algiers. Patients are recruited in the sampling room after having completed an information sheet in the reception room, it contains the parameter or recommended analysis “Hcy assay” sent from several departments concerned. Patient files constitute the source of information, their exploitation is carried out on site, because the selection of samples is subject to several criteria to be accepted such as the complete information sheet and the reason for the prescription.

Material

Biological Material:

- For our experiment, we used 140 blood samples from 140 different patients (adults/children, female/male), suffering from various pathologies (VTE, KF, stroke).

Methods

Hcy dosage techniques used:

The quantitative determination of tHcy in plasma constitutes an aid in the diagnosis and monitoring of patients suspected of suffering from hHcy or homocystinuria, it is done by:

- A competitive chemiluminescence immunoenzymatic technique, this test is reserved for in vitro use with the IMMULITE 2000 Homocysteine analyzer.
- An enzymatic assay for Hcy, this test is reserved for in vitro use with the COBAS INTEGRA Homocysteine analyzer.

Most studies use plasma tHcy as a marker, which corresponds to all free and albumin-bound Hcy. It varies with age, sex, medications interfering with folate metabolism, serum creatinine level, level of liver function, and hormonal status in women. Normal fasting homocysteinemia values are between 5 and 12 µmol/L.

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Statistical Analyses

Statistical calculations were carried out with Microsoft Excel pro plus 2019 software and IBM SPSS Statistics software.

RESULTS AND DISCUSSION

Correlation between hyperhomocysteinemia and age:

This study was carried out on a population of 140 patients including 131 adults (p = 94 ± 4%) and 9 children (q = 6 ± 4%) (Tab.1), suffering from various pathologies.

Table 1: Number and Percentage of Adults and Children Accurately

Age	Number of patients	Percentage (%)	Précision (±)
Adults	131	94	4
Children	9	6	4
Total	**140**	**100**	

According to the result of the comparison test between the observed percentages and the theoretical percentages (|ε|cal = 5.25 > 1.96): From a statistical point of view, the difference is highly significant. It seemed that adults were most affected by acquired hHcy.

Correlation between Hyperhomocysteinemia and Sex

This study was carried out on a population of 140 subjects including 87 female patients (p=62%) and 53 male patients (q=38%), see (Tab.2).

Table 2: Number and Percentage of Patients' Gender Accurately

Sex	Number of patients	Percentage (%)	Précision (±)
female patients	87	62	8
male patients	53	38	8
Total	**140**	**100**	

According to the result of the comparison test between the observed percentages and the theoretical percentages (|ε|cal = 1.97 > 1.96): From a statistical point of view, the difference is slightly significant. It seemed that male patients were more affected by hHcy compared to female patients.

The average of the tHcy values was calculated for both sexes, that of female patients is “m1 = 21.93 ± 7.94 µmol/L” and that of male patients is “m1 = 21.93 ± 7.94 µmol/L” and that of male patients is

“m2 = 37.02 ± 12.32 µmol/L”, see (Tab. 3).

Table 3: Accurately Averaged Plasma tHcy of both Sexes

Sex	Average	Précision (±)
female patients	21,93	7,94
male patients	37,02	12,32

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According to the result of the Z test for comparison between two observed means (Z=2.02>1.96): From a statistical point of view, the means of tHcy values are significantly different between the two sexes (m1 < m2), with a risk of error of 0.047 (α=4.7%). It seemed that male patients were most affected by hHcy, which confirms the result of the previous statistical study.

The increase in serum Hcy concentrations is associated with different factors, notably the difference in muscle mass, hormonal status and vitamin status; men appear to have more hHcy than women **(Refsum and al. 2004).** Several authors confirm the male dominance in the prevalence of hHcy, a recent study in hypertensive patients showed that the level of tHcy in women was significantly lower than that in men **(Pang and al. 2019).**

Gender differences were found with a higher mean value in men. These observations are consistent with previously published data; we give the example of a Beninese study which suggests that the average serum Hcy in men was 5.1 µmol/L higher than that in women ($p < 0.001$) **(Mabchour and al. 2010).**

It has been confirmed that Hcy production is linked to creatinine synthesis **(Brattström and al., 1994).** Men generally have greater muscle mass and therefore a greater demand for creatinine biosynthesis, which leads to greater Hcy production **(Rauh and al. 2001).** In addition, the vitamin status of Vit B9, Vit B12 and vitamin B6 are different between the two sexes, which could partly explain this difference **(Refsum and al. 2004).** Most vegetarians develop hHcy and Vit B12 deficiency since the main food source of Vit B12 is meat **(Obersby and al. 2013).**

Il est intéressant de noter que les taux plasmatiques d'Hcy sont plus faibles chez les femmes en âge de la procréation que chez les hommes du même âge, bien qu'ils augmentent après la ménopause, donc on constate que l'Hcy peut être affecté par les concentrations des hormones sexuelles **(Tinelli et al. 2019)**. D'autres hypothèses expliquent la baisse du taux d'Hcy chez les femmes enceintes par l'activation des enzymes du métabolisme de l'Hcy due à l'augmentation accrue du cortisol et de l'œstrogène, une autre explication possible à ce phénomène est l'utilisation de l'Hcy maternel par le fœtus **(Aubard et al. 2000)**.

