4-Discussion

4:1-Homocystiene and IDDM

Diabetes mellitus is a chronic and complex disease, requiring continued life long management aimed at reducing the high morbidity and premature mortality caused by chronic complications associated with long standing hyperglycemia [115].

One hypothesis that explains how hyperglycemia might leads to the chronic complications of diabetes mellitus is that increased intracellular glucose leads to the formation advance glycation end products (AGEs) via the nonenzymatic glycosylation of cellular proteins results from the interaction of glucose with amino-groups of proteins. AGEs have been shown to cross links proteins (eg. collagen) and accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction and alter extracellular matrix composition and structure. The serum level of fast blood sugar (FBS) correlates with level of glycaemic control. The clinical implication of FBS is that the goal of therapy is to achieve an FBS level as close to normal as possible to prevent many of early complications of DM [116,117].

In the present study homocysteine increased significantly among diabetes patients when compared to healthy control subjects by 37% as shown in figure (3-2) and this result showed the possibility of increasing complications in diabetes patients such as cardiovascular disease because of homocysteine has emerged as a novel independent marker of risk for the development of cardiovascular disease over the past three decades [115].

Baushey, *et al.* (1995) have found a linear relationship between homocysteine levels and vascular risk, that a 5 µmol/L increase in homocysteine concentration was associated with an increase in vascular risk of about one thirds [58].

There are several prospective studies that have investigated the relation between homocysteine and cardiovascular disease. Many [118-129] but not all [130-132] found a positive relation .Non of the previous studies, however, investigated the possibility of interaction between hyperhomocysteinemia and diabetes with regard to risk of death. The strength of the relation between hyperhomocysteinemia and death appeared to be stronger among those with diabetes. An interaction of hyperhomocysteinemia with diabetes is biologically plausible. High homocysteine concentrations may exert an atherothrombotic effect through increasing oxidative stress, which may induce endothelial dysfunction.

Hoogeveen, *et al.(*1998) showed that high homocysteine concentration effect the properties of the extracellular matrix and increase smooth muscle cell proliferation [133-135]. Oxidative stress is thought to be increased in diabetes [136], and matrix alterations are a prominent feature of diabetes in general, both of which might make diabetes patients more susceptible to the adverse affect of hyperhomocysteinemia.

Jakubowski, *et al.* (2002) showed the important role of oxidative – redox stress and Hcy is biologically plausible because Hcy promotes oxidant injury to vascular cells (particularly the endothelium) through the auto-oxidation of Hcy, formation of Hcy mixed disulfides, interaction of Hcy thiolactones, and protein homocysteinylation, also formation of mixed disulfides contributes to the additional formation of ROS [137].

Misra *et al.*(1994) estimated that Hcy may undergo complicated rearrangements to form Hcy thiolactone (a cyclic thioester), which is chemically reactive and acylates free amino groups such as the side-chain lysine groups in proteins. In the process of forming homocysteinylated proteins further oxidative stress develops and homocysteinylated proteins become damaged and may lose their biological activity. This results in the modification of proteins and in particular the modification of low-density lipoproteins (LDL-cholesterol), which contribute to their retention within the intima and subsequent inflammatory foam cell formation associated with atherogenesis [138,139].

Melvin, *et al.* (2004) demonstrated increasing of serum homocysteine in diabetic, metabolic syndrome and atherosclerosis and concluded that homocysteinemia being an independent risk factor for cardiovascular disease as occurs in thrombotic events (such as arterial and venous occlusion) and ischemic disease such as stroke and myocardial infarction (two of the most common causes of death and disability). With an aging population who are

at greater risk of developing these morbid and mortal cardiovascular diseases associated with metabolic syndrome, diabetic, and atherosclerosis, there should exist a dedicated consideration for folate and possible cobalamin supplementation, in addition too global risk reduction.among them [140].

4:2- Antioxidant vitamins and IDDM

In this study indirect method used to individuate oxidative stress and it is dangerous among diabetes patients, by measuring of antioxidant vitamins to control diabetes complications.

According to our results, there was significantly reduction of vitamins A, C, &E concentration in diabetes when compared with healthy control. And there was positive correlation between vitamins level and age $p<0.001$ in both healthy control and diabetes patients and these findings illustrated in figures (3.12- 3.17).

The results showed the significant correlation between antioxidant vitamins and homocysteine as shown in figures (3.18- 3.23). This indicates that these parameters are interrelating with each other and confirms the role of oxidative stress in diabetes.

Toxic oxygen free radicals are generated continuously in humans. Under physiological condition, the human body has developed a complex antioxidant defense system sufficient to protect the cells against oxidative damage. However, oxidative stress could result from loss of this protective

balance under abnormal pathological condition by overproduction of free radicals or inadequate antioxidant defenses [141].

Several different mechanisms have been proposed to explain why oxidative stress is increased in diabetes mellitus. These mechanisms fall into two general categories: increased production of reactive oxygen species (ROS) and decreased antioxidant defenses. Hyperglycemia in diabetes mellitus may increase ROS production via changes in the redox potential of glutathione [142], though nonhyperglycemic mechanisms have also been reported, e.g., increased activity of xanthine oxidase, a superoxide-generating enzyme [143].

Decreased antioxidant defenses have also been observed in diabetes mellitus, including reductions in serum paraxonase [144] and in total antioxidant capacity in plasma [145]. Some of these mechanisms may possibly operate simultaneously in a synergistic fashion.

In addition to an increase in ROS, a decrease in antioxidant capacity occurs in diabetes mellitus [146-148]. A decline in important cellular antioxidant defense mechanisms, including the glutathione redox system & vitamin C-vitamin E cycle, significantly increases susceptibility to oxidative stress. Thus, attempts have been made to reduce oxidative stress-dependent cellular changes in patients with diabetes by supplementation with naturally occurring antioxidants, especially vitamin E [149-151], vitamin C, and vitamin A.

The putative role of ROS in the development of diabetic complications has been investigated for several decades [152-154]. Evidence of ROS involvement in hyperglycemia-induced embryopathy was first obtained in studies, in which antioxidant enzymes proved to be protective in vitro [153, 155]. Increased ROS production [156,157] and lipid peroxidation [158] have subsequently been found in rat embryos cultured in high glucose and in embryos of diabetic rat mothers. Enzymatically produced ROS can disturb embryo development in vitro similarly to the effect of high glucose [159], and the resulting maldevelopment can be blocked by vitamins E and C separately [160]. Apart from increased production, the putative ROS excess can be attributed to impaired embryonic defense in response to an oxidative environment [161].

Oral vitamin E treatment appears to be effective in normalizing abnormalities in retinal hemodynamics and improving renal function in patients with type 1 diabetes of short disease duration [162] Vitamin E was beneficial in those individuals with the poorest glycemic control and the most impaired retinal blood flow. These data suggest that vitamin E and perhaps supplementation with other antioxidants may provide an additional benefit in the treatment of either diabetic retinopathy or nephropathy.

The favorable action of vitamin E on diabetes shown in this study could be mainly due to two different mechanisms. Vitamin E is a potent scavenger of superoxide and other reactive species. In diabetes mellitus the presence of hyperglycemia leads to an abnormal production of superoxide anion and hydroxyl radicals and to decreased vitamin E level in tissue and blood [163];

in contrast, chronic vitamin E administration restores plasma antioxidant defenses such as the GSH/GSSG ratio [164]. Thus, chronic vitamin E administration may lower the quenching effect of free radicals on nitric oxide.

Study by Stephen, *et al.(*1997) showed the dangerous effect for increasing ROS on diabeties patient and decrease in the level of all major lipophilic antioxidant, peroxidation of lipids, and reported that ascorbate enhance the cytoprotective effects of quercetin and rutin against oxidative stress-enduced death of human skin fiberblasts [165] .

An investigation by Julie, *et al.* (1999) agreed with our results that mean serum Vitamin C concentrations were significantly lower in persons with newly diagnosed diabetes than in those without diabetes. After adjustment for age, sex, dietary intake of vitamin C and other important covariates, however, mean concentrations did not differ according to diabetes status [166].

As well as, Pfleger and Scholl (1937) observed vitamin C deficiency in diabetics and reported that ascorbic acid administration reduced the amount of insulin needed to control the blood sugar [167].

While, Cai and Harrison (2000); Laigh *et al*. (2000) observed that a damaged endothelium (endothelial dysfunction) is a key event in the development of diabetic macroangiopathy and is associated with oxidative stress-mediated blunting of nitric oxide action. Endothelial dysfunction has been documented in individuals who are insulinresistant and in those at risk for developing in both type I and type II diabetes [168, 169].

Bravenboer, *et al.* (1992) and Cameron *et al*. (1993) used antioxidant treatment to reduce the hard effect of free radicals and prevent the development of diabetic neuropathy. They observed there was significantly decreased in antioxidant vitamin C, E, and β- carotene before the treatment, [170, 171].

Olemedilla (1997) studied the fat soluble antioxidant status in diabetic patients. The team examined and compared concentrations of serum retinol, tocopherol, and main carotenoids. And they found that β-carotene were the only nutrients positively associated with the disease, while retinol was the only nutrient that indicate 4 a significant negative association with diabetes [172].

Conflicting results were reported by some studies [173-175]. In fact the investigators observed the serum fat-soluble antioxidant levels equal to or higher than those in the controls, and concluded that supplementation with fat-soluble antioxidants is not necessary for patients with diabetes [175].

Although, some reports mentioned that antioxidant supplements (vitamin E, vitamin C or beta-carotene) have not demonstrated beneficial effect in cardiovascular disease outcomes or glycemic control [176-178] and there is evidence that long-term beta-carotene supplementation may be harmful when consumed by smokers antioxidants supplementation should be discussed with patients who smoke [177-179].

Finally, an exaggerated oxidative stress degree has been postulated as the link between diabetes mellitus and endothelial function [180]. In fact, in these patients hyperglycemia [181], hyperinsulinemia [182,183], and hypertriglyceridemia [184] lead to increased oxidative stress, which, in turn, might be responsible for an inactivation of nitric oxide. As it is now widely demonstrated that flow-dependent changes in arterial diameter are mediated by endothelium-dependent mechanisms, i.e. by the availability of nitric oxide [185,186], the presence of elevated oxidative stress in diabetic patients results in endothelial dysfunction [187].

Conclusion

In this study we have found that :

1- Serum homocysteine was increased in diabetic compared with healthy control subject, this give an alarm of incidence of coronary heart disease in IDDM.

2- Serum vitamin A level decreased in diabetic compared with healthy control subjects.

3- Serum vitamin E (α-tocopherol) decreased significantly in diabetic compared with healthy control subjects.

4- Serum vitamin C (ascorbic acid), which is water soluble vitamin significantly decreased in diabetic compared with healthy control subjects.

5- The age of the studied subject have been found to be significantly effects in the levels of serum homocysteine ,vitamin A, vitamin C, &vitamin E in diabetic patiants compared to healthy control subjects.

6- There was no significant difference in level of serum homocysteine and antioxidant vitamins (A, C, &E) between females and males in both groups diabetic and healthy control.

Future work

1- Determination of folic acid, vitamin B6 (coblamine) &B12 which related directly to homocysteine metabolism in children with IDDM.
2- Study the relationship between homocysteine & enzymes (GSH, SOD &CAT) in diabetic.
3- Study the relationship between vitamins & enzymes (GSH, SOD & CAT) in diabetes.
4- Assessment of lipids profile and some trace elements such as (Zn, Se, Cr & Mg) in diabetic patients.

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1:1- Diabetes Mellitus (DM)

1:1:1- Historical Perspective

The name diabetes derived from the Greek verb meaning to pass through. Diabetes is undoubtedly an ancient medical problem for more than 2000 years. Polyuria was recorded in the Ebers papyrus from ancient Egypt even before Celsius (30 B.C to 50 A.D). Diabetes was described unmistakably as a disease of polyuria without pain but with emaciation. Aretaeus in the first century named it and outlined its principal presenting symptoms, progressive nature and fatal outcome. The ancient Hindu Vedas, however, include information (attributed to Susruta) which showed that diabetes was known as the honey-urine disease dating from the sixth century. The Moslem physician Avicenna (980-1037A.D) first described diabetic gangrene and some translations credit him also with the first hypothesis of a nervous origin of diabetes and the first theory of the role of the liver [1].

Thomes Willis (1775) introduced a taste for urine as a part of its qualitative examination, discovering it to be wonderfully sweet as if imbued with honey or sugar. Mathew Dobson (1775) recognized that the sweet material in the diabetic urine was indeed sugar which was identified as glucose by M.Cherreul in the eighteenth Century. After scientific advances in chemistry and medical science, Trommer (1841) developed a qualitative test while, Fehling (1850) developed a quantitative test for urinary sugar.

Claude Bernard (1813-1878) defined the renal threshold for glucose by showing that glucose is excreted in the urine when either the blood concentration is too high or when the renal threshold is too low (renal

glycosuria). He demonstrated also a cerebral effect on the blood sugar concentration and the possibility of nervous glycosuria.

Appolinaire Bouchardat (1806-1886) in France, Amold Cantani (1837-1893) in Italy and Bemard Naunyn (1839-1925) in Germany, had a method of diabetes treatment which was a general hygienic approach to life, including diet and exercise. The discovery by Von Mering & Minkowski, in 1889, that pancreatectomy causes a metabolic disorder like that of spontaneous diabetes, followed by the discovery of insulin by Banting & Best (1921), led to the conclusion that diabetes mellitus was a result of insulin deficiency.

Insulin is a protein, can not be taken orally without being broken down and destroyed by the digestive juices. Research continued for other substances which might reduce hyperglycemia. Eventually, however, the antibacterial effect of sulfonamide compound, Para-aminobenzene sulfonamide isopropylthiadiazole, in typhoid fever, M. Janbon in France (1942) observed certain neurological reactions which could be counteracted by prompt increased glucose levels. Further studied of this and related compounds in experimental animals, Karl J. Fuchs in 1955 tested the antibacterial properties of 1-butyl-3- sulfanilyl urea (carbutamide) and observed insulin-like reaction by lowering blood glucose, which led Laboratories to publish his preliminary clinical report on the original compound (Para-aminobenzene sulfonamide isopropyl- thiadiazole) concluding, that since some insulin producing tissue was needed for the activity of the drug, it must act by stimulating the secretion of endogenous insulin.

A series of compounds have been developed to take advantage of these effects in treating diabetes mellitus [2].

1:1:2- Introduction

Diabetes mellitus (DM) can be defined as an almost in born error of carbohydrate metabolism occurring during varying periods of life [3]. It is a systemic disease characterized by chronic hyperglycemia usually accompanied by glucosuria. This malfunction is due to an absolute or relative deficiency of normally functioning insulin [4,5].

Diabetes is a major health problem that seriously effects morbidity and mortality [6]. It is the main cause of blindness [7], end stage renal failure [8], and amputation of lower extremities [9,10].

1:1:3- Classifications of DM:

In 1970 the National Institutes of Healthy (NIH) developed a classifications scheme for diabetes mellitus and other types of glucose intolerance based on current knowledge of the biochemistry of the diseases. This new standardized approach for naming diabetic states replaces an inexact nomenclature that developed pre diabetes, Iatent diabetes, covert diabetes, and juvenile onset and adult-onset diabetes [11].

1:1:3:1- Type I: Insulin Dependent Diabetes Mellitus (IDDM)

This type characterized by onset childhood or adolescence under the age of 20 years [12,13]. IDDM is usually due to immunologic destruction of the β-cell of the pancreatic islets and requires treatment with insulin injection, exercise, and a diabetic diet [14]. The failure to produce insulin is the primary pathogenic factor. Type I Diabetes Mellitus is also associated with the excessive production of acetoacetate and β-hydroxybutyrate and the development of acidosis. Individuals with this type of diabetes are especially

prone to diseases of nerves (neuropathy), kidney disease (nephropathy), and blood vessel diseases [15,16].

1:1:3:2- Type II: Non-Insulin Dependent Diabetes Mellitus (NIDDM)

NIDDM is the more common form, is due to insensitivity to insulin (insulin resistance). Type II Diabetes Mellitus is not associated with acidosis, this type arise in adults usually after the age of 40 years [16].

Type II is first treated with weight reduction, a diabetic diet, and exercise. When these measures fail to control the elevated blood sugars, oral medications are used. If oral medications are still insufficient, insulin injection are considered [18].

1:1:3:3- Secondary diabetes

DM caused by other conditions and diseases is called secondary diabetes [19]. Secondary diabetes can be caused by pancreatic disease, a cromegaly (growth hormone deficiency), Cushing's syndrome (elevated cortisol), pheochromocytoma (excessive catecholamines), glucagonoma (excessive glucagon because of a tumor), Somatostatinoma (excessive somatostain because of tumor), primary aldosteronism, severe liver disease, and administration of certain drugs, hormones and chemicals [20].

1:1:3:4- Gestational Diabetes

Gestational diabetes refers to diabetes that occurs during pregnancy [21]. A study in 1984 estimates that 39% of women with gestational diabetes manifest type II diabetes mellitus 20 years after delivery. Screenings of pregnant women for gestational diabetes, to prevent prenatal complications

associated with maternal hyperglycemia, has become a wide spread, accepted practice [20].

1:1:4-Clinical and Metabolic Features of IDDM

Most of the metabolic changes of diabetes mellitus are a consequence of insulin deficiency. Hyperglycemia is, by definition an invariable finding. If plasma glucose concentration exceeds about 11 µmol/L and renal function is normal, there will be glycosuria. High urinary glucose concentrations produce on osmotic diuresis and therefore polyuria [22]. Cerebral cellular dehydration due to hyper osmolality, secondary to hyperglycemia, causes thirst (polydipsia). A prolonged osmotic diuresis may cause excessive urinary electrolyte loss.

Abnormalities in lipid metabolism may be secondary to insulin deficiency. Lipolysis is enhanced and plasma Free Fatty acid (FFA) concentrations rise. In the liver FFA are converted to acetyl CoA and ketones, or are re-esterified to form endogenous triglycerides and incorporated into Very Low Density Lipoprotein (VLDL); the latter accumulate in plasma because lipoprotein lipase, which is necessary for VLDL catabolism requires insulin for optimal activity. If insulin deficiency is very severe there may also be chylomicronaemia. The rate of cholesterol synthesis is also increased, with an associated increase in plasma LDL concentrations. Increased breakdown of protein may cause muscle wasting [23, 24].

1:1:5- The insulin

Insulin is a peptide hormone, synthesized in the β-cell of the islet of langerhans of pancreas. It is a two peptide chains (A and B), Chain (A) which contains 21 amino acids and chain (B) contains 30 amino acids. The two chains are linked by disulphide bridges. The first one is between amino acid numbers 20 from chain (A) with amino acid number 19 from chains (B), as shown in figure (1-1) [25].

Insulin is important in the metabolism of carbohydrate, fat, and protein. Any insulin deficiency may cause an increase in the release of glucose from the liver and a decrease in the uptake of glucose by muscle, as well as an increased in the lipolysis process in liver and adipose tissue. The first two may lead to an increase in extracellular glucose while the last increases the blood lipids and ketone bodies [5, 29].

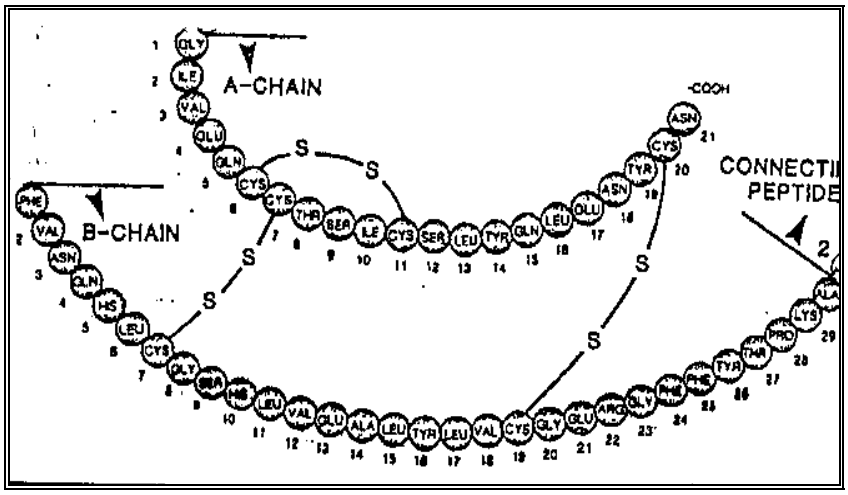


Fig.(1-1): Human Insulin (Foster, 1998)[25].

1:1:6- Diabetes and Heart Disease

Cardiac disease, especially Coronary Artery Disease (CAD) eventually occurs in a majority of diabetic patients. Diabetes is basically a metabolic cum vascular disease with genetic and environmental components, producing a specific microanglophathy and also increased tendency for macro vascular changes. The increased macro vascular changes place the diabetic at an increased special coronary vascular risk [27].

Moses in 1990 found that most people with diabetic have health problems or risk factors, such as, high blood pressure and cholesterol that increase one's risk for heart disease and stork when combined with diabetes. In fact, more than 65% of people with diabetes die from heart disease or stroke with diabetes. Heart attacks occur earlier in life and often result in death. By managing diabetes, high blood pressure and cholesterol, people with diabetes can reduce their risk [28, 29].

1:2- Homocysteine [HCY]

Homocysteine is nonessential amino acid of molecular weight (268 dalton), formed during the metabolism of methionine [30, 31]. High dietary consumption of methionine, which can be found in meats and dairy products, can result in the overproduction of homocysteine [32].

Abnormally high blood levels of homocysteine are strongly linked to an increased of CAD and stroke.

Homocysteine may harm the lining of the arteries and contribute to blood clotting [33]. Homocysteine is metabolized by one of the two pathways:-

1-Remethylation and 2-Transsulfuration as shown in figure (1-2) [34].