Interestingly, plasma Hcy levels are lower in women of reproductive age than in men of the same age, although they increase after menopause, so it is seen that Hcy may be affected by the concentrations of sex hormones **(Tinelli and al. 2019).** Other hypotheses explain the drop in Hcy levels in pregnant women by the activation of Hcy metabolism enzymes due to the increased increase in cortisol and estrogen, another possible explanation for this phenomenon is the use of maternal Hcy by the fetus (Aubard et al. 2000).

Correlation between Hyperhomocysteinemia and Pathologies

The results of calculating the average plasma tHcy for each pathology are represented in (Tab.4).

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Table 4: Moyennes de l'tHcy Plasmatique de Chaque Pathologie Avec Précision

	Average	Précision (±)
Venous thromboembolism diseases	19,57	6,02
Kidney Failure	41,31	7,52
Stroke	13,95	1,79

Venous thromboembolic diseases

In our study we associated the term "Venous thromboembolism diseases" with several thrombotic vascular pathologies: DVT, TVS, TVC, pulmonary embolism and thrombophlebitis.

After calculating the average of the hHcy of the two sexes suffering from VTE (Tab.5) and according to the result of the Z test for comparison between two averages ($|\varepsilon|=0.87<1.96$): From from a statistical point of view, the difference is not significant for $\alpha=5$. So it seemed that in VTE sufferers, there is no significant difference between the mean tHcy of female patients and that of male patients for $\alpha=5$.

Table 5: Average plasma tHcy of both Sexes in VTE Sufferers

Venous thromboembolism diseases			
Sex	**Number of patients**	**Average of tHcy**	**Précision (±)**
female patients	39	17,59	7,13
Male patients	20	23,44	11,81

Hcy could promote the development of thromboses by different mechanisms: the inhibition of anticoagulant molecules (Heparin-like glycosaminoglycan, AT III, CRP, TM) or a reduction in fibrinolysis **(Perła-Kaján and al. 2007).** hHcy caused by folate deficiency is the main cause of thrombosis **(Chen, 2018).**

Among the suggested mechanisms, the production of oxidative stress by generating ROS by the oxidized sulfhydryl groups of Hcy which react on the metabolism of nitric oxide (protective agent of the vascular endothelium and vasodilator) by reducing bioavailability NO and interfere with glutathione synthesis, induction of a hypercoagulable state by activating coagulant factors and inhibiting anticoagulant factors, mainly activation of factor V, inhibition of protein C and thrombomodulin (TM), platelet production of thromboxane A2 (TXA2), increased proliferation of vascular smooth muscle cells, irregular ECM collagen formation, and LDL aggregation **(Guilland and al. 2003; Kim and al . 2018).**

Increased Hcy levels have shown a predilection for promoting platelet adhesion to endothelial cells and have also been associated with higher levels of prothrombotic factors, e.g., β-thromboglobulin, the activator of tissue plasminogen and factor VIIc. These lead to increased thrombus formation **(Ganguly and Alam, 2015)**. A strong correlation has been demonstrated between high Hcy level and the incidence of venous thrombosis in case control studies **(M. Cattaneo, 2001; M. Cattaneo and al. 2001).** The possible mechanism was described due to Hcy-induced platelet adhesion to endothelial cells, LDL atherogenesis, and the formation of pro-coagulant red blood cell-derived microparticles **(Xie and al. 2014; S. Zhang and al. 2014).**

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A meta-analysis showed that an increase in tHcy of 5 µmol/l was associated with a 27% higher risk of venous thrombosis **(Den Heijer and al. 2005).** However, meta-analyses using the MEGA 1999-2004 case control study from the Netherlands showed no association between elevated Hcy concentrations and venous thrombosis risk **(Ospina-Romero and al. 2018).** It is not yet clear whether high concentrations of Hcy can cause venous thrombosis **(Ostrakhovitch and Tabibzadeh, 2019).**

Kidney Failure

In our study we associated the term “Kidney failure patients” with hemodialysis patients, kidney transplant recipients and pre-transplant recipients, as well as kidney donors.

After calculating the average of the hHcy of the two sexes in IR (Tab.6) and according to the result of Z comparison between two averages ($|\varepsilon| = 2.52 > 1.96$): There is statistically a significant difference between the mean tHcy of female patients and that of male patients in those with renal insufficiency for $\alpha=5$. It appeared to be that male KF have a higher average tHcy than female IRs.