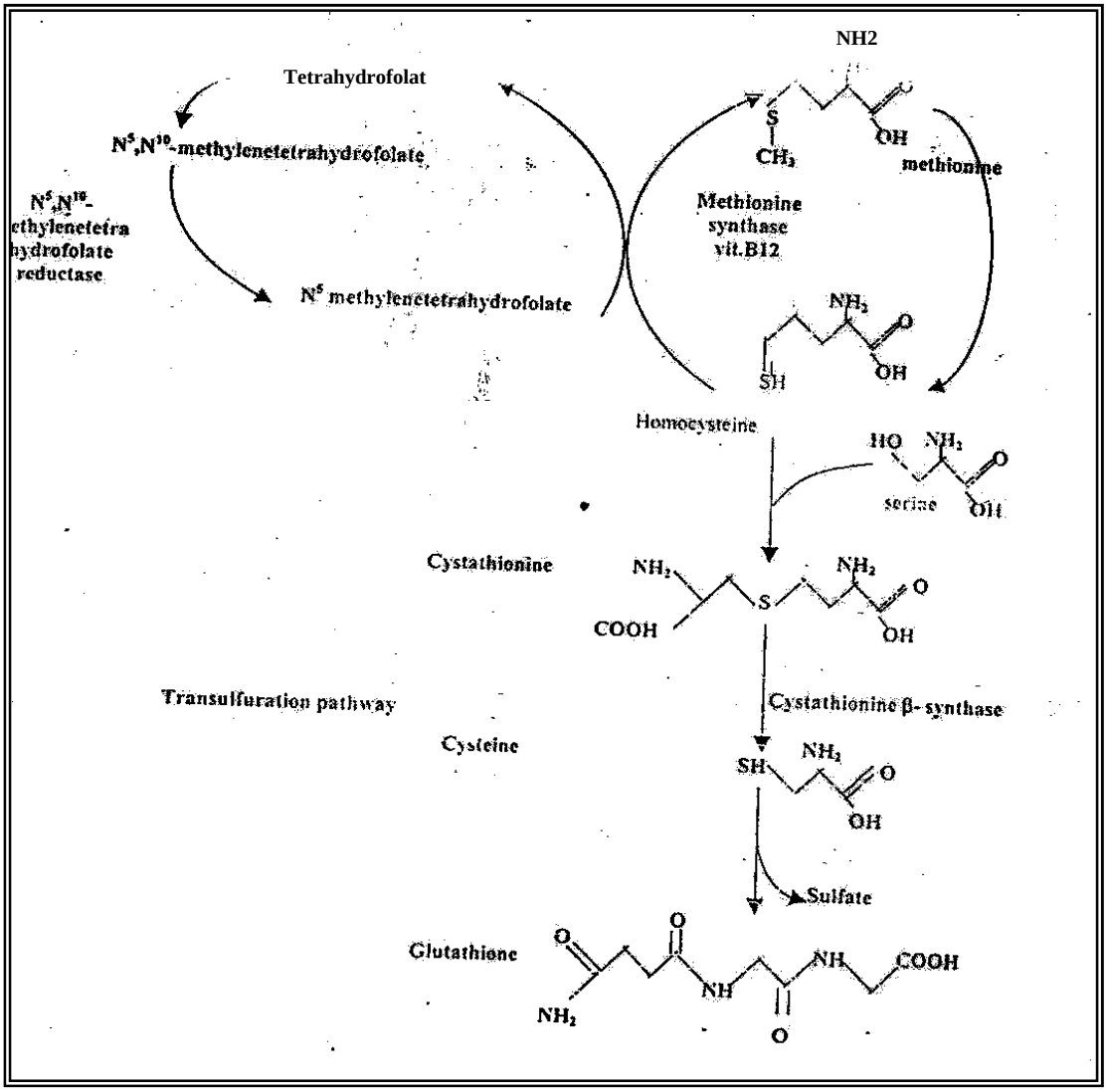


Fig .(1-2): Homocysteine metabolism (Kruger, 2000)[34].

1-Remethylation

In the remethylation cycle, homocysteine is salvaged by the acquisition of methyl group in a reaction catalyzed by methionine synthase. Vitamin B12 (Cobalmine) is an essential cofactor for methionine synthase, N^5-Methyl tetrahydrofolate is the methyl donor in this reaction, and N^5, N^{10} methylene tetrahydrofolate reductase functions as catalyst in the remethylation process [35,36].

2-Transsulfuration

Under conditions in which an excess of methionine is present or cysteine synthesis is required, homocysteine enters the transsulfuration pathway. In this pathway, homocysteine condenses with serine to form cystathionine in a reaction catalyzed by vitamin B6-dependent enzyme (systathionine β-synthase) [37]. Cystathionine is subsequently hydrolyzed to form cysteine which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in urine [38].

1:2:1-Plasma Homocysteine

The majority of the clinical studies involving homocysteine have relied on the measurement of total homocysteine, which includes homocysteine, mixed disulfide involving homocysteine thiolactone, free homocysteine, and protein–bound homocysteine. Protein bound (i.e., disulfide-linked) homocysteine accounts for 70-80% of the total pool [39]. Normal total homocysteine concentrations range from 5 to 15 μmol per liter in the fasting test [40]. Kang *et al*. have classified hyperhomocysteinemia as moderate (homocysteine concentration 15 to 30 μmol per liter), intermediate (>30 to 100 μmol per liter), and severe (>100 μmole per liter) on the basis of concentration measured during fasting [41].

1:2:2- Causes of Hyperhomocysteinemia

Elevations in plasma homocysteine are typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors [42]. Homocysteineuria is a rare, autosomal recessive in born error of metabolism that results in early death from rapidly progressive atherosclerotic vascular disease [43]. Homocysteine levels in patients with homocysteineuria have been reported as high as 200μmole per liter [44].

The most common genetic defect resides in the cystathionine *β*-synthase locus and causes decreased transsulfuration of homocysteine. Deficiencies of other enzymes such as methionine synthase and 5,10-methylentetrahydrofolic acid reductase have also been implicated [45]. The heterozygous state for the cystathionine *β*-synthase gene defect has also been

associated with elevated homocysteine levels. It is prevalence in the general population has been estimated at approximately 1 in 300 [44].

Enzyme deficiencies are not only a cause of hyperhomocysteinemia. Dietary deficiencies of vitamin B6, vitamin B12, or folic acid may also result in elevated plasma homocysteine levels because these are essential cofactors for the previously mentioned enzymes.

Persons homozygous for thermolabile methylene tetra-hydrofolate reductase deficiency have moderate hyperhomocysteinemia and a greater risk of CAD, which can be modified with folate in the diet or from supplements [46-48]. Persons heterozygous for cystathionine β-synthase deficiency also have moderate hyperhomocystienemia that can be modified with vitamin B6 supplementation, however, heterozygous cystathionine β-synthase deficiency has not been consistently linked with vascular disease [49].

1:2:3- Pathophysiologic mechanism of hyperhomocysteinemia

Experimental evidence suggests that the atherogenic propensity associated with hyperhomocysteinemia that results from endothelial dysfunction and injury followed by platelet activation and thrombus formation [50].

Homocysteine is rapidly auto-oxidized when added to plasma forming homocysteine, mixed disulfides homocysteine thiolactone [51], potent reactive oxygen species including superoxide and hydrogen peroxide are produced during the auto-oxidation of homocysteine which has been implicated in the vascular toxicity of hyperhomocysteinemia [52].

Auto-oxidation of homocysteine produces other cytotoxic reactive oxygen species including the superoxide anion radicals and hydroxyl radical [53], which can initiate lipid peroxidations [54], an effected that accurate level of the endothelial plasma membrane and within lipoprotein particles [55].

Homocysteine thiolactone which is a highly reactive on hydrous by homocysteine oxidation, combines with low density lipoprotein to form aggregates that are taken up by intimal macrophages and incorporated into foam cells with nascent atheromatous plagues [56].

Also homocysteine thiolactone impair oxidative phosphorylation and promote the proliferation and fibrosis of smooth muscles [57].

1:2:4- Association Between Hyperhomocysteinemia and CAD

Interestingly, an elevated plasma homocysteine concentration conferred an independent risk of vascular disease similar to that of smoking or hypercholesterolemia and also had a multiplicative effect on risk among cigarette smoker and patients with diabetes. Therefore controlling hypertension, diabetes and smoking may be particularly important in patients with hyperhomocysteinemia. In a meta-analysis, Boushey *et al.* [58] estimated that 10% of the risk of CAD in the general population is attributable to homocysteine. They reported that an increase of 5μmol per liter in the plasma homocysteine concentration raises the risk of CAD by as much as an increase of 20 mg/100/(0.52mmol/L) in the cholesterol concentration. They suggested that increasing folate consumption by approximately 200μg per day would reduce total homocysteine concentration by approximately 4 μmol per liter, a reduction that could potentially a major effect on cardiovascular mortality [24].

Den Heijer *et al.* [59], have demonstrated that mild hyperhomocysteinemia is also an independent risk factor for venous thromboembolism. They found a marked increase in the risk of venous thrombosis at the highest plasma homocysteine concentrations. A plasma homocysteine concentration of more than 22 μmol per liter increased the risk for deep venous thrombosis to 4 times. It is unknown whether homocysteine lowering therapy reduces the risk of venous thrombosis in patients with such high concentrations. Ridker *et al.* [60] showed that the combination of hyperhomocysteinemia and factor VLeiden further increase the relative risk of venous thromoembolism up to 3.6 folds.

1:2:5-Treatment of Hyperhomocysteinemia

The treatment of hyperhomocysteinemia varies with the underlying cause; however, vitamin supplementation (with folic acid, pyridoxine and vitamin B12) is generally effective in reducing homocysteine concentrations [24].

Folic acid alone or combined with vitamins B6 and B12 vitamins B6 and B12 have all been shown to reduce homocysteine concentrations. Normalization of the plasma homocysteine concentration usually occurs within four to six weeks after the initiation of therapy, but may occur in as little as two weeks.

Interestingly, the reduction in mortality from cardiovascular causes since 1960 has been correlated with the increase in vitamin B6, B12 supplementations in the food supply [61].

1:3-Free Radical

Chemically speaking, a free radical is any molecule that contains one or more unpaired electrons.

Normally, these electrons exist in stable pairs; however, when unpaired, they make molecules extremely reactive. They attempt to "Steal" electrons from other molecules to reform a stable structure. This can initiate a destructive chain reaction which can ultimately lead to cell injury and death. Typically cell components damaged by free radicals include:

Poly unsaturated fatty acids in cell membrane, proteins such as enzymes and membrane ion transporters, and DNA [62,63].

Free radicals are continually produced by the body, mostly by:

- Biochemical redox reqactions involving oxygen, which occur as part of normal cells metabolism.
- Phagocytes as part of controlled inflammatory reaction free radicals disappear from the body following reactions with other free radicals, or more commonly due to the actions of the antioxidant system [64].

1:3:1- Sources of Free Radicals

1-Endogenous Sources

This source include mitochondria, lysosomes, peroxisomes, nucleus, endoplasmic reticulum, plasma membrance and cytosol [65,66].

2-Exogenous Sources

Radiation, cigarettes smoke, some of organic solvents, herouess, pesticides and some drugs which metabolized to free radicals that cause damages for tissue such as liver and reins [67,68].

1:3:2- Source of oxidative stress in diabetes

Possible source of oxidative stress in diabetes include altered carbohydrate and lipid metabolism, decreased level of antioxidant defenses such as glutathione (GSH), and antioxidant vitamins, and most importantly increased generation reactive oxygen species (ROS) lipid peroxidation and glycation [69,70]. Glucose can react slowly with proteins, amino groups of phospholipids and DNA, modifying them by non-enzymic glycation, generating reactive oxygen species during this process. Further oxidation of glycation products by ROS leads to the formation of advanced glycation end products (AGEs), which damage biological tissues. In diabetics, advanced glycation end products are present on circulating LDL, and also in atherosclerotic lesions, and are thought to contribute to endothelial injury [71,72].

In particular, diabetes mellitus is strongly associated with increased oxidative stress, which could be a consequence of either increased production of free radical, or reduced antioxidant defenses [73,74].

In both type I and type II diabetes, late diabetic complications in nerve, vascular endothelial, and kidney arise from chronic elevations of glucose and other metabolites including free fatty acids (FFA) as illustrated in figure (1-3)[75].