Table 6: Average plasma tHcy of both sexes in KF sufferers

Kidney failure			
Sex	Number of patients	Average of tHcy	Précision (±)
female patients	17	31,94	5,81
male patients	21	48,90	12,80

hHcy is the most common cardiovascular risk factor found among patients in chronic kidney failure, on dialysis and after kidney transplantation. Decreased renal Hcy metabolism, and not decreased urinary excretion, is the cause of hHcy in renal failure **(Halabi and al. 1998).**

The assessment of renal function is based on the measurement of several parameters combined in complex formulas. The estimated glomerular filtration rate (eGFR) reflects filtration through all functioning nephrons in a healthy adult. It depends on age, gender and height. For example, the eGFR rate naturally decreases with age. It also indicates the diagnosis and prognosis of renal failure **(Stevens and Levey, 2009).** The eGFR expressed in “ml/min/1.73m2”. hHcy is significantly associated with decreased eGFR and risk of renal impairment. hHcy increased the risk of kidney failure by 0.16 times per 1 µmol/L **(Xiao and al. 2018).** Hcy is linked to GFR, so when kidney function decreases, Hcy values increase (glomerular filtrate falls below 75 mL/Min) **(Tinelli and al. 2019).**

It also seems that renal failure contributes to the inactivation of crucial enzymes in Hcy metabolism, mainly trans-sulfuration and remethylation enzymes, which will lead to hHcy (**Kim and al. 2018).**

An increase in methylmalonic acid (MMA) is observed in parallel with the increase in plasma tHcy, it has the capacity to impair renal function and therefore induce chronic renal failure **(Martinelli and al. 2011; Huemer and al. 2014).**

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Strokes:

After calculating the average of the hHcy of both sexes in patients having suffered a stroke **(Tab.7)** and according to the result of the Student's T test (t = 3.29 > 1.96): In patients having suffered strokes, there is statistically a significant difference between the mean tHcy of female patients and that of male patients for α=5. It appeared that male stroke patients had a higher mean tHcy than female stroke patients.

Table 7: Average Plasma tHcy of both Sexes in Stroke Patients

Stroke			
Sex	Numbre of patients	Average of tHcy	Précision (±)
female patients	11	12,50	1,92
male patients	5	17,14	2,04

Homocysteine at a concentration of 10 µmol/L or higher is considered a risk factor in the development of cardiovascular diseases and ischemic heart disease **(Ostrakhovitch and Tabibzadeh, 2019).** A recent meta-analysis showed that stroke patients had higher Hcy levels than controls, so tHcy could be a risk factor for stroke in China **(T. Zhang and al. 2020).**

Additionally, some studies have shown that patients who have high Hcy levels are approximately twice as likely to have a stroke as normal people **(Han and al. 2015; Iso and al. 2004).** In the context of clinical prevention, a meta-analysis showed that a 25% reduction in Hcy is associated with a reduction in cardiovascular risk of 11% and stroke risk of 19% **(Clarke and al. 2002).** Conversely, the risk of stroke and myocardial infarction would increase by 6 to 7% for each step of 1 µmol/L in the Hcy level, knowing that these studies were carried out in young and elderly subjects **(Song and al.2006).**

GENERAL CONCLUSION

Based on recent retrospective and prospective studies, it is now widely accepted that increased plasma tHcy is a risk factor for various diseases. Impaired enzyme function due to genetic mutation or deficiency of essential folic acid, vitamins B12, B6 and B2 due to poor diet, can lead to hyperhomocysteinemia. However, literature data show that plasma Hcy may have an important role in the pathogenesis of several diseases through Hcy-induced oxidative stress, endothelial dysfunction, inflammation and excitotoxicity. Although several complex molecular mechanisms are not yet fully understood, several studies confirm that supplementation with vitamin B12, B6 and folic acid effectively reduces Hcy concentration. It would be imperative to administer homocysteine-lowering vitamins.

In our work we studied the association of hHcy with age, sex and the evaluation of the relationship between hHcy and several pathologies mentioned previously, as well as the sex difference of hyper-homocysteinemic patients in various pathologies studied. We found that Hcy level correlates with age, therefore hHcy is associated with aging, which implies the impact of age on Hcy metabolism. As well as hHcy is more significant in male

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patients than female patients. Men are more susceptible than women. We also found that Hcy is positively associated with several pathologies.

All of these results stimulate our reflection on the advisability of effective vitamin supplementation (mainly vitamins B6/9/12) which allows, on the one hand, the reduction of tHcy levels and, on the other hand playing a role protective and preventive against certain pathologies.

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