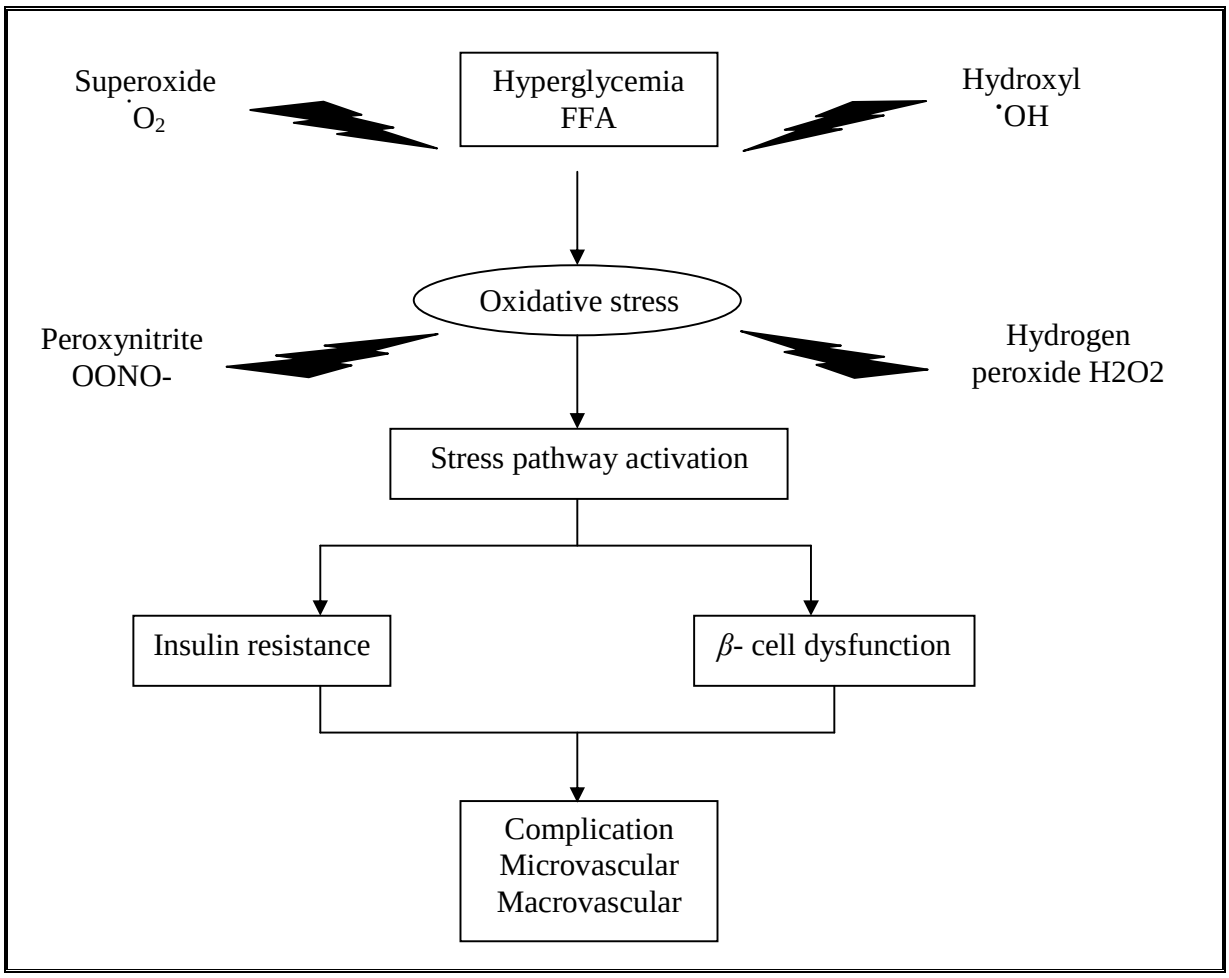


Fig.(1-3): Link between Hyperglycemia and Oxidative stress with their complications (Evans, 2002)[75].

1:4- Antioxidants

Any substance, when present at low concentration compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate [76]. The term oxidisable substrate includes almost everything founds in livings cell, including proteins, lipids, carbohydrates and DNA [77].

Several types of reactive species are generated in the body as a result of metabolic reactions in the form of free radicals or non radicals. These species may be either oxygen derived or nitrogen derived and called prooxidants, the body is endowed with another category of compounds called antioxidants to counter their effect [78].

These antioxidants remove free radical once it formed, thus preventing the radical chain reaction. Chain breaking antioxidants are electron donors which react with free radical. The antioxidant is oxidized to relatively unreactive antioxidant radical, which is unable to attack further molecules as shown below [79,80].

$$R^{\bullet} + AH \rightarrow RH + A^{\bullet}$$

$$RO^{\bullet} + AH \rightarrow ROH + A^{\bullet}$$

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$$

$$R^{\bullet} + A^{\bullet} \rightarrow RA$$

$$RO^{\bullet} + A^{\bullet} \rightarrow ROA$$

$$ROO^{\bullet} + A^{\bullet} \rightarrow ROOA$$

These antioxidants are produced either endogenously or received from exogenous sources and include:

- ❖ Enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.
- ❖ Minerals like Se, Mn, Cu, and Zn.
- ❖ Vitamins like Vit. A, C, and E.

And other compounds with antioxidant activity include glutathione, flavonoids, bilirubin and uric acid [81,82].

In a healthy body, prooxidants and antioxidant maintain a ratio and a shift in this ratio towards prooxidant gives rise to oxidative stress. This oxidative stress may be either mild or severe depending on the extent of shift and remains the cause of several diseases such as cardiovascular disease, neurological disease, malignancies, renal diseases, diabetes, inflammatory problems, skin diseases, aging, respiratory diseases, liver diseases and different types of viral infections [78].

1:4:1- Vitamin A

First described in 1909 and found to prevent night blindness in 1925. Vitamins A is known to be made up of three biologically active forms, retinol, retinal, and retinoic acid. These major vitamin A compounds all contains a trimethyl cyclohexenyl group and an all-transpolyene chain with four double bonds as shown in figure (1-4). These compounds are derived directly from dietary sources, primarily as retinyl esters, or from metabolism of dietary carotenoids (Provitamin A) [83].

Major dietary sources of these compounds include animal products pigment fruits and vegetables. Each of these compounds is soluble in organic solvents, with retinoic acid being more polar than the others. Oxidation of retinol or retinal by peripheral cells is irreversible: thus neither retinoic acid nor retinal is metabolically converted to retinol [84].

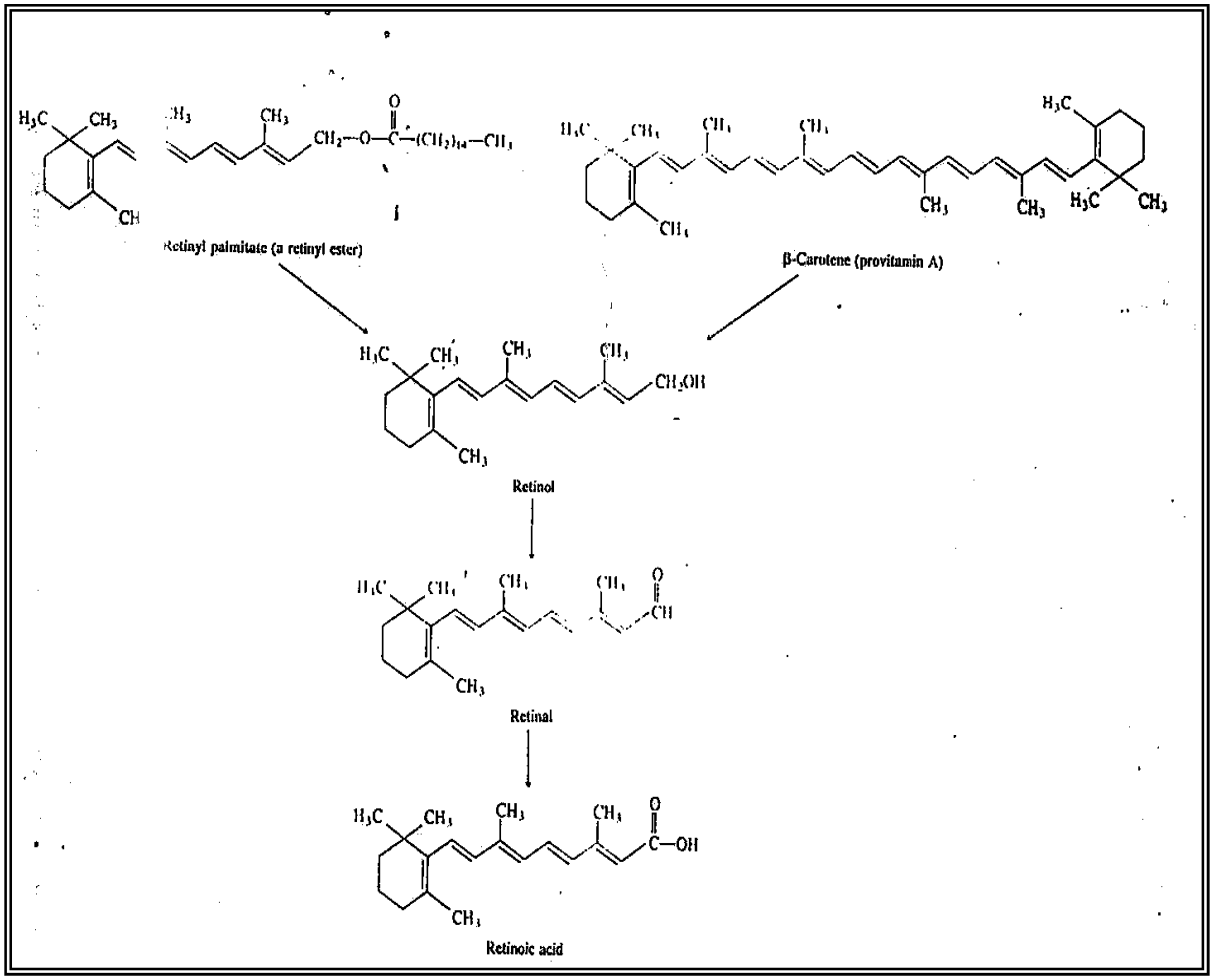


Fig.(1-4): Structures of β-Carotene and its conversion to Vitamin A derivatives (Robert Roskoski, 1996)[83].

1:4:1:1- Function

The only clearly defined physiological role for retinol is it's role in vision. Retinol is oxidized in the rods of the eye to retinal, which, when complexed with opsin, forms rhodopsin, allowing dimlight vision [85].

Vitamin A has important antioxidant properties which translate into anticancer and anti atherosclerosis effects [86].

Vitamin A is an antioxidant and play a role in trapping peroxy free radicals in tissues at low partial oxygen pressures. The ability of retinol to act as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure as shown in scheme (1-5). Since retinols is effective at low oxygen concentrations [87-89].

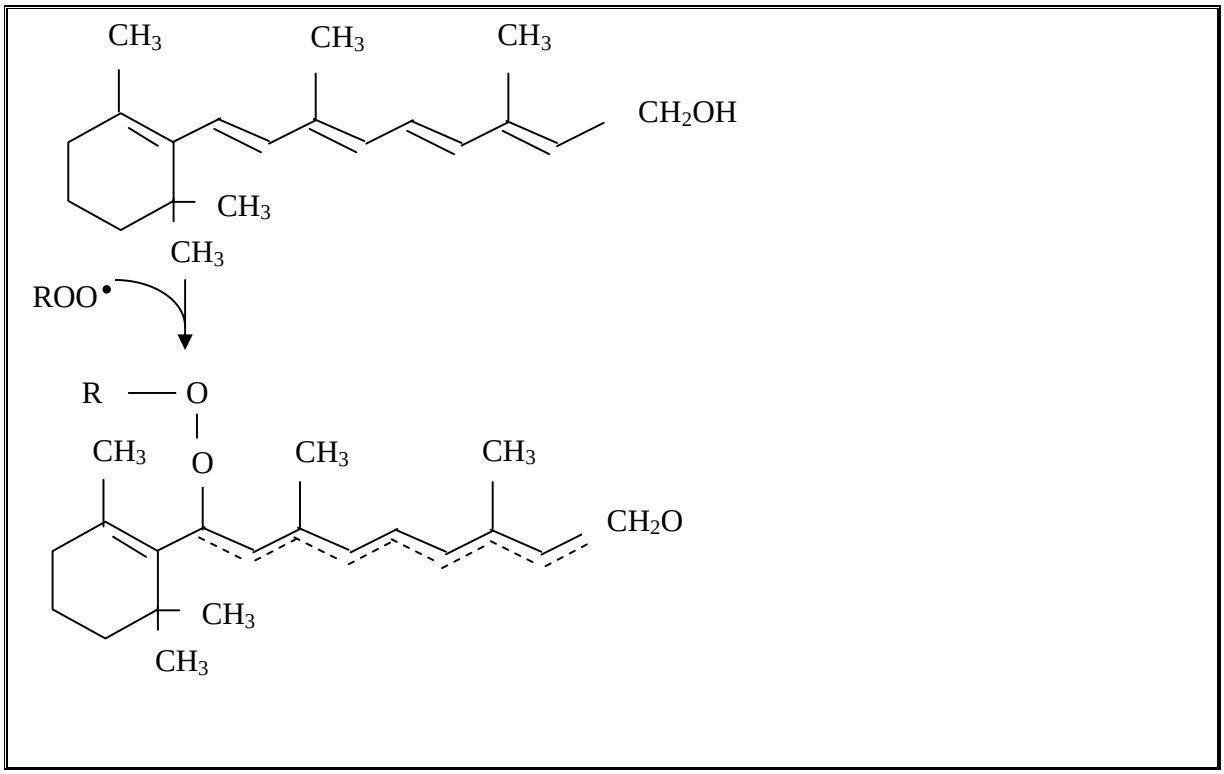


Fig.(1-5): The formation of resonance-stabilized carbon-centered radical from a peroxyl radical ($ROO^{\bullet}$) and retinol (Burtan, 1984)[89].

1:4:1:2- Clinical and chemical deficiency signs

Clinical signs of vitamin A deficiency can be separated into early and late signs.

Early signs:

- Night blindness, growth retardation, appetite loss, reduced taste, recurrent infections, dermatitis and dry mucous membranes.

Late Signs:

- Bone growth failure, aspermatogenesis, xerophthalmia (dry, thickened, luster less eyeballs, and blindness) [90].

The chemical deficiency sign is reduction in plasma or serum vitamin A. Generally retinol values below 0.7μmol/L are associated with clinical symptoms, and values above 1.75 are not [91]. Vitamin A itself is not excreted in human urine.

1:4:1:3- Pathophysiology

Because retinol and retinol-binding protein (RBP) are secreted from liver as a 1:1 complex, low plasma concentrations of both are seen in vitamin A deficiency. Adequate concentration of vitamin A usually indicates dietary and tissue adequacy, but low concentrations do not always indicate dietary deficiency. Factors that reduce hepatic synthesis of RBP, or secretion of the RBP-retinol complex, lower concentrations of retinol and RBP, even though dietary intake and the hepatic retinol store are adequate. These states are primarily recognized by the absence of an increase in retinol after oral therapy with vitamin A and include protein-calorie malnutrition, liver disease, zinc deficiency, and cystic fibrosis.

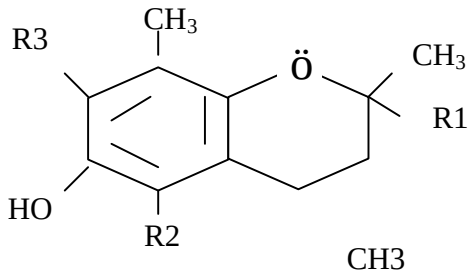
Pathophysiological conditions that can result in increased retinol and RBP include chronic renal disease and use of oral contraceptives [92].

1:4:2- Vitamin E

A factor in vegetable oils that restored fertility to rats was isolated in the early 1920S as vitamin E, later it was given the generics name tocopherol and was shown to include several biologically active isomers as shown in figure (1-6)[93].

The word tocopherol is greek derivation, meaning an oil that "bringsforth in childbirth," but the fertility role of these compounds is still questionable. α-Tocopherol is the predominant isomer in plasma and is the most potent isomer by current biological assays.

Dietary sources of tocopherols include vegetable oils, fresh leafy vegetables, eggs yolk, legumes, peanuts, and margarine. Diets suspect for vitamin E deficiency are those low in vegetable oils or fresh green vegetables or those high in unsaturated fats [85].



R1= — (CH_2— CH_2— CH— CH_2)$_3$H Tocopherol

R2	R3	
CH_3	CH_3	α
CH_3	H	β
H	CH_3	γ
H	H	δ

Fig.(1-6): Vitamin E isomers (Manohar, 2003)[93].

1:4:2:1- Function

Vitamin E function as an antioxidant, protecting unsaturated lipids from preoxidation (Cleavage of fatty acids at unsaturated sites oxygen addition across the double bond and formations of free radicals) [94].

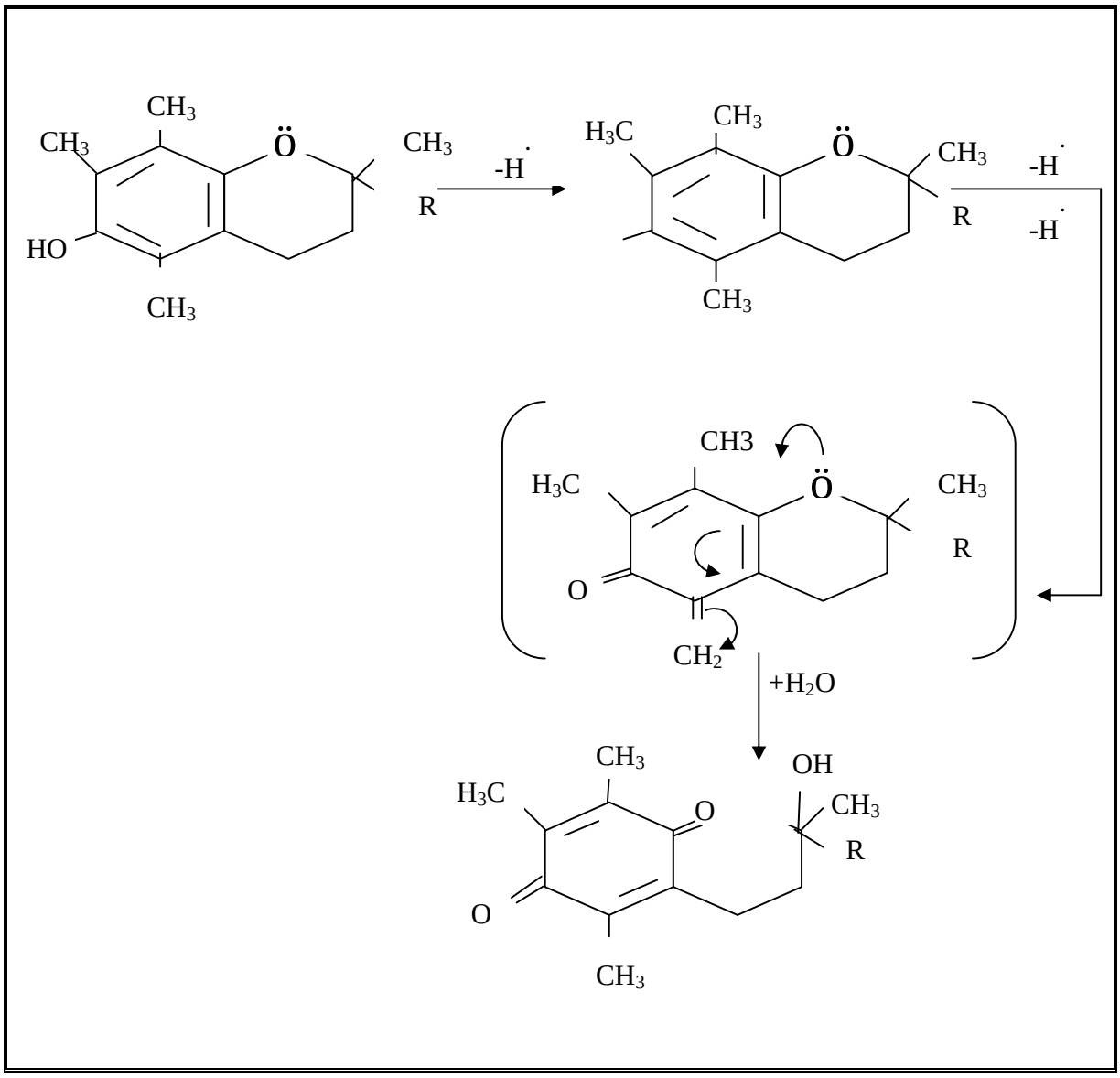
Vitamin E is the major chain-breaking in body tissues and is the first line of defense against lipid preoxidation, protecting cell membranes from free radical attack through its free radical quenching activity by participating in the following reactions [95].

$$RH^{\bullet} + \alpha TH \rightarrow H_2O + \alpha T^{\bullet} \qquad LOO^{\bullet} + \alpha TH \rightarrow LOOH + \alpha T^{\bullet}$$

$$RO^{\bullet} + \alpha TH \rightarrow ROH + \alpha T^{\bullet} \qquad L^{\bullet} + \alpha TH \rightarrow LH + \alpha T^{\bullet}$$

$$ROO^{\bullet} + \alpha TH \rightarrow ROOH + \alpha T^{\bullet}$$

Chemically, α-tocopherol (αTH) may undergo the following sequence of reactions leading to the formation of α-tocopherol quinone:



Vitamin E regulates platelet aggregation by inhibiting platelet cyclooxygenase activity and thus decreases prostaglandin production. It has a role in regulation of protein kinase C activation[96].

1:4:2:2- Clinical and Chemical deficiency signs

Vitamin E clinical deficiency signs are mild hemolytic anemia, ataxia loss of tendon reflexes, pigmentary retinopathy. Patients with conditions resulting in fat malabsorption, especially cystic fibrosis and abetalipoproteinemia are suspect for vitamin E deficiency. A relationship has been recognized between vitamin E deficiency and progressive loss of neurological function infants and children with chronic cholestestasis [91]. The chemical deficiency sign is reduction in serum vitamins E. Plasma concentrations of α-tocopherol below 5 mg/L are associated with increased erythrocyte hemolysis in the presence of hydrogen peroxide and are thus designated "deficient". There is a strong correlation between plasma α-tocopherol and plasma lipids, suggesting that plasma concentrations should be interpreted relative to plasma lipids level; 0.8 mg of α-tocopherol per gram of total plasma lipids appears to indicate adequate levels of vitamin E infants. Elevation of plasma total lipids above 15 g/L can apparently shift erythrocyte α-tocopherol to plasma, potentially altering erythrocyte susceptibility despite "adequate" plasma concentrations of α-tocopherol in hyperlipidemic states [97].

1:4:2:3- Pathophysiology

At the present time assessment of vitamin E status is primarily indicated in newborns, in persons with potential fat malabsorption states, and in persons receiving synthetic diets.

Elevated values of serum vitamin E have been reported during pregnancy and in patients with Battent's disease (a progressive childhood

encephalopathy with disturbed Poly Unsaturated Fatty Acid (PUFA) metabolism). Decreased serum values have been reported in patients with grand mal Seizures, and in persons exposed to non symptomatic doses of organophosphates [98, 99].

1:4:3- Vitamin C

The symptom cluster known as scurvy (swollen gums with loss of teeth, skin lesions, and pain and weakness in the lower extremities) was clearly described during the crusades and became common place when long seavoyages began [100].

This anti scurvy agents, Vitamin C, was isolated in 1932 and later given the name ascorbic acid (from its anti scorbutic effect). The structures of this water-soluble vitamin and its related compounds, are shown in figure (1-7).

Major dietary sources include fruits (especially citrus fruits) and vegetables (tomatoes, green peppers, cabbage and potatoes). A quantitatively significant dietary source is ascorbate added to other foods as a preservative.

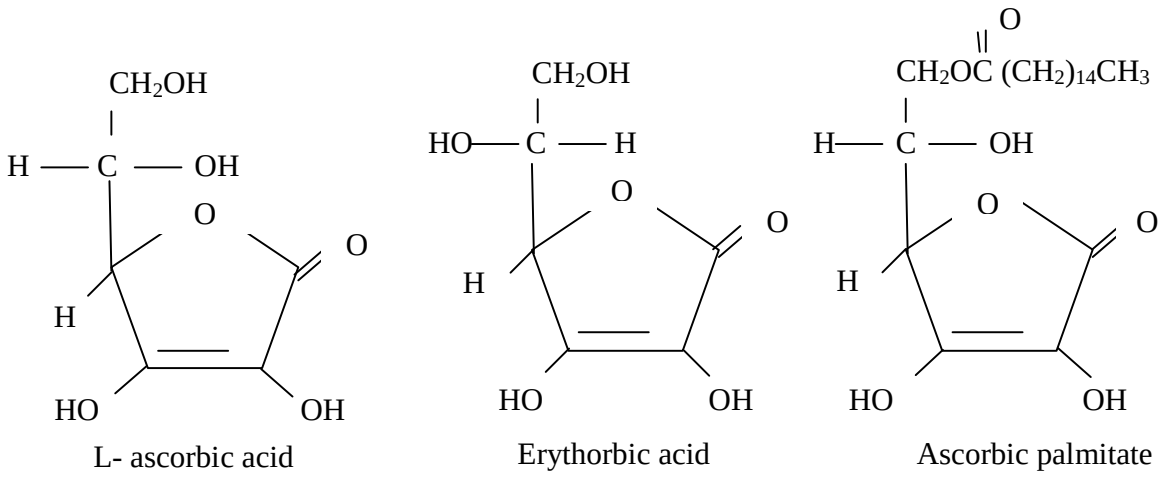


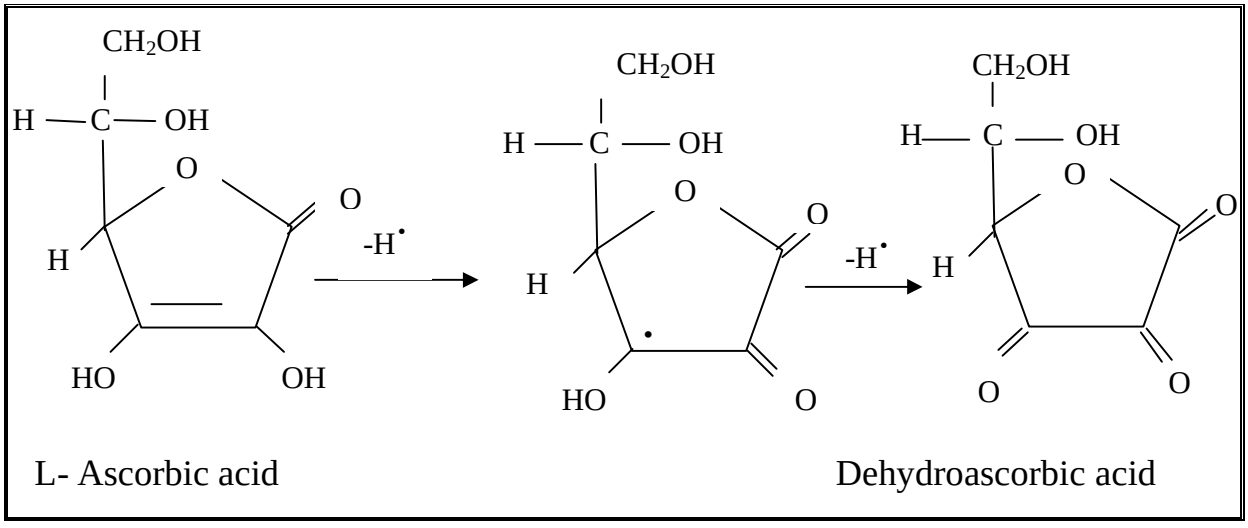
Fig.(1-7): Structures of ascorbic acid and related compounds (Devaraj, 2000)[101].

1:4:3:1- Function

There are numerous benefits that can be attributed to vitamins C, including strengthening of bones and connective tissue, aiding in the healing of wounds, and increasing the performance of the immune system. One of vitamin C best attributes is its amazing anti-oxidant ability [102-104].

It protects the fluids of the body such as blood from damage by free radicals, by strengthing arterial walls.

Ascorbic acid act as quenching of singlet oxygen, removal of molecular oxygen and hydrogen donations to lipid radicals as shown in the followings sequence of reactions leading to the formation of dehydro-ascorbic acid from L-ascorbic acid by losing two radicals hydrogen [105].



L- Ascorbic acid Dehydroascorbic acid

Ascorbic acid act as a general water soluble antioxidant, e.g. in reducing oxidized α- tocopherol in membranes as shown in figure(1-8) and may inhibit the formations of nitrosamines during digestion[106].

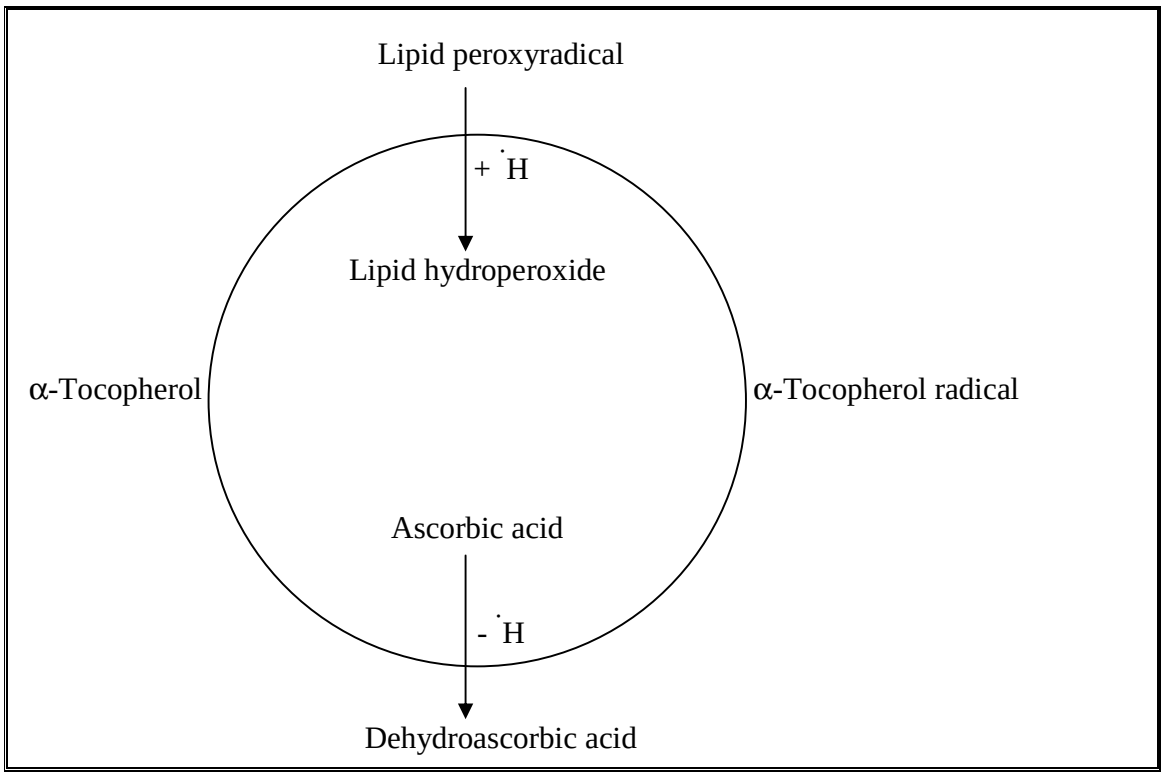


Fig.(1-8): Synergistic effect of α- Tocopherol & Ascorbic Acid (Stoyanavsky, 1995)[106].

In addition there is evidence for ascorbate involvement in hydroxylation reaction (including conversion of cholesterol to bile acids), and act as a prooxidant by converting the reducing ferric to ferrous ion, which become more active catalysts of oxidation in the aqueous system [107].

1:4:3:2- Clinical and Chemical deficiency Signs

Clinical signs of ascorbic acid deficiency can be separated into early and late signs.

Early signs:

- ❖ weakness, lassitude irritability vague aches, and pains.

Late signs:

- ❖ Scurvy (hemorrhages into skin alimentary and urinary tracts, other tissues, osteoporotic bones, defective tooth formation anemia, pyrexia, and delayed wound healing).

The chemical signs of scurvy have been associated with serum ascorbate values below 2.4mg/L or whole blood ascorbate levels below 3mg/L. The reference range for ascorbic acid is between 28-84 μmol/L [108].

1:4:3:3- Pathophysiology

Ascorbic acid requirements are increased in chronic illness, during pregnancy, and during oral contraceptive use. Deficiency has been observed in infant receiving breast milk from deficient mothers. There is controversy as to whether the transient tyrosinemia observed in many infant is a harmless condition or one needing therapy with ascorbate to avoid impaired mental development [109].

1:5- Aim of study

The aim of this study is conduct for children with IDDM to measure:

1- The concentration of the antioxidant vitamins (A,C ,and E).

2- The concentration of homocysteine .

3- Effect of age and sex on the level of vitamins and homocysteine.

4- Comparison between two levels of vitamins and homocysteine in healthy control groups& diabetic patients

3 –Results

3:1- Biochemical changes of healthy control and diabetic subjects:

3:1:1- Fasting Blood Sugar (F.B.S)

Fasting blood sugar (F.B.S) was measured for healthy control and diabetic subjects, its mean values and SD at 95 % Confidence Interval (CI) were given in table (3-1) and showed graphically in figure (3-1).

Table (3-1): Concentration of F.B.S for healthy control and diabetic.

Sample	Fasting blood sugar µmol/L			
	n	Mean ± SD	95% CI of mean	p-value
Control	30	5.373±0.911	4.713- 6.033	< 0.001
Diabetic	34	13.636±5.021	10.231-17.040	

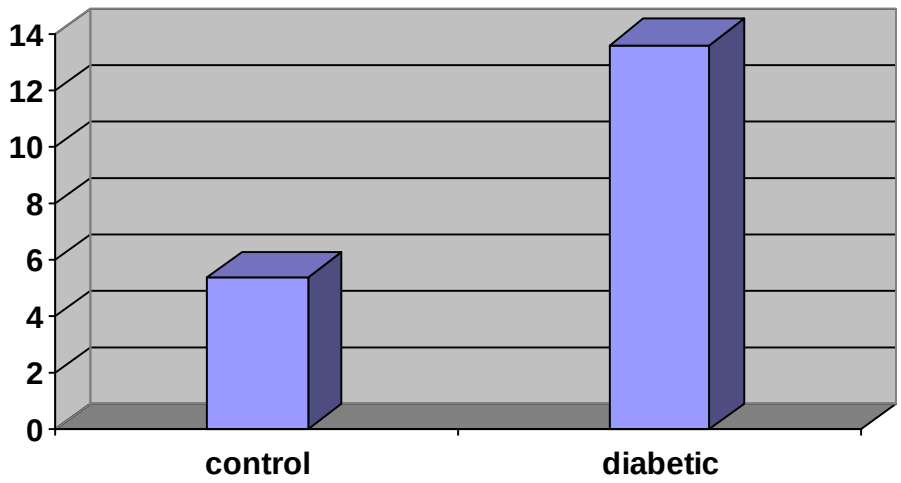


Fig.(3-1): Concentration of F.B.S for healthy control and diabetic.

3:1:2- Homocysteine

This study showed a significant higher homocysteine concentration between healthy control (6.498±1.739) and diabetic subjects (10.249±1.886) with p-value <0.001 as shown in table (3-2) with values of 95% CI for healthy control and diabetic.

Table (3-2): Homocysteine concentration in healthy control and diabetic subjects.

Sample	Homocysteine µmol/L			
	n	Mean ± SD	95% CI	p-value
Control	30	6.498±1.739	5.8-7.1	< 0.001
Diabetic	34	10.249±1.886	9.6-10.9	

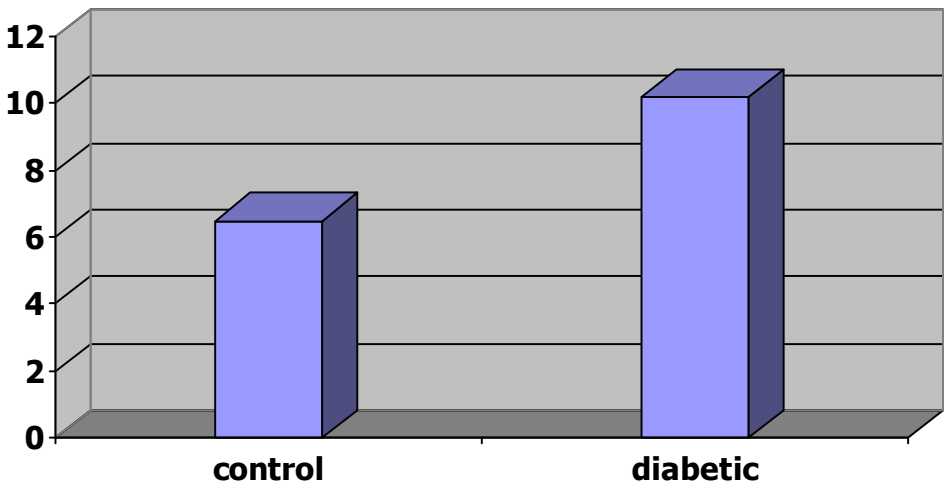


Fig.(3-2): Homocysteine conc. in healthy control and diabetic subjects.

3:1:3 -Serum Vitamins

Table (3-3) & figure (3-3) show highly significant difference between concentration of antioxidant vitamins in healthy control and diabetic. Vitamin A significantly decreased in diabetes patients 0.693±0.125 compared with healthy control subjects 1.176±0.291. The results of vitamin C showed there were a highly significant difference ($p<0.001$) between healthy control 38.222±1.237 and diabetic subjects 22.293±5.296. Serum vitamin E levels were decreased significantly in diabetes 9.341±1.902 compared with healthy control subjects 16.342±4.089 with $p<0.001$.

Table (3-3): Vitamins concentration in healthy control and diabetic subjects.

Vitamins µmol/L	Control n=30	Diabetic n=34	p- value
	Mean ± SD	Mean ± SD	
Vit. A	1.176±0.291	0.693±0.125	<0.001
Vit. C	38.222±1.237	22.293±5.296	<0.001
Vit. E	16.342±4.089	9.341±1.902	<0.001

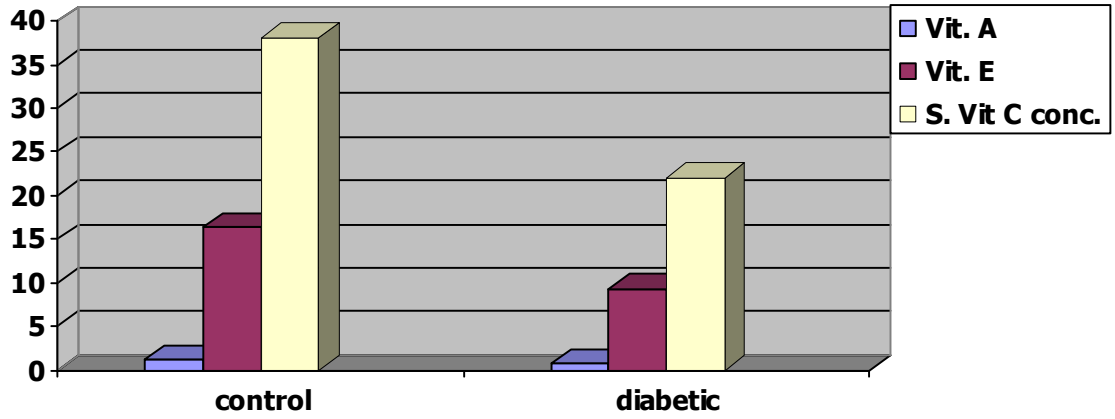


Fig.(3-3): Vitamins conc. in healthy control and diabetic subjects.

3:2 -The effect of age on biochemical changes

The thirty healthy control subjects and thirty four diabetes were divided into three groups according to their ages. The first group ranged from 1.5-5 years. The second group from 6-10 years, while the third group ranged from 11-15 years.

This part of the study demonstrates in general a clinically useful relationship between serum homocysteine, vitamin A, vitamin C, and vitamin E in diabetes patient with their age as compared to healthy control subjects.

3:2:1 -Serum Homocysteine

In the first group, the serum levels of homocysteine for healthy control and diabetic subjects were 4.848±1.296 and 8.819±0.975, respectively. These results indicated that there was highly significant differences in serum levels between healthy control homocysteine and diabetic subjects $p< 0.001$. Serum levels of homocysteine were increased significantly in diabetic subjects 9.255±1.105 compared to healthy control 6.944±0.879 with $p<0.001$. In the third age group there was significantly increased in diabetes patients 11.95±1.462 than in control subjects 8.153±1.148 with $p<0.001$.

These result demonstrated that serum homocysteine concentrations were increased in diabetic in all age groups compared to healthy control subjects.

Table (3-4): A comparison of the variation in serum homocysteine concentration for healthy control &diabetic with their age.

Range of age	No. of patient	Homocysteine concentration µmol/L		
		Mean ± SD of control	Mean ± SD of diabetic	P- value
(1.5-5)year	9	4.848±1.296	8.819±0.975	<0.001
(6-10)year	11	6.944±0.879	9.255±1.105	<0.001
(11-15)year	14	8.153±1.148	11.95±1.462	<0.001

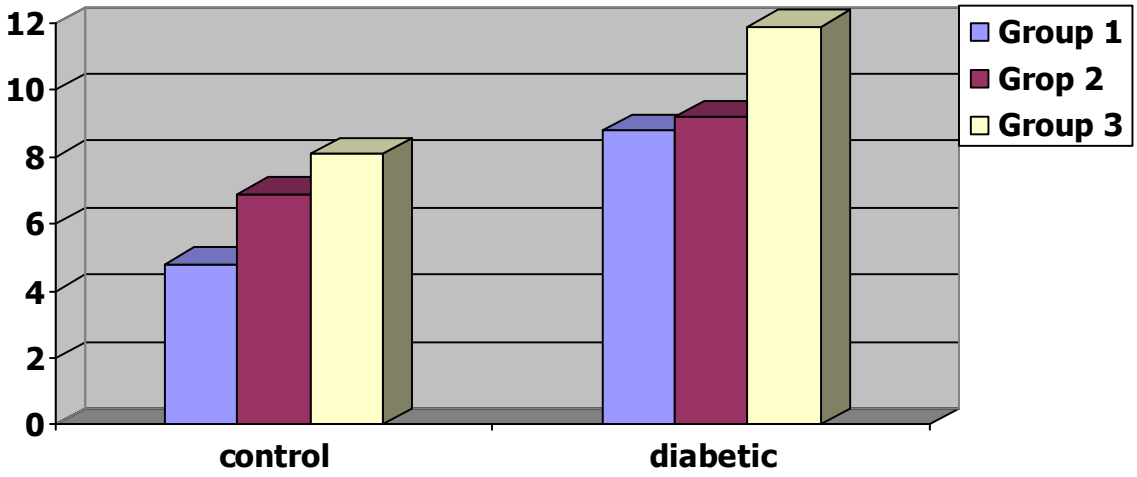


Fig.(3-4): Serum homocysteine level in diabetic compared to healthy control according to their age groups.

3:2:2- Serum Vitamin A

In group one (1.5-5)year, vitamin A levels was decreased significantly in diabetic 0.613±0.122 comparing to healthy control subjects 1.076±0.202 p<0.001. Serum vitamin A concentration was highly significant difference in healthy control 1.174±0.263 than in diabetic 0.682±0.110 in group two(6-

10) year. In the last age group (11-15)year there was significantly difference between the concentration of vitamin A in healthy control and diabetic subjects $p<0.001$, mean values ±SD were 1.314±0.388, 0.753±0.112 for healthy control and diabetic subjects respectively. The serum vitamin A levels in diabetes patients were found to decrease when compared to healthy control subjects in all age groups. These findings are listed in table(3-5)and shown graphically in figure(3-5).

Table (3-5): Means serum vitamin A concentration in healthy control and diabetic subjects with different age groups.

Range of age	No. of patient	Vitamin A concentration μmol/L		
		Mean ± SD of control	Mean ± SD of diabetic	P- value
(1.5-5)year	9	1.076±0.212	0.613±0.122	<0.001
(6-10)year	11	1.174±0.263	0.682±0.110	<0.001
(11-15)year	14	1.314±0.388	0.753±0.112	<0.001

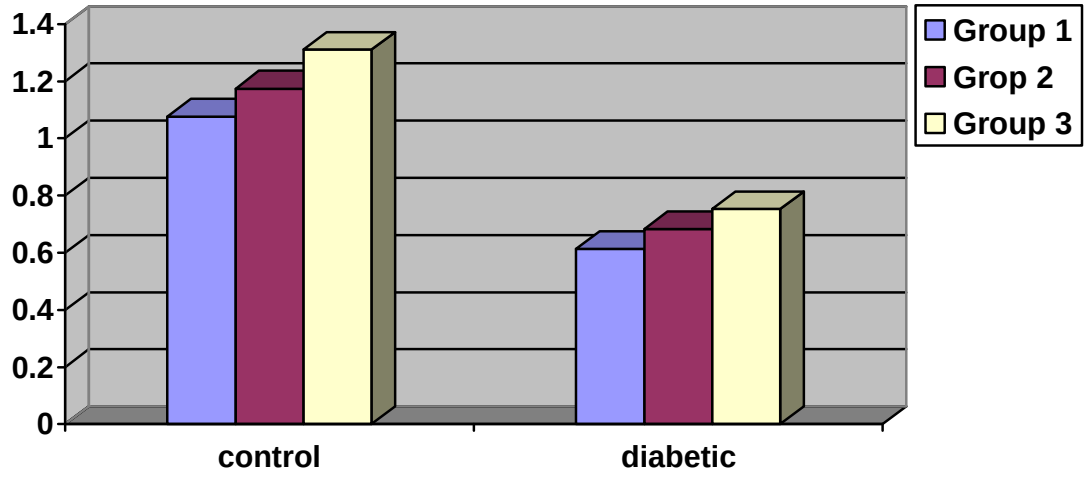


Fig.(3-5): A comparison of the variation of vitamin A level for healthy control and diabetic according to the age groups.

3:2:3- Serum Vitamin C

In the first, second, and third groups there was highly significant difference between the healthy control and diabetic subjects, P< 0.001. In the first group (1.5-5) year. Vitamin C concentration 31.716±3.636, 17.143±3.095 for healthy control and diabetic subjects ,respectively. Second group (6-10) year, vitamin C concentration in healthy control 36.951±1.223 and decreased significantly in diabetic to 20.845±5.192. In the last group (11-15) year, the concentration of vitamin C in healthy control 49.410± 8.778 and in diabetic subjects 26.742± 1.763. These findings listed in table(3-6) and shown graphically in figure (3-6).

Table (3-6): Means concentration of serum vitamin C in healthy control and diabetes patients according to their age groups.

Range of age	No. of patient	Vitamin C concentration µmol/L		
		Mean ± SD of control	Mean ± SD of diabetic	P- value
(1.5-5)year	9	31.716±3.636	17.143±3.095	<0.001
(6-10)year	11	36.591±1.223	20.845±5.192	<0.001
(11-15)year	14	49.410±8.778	26.742±1.763	<0.001

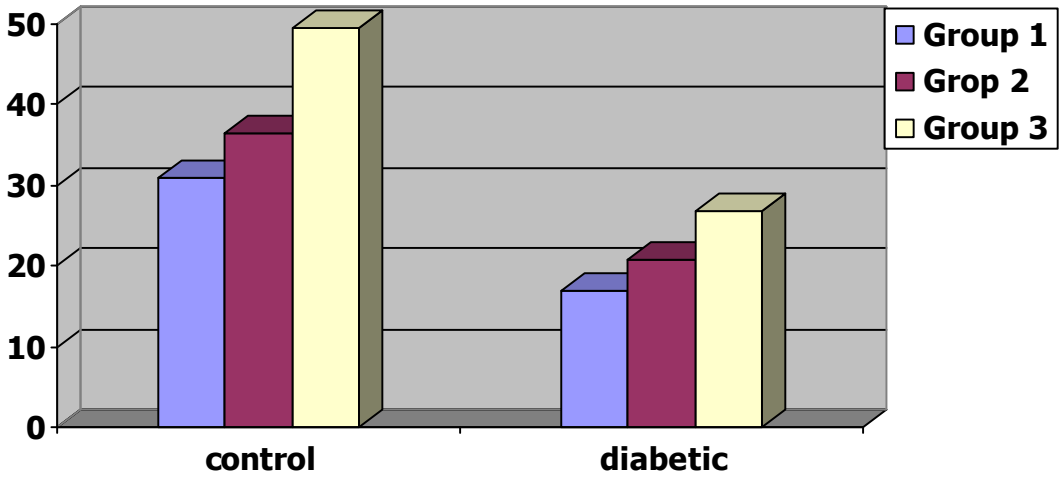


Fig.(3-6): Serum vitamin C level in diabetic compared to healthy control according to their age groups.

3 :2:4 -Serum vitamin E

Serum vitamin E levels in the first age group were found to decrease significantly in diabetic 7.019±1.047 compared to healthy control 13.986±1.582 with p< 0.001. In the second group we have found that serum vitamin E levels for healthy control 14.793±3.601 and in diabetic 9.315±1.292, also there was highly significant difference between them p<0.001. Vitamin E levels were observed highly significant difference in diabetic subjects 10.855±1.013 compared to healthy control subjects 21.714±1.311 with p<0.001. Serum vitamin E concentration was found to decrease significantly in diabetes patients compared to healthy control subjects in all age groups as shown in table (3-7) and figure (3-7).

Table (3-7): Means concentration of serum vitamin E in healthy control and diabetes patients according to their age.

Range of age	No. of patient	Vitamin E concentration μmol/L		
		Mean ± SD of control	Mean ± SD of diabetic	P- value
(1.5-5)year	9	13.986±1.582	7.019±1.047	<0.001
(6-10)year	11	14.793±3.601	9.315±1.292	<0.001
(11-15)year	14	21.714±1.311	10.855±1.013	<0.001

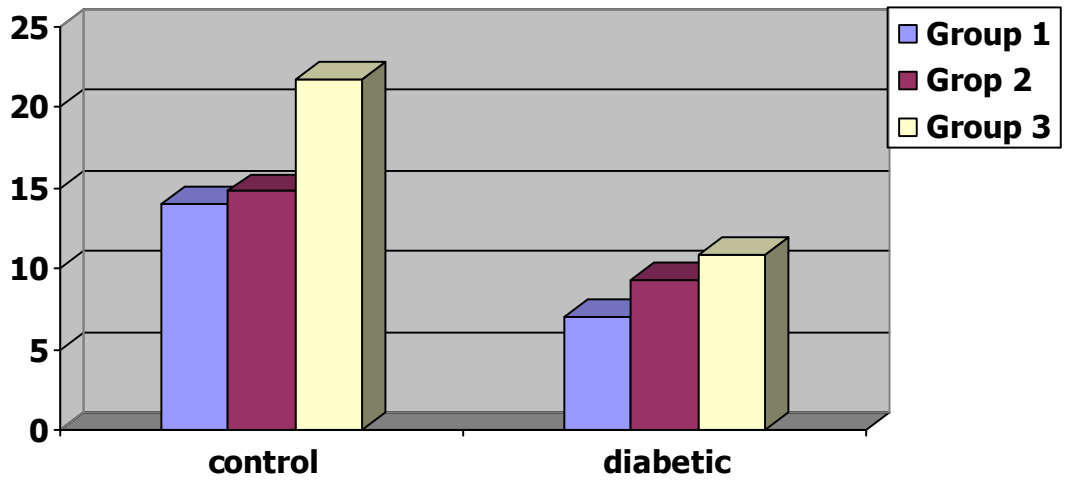


Fig.(3-7): Serum vitamin E level in diabetic compared to healthy control according to their age.

3:3 –The biochemical changes and sex

In this study there was no effect observed for sex on biochemical changes (homocysteine, vitamin A, vitamin C, & vitamin E) in diabetes (16 females, 18 males). Homocysteine levels were 9.804±1.394µmol/L in diabetic females and 10.644 ±2.199 µmol/L in diabetic males and there was no significant difference between them p>0.05. Vitamin A concentration was no significantly differ in diabetic female 0.685±0.119µmol/L compared to diabetic males 0.700±0.132µmol/L. Vitamin C level was 21.774±4.642 µmol/L and 22.754±5.912µmol/L in diabetic females and males, respectively p>0.05. Serum vitamin E concentration was increased in diabetic males 9.548±2.174µmol/L but it was not significantly differ when compared to diabetic females 9.108±1.579µmol/L.

All these findings compared to healthy control subjects(13 females, 17 males), listed in table (3-8) and shown graphically in figure (3-8).

Table (3-8): The biochemical changes level in female and male for healthy control &diabetic subjects.

Biochemical changes µmol/L	Control		Diabetic	
	Female n=13	Male n=17	Female n=16	Male n=18
Homocysteine	5.668±1.752	5.695±2.112	9.804±1.394	10.644 ±2.199
Vit. A	1.125±0.205	1.153±0.339	0.685±0.119	0.700±0.132
Vit. C	32.977±8.682	39.459±9.475	21.774±4.642	22.754±5.912
Vit. E	14.457±2.940	17.437±3.948	9.108±1.579	9.548±2.174
p- value	>0.001		>0.001	

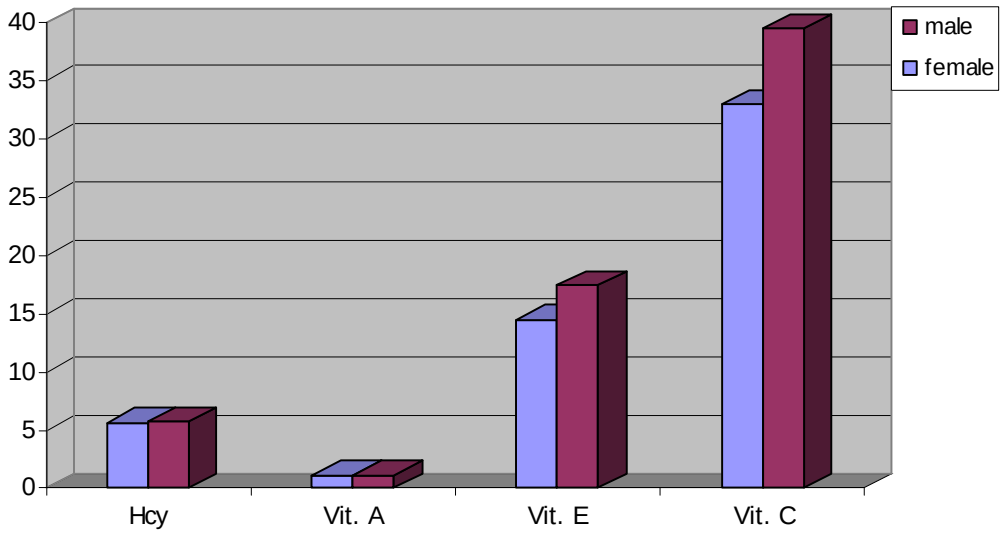


Fig.(3-8): Mean levels of biochemical changes in female and male for healthy control subjects .

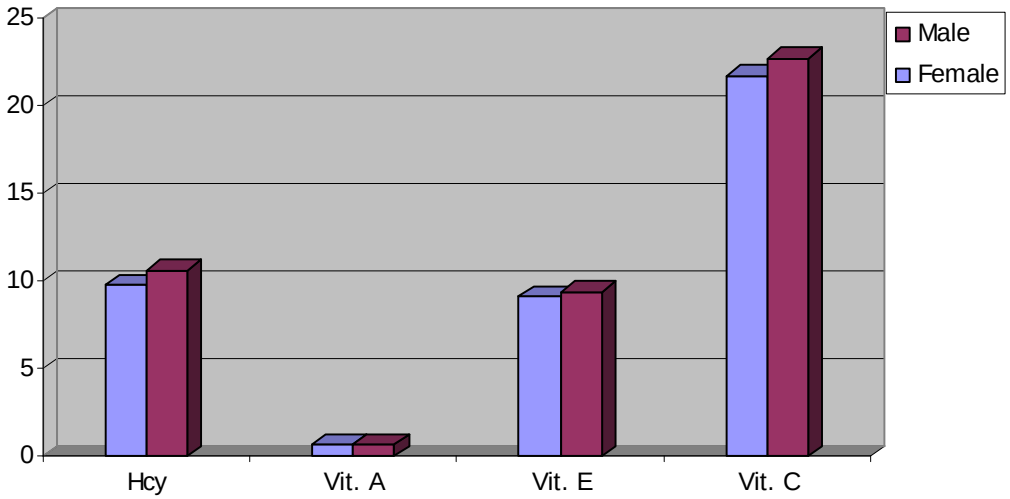


Fig.(3-9): Mean levels of biochemical changes in female and male for diabetes patients.

3:4-Correlation between age and biochemical changes

3:4:1- Age and serum homocysteine

There was a significant positive correlation between homocysteine level and age in healthy control subjects ($r=0.84$, $p<0.001$) and diabetic ($r=0.83$, $p < 0.001$) as shown in figures (3-10 and 3-11 respectively).

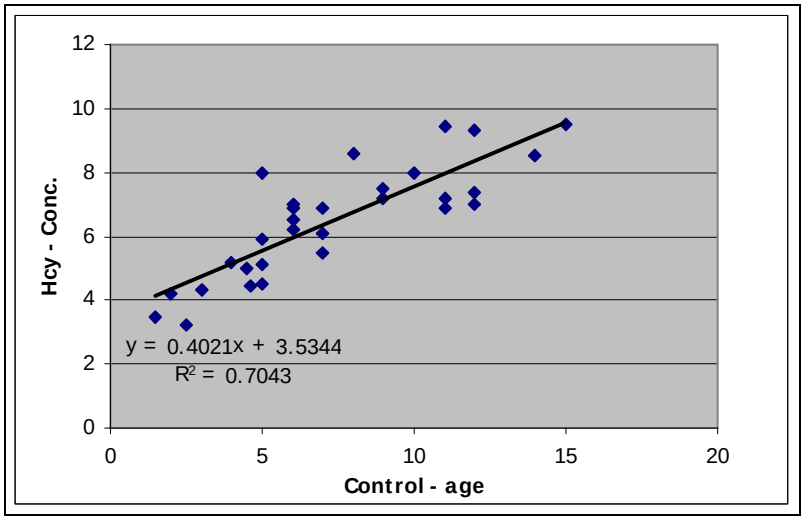


Fig.(3-10): Correlation between serum homocysteine level and age for healthy control subjects (n=30) $p<0.05$.

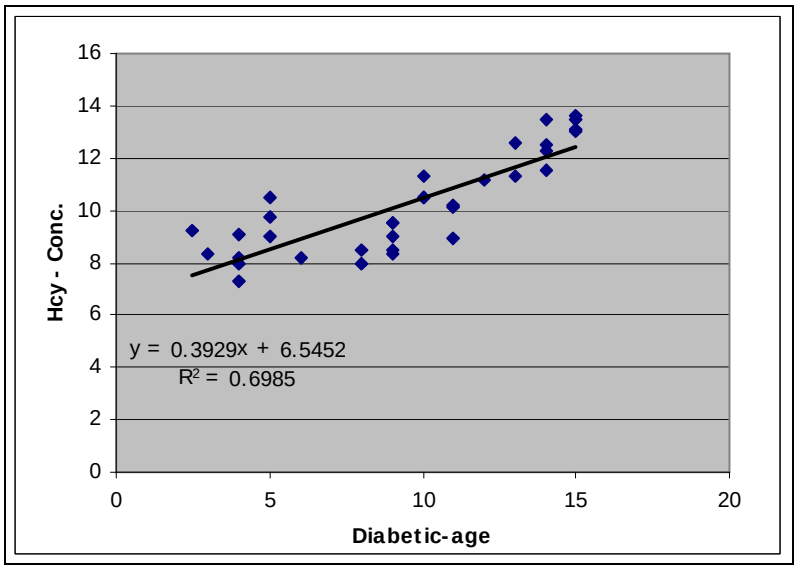


Fig.(3-11): Correlation between serum homocysteine level and age for diabetic subjects (n=34) $p<0.05$.

3:4:2- Age and serum vitamin A

Figures (3-12 and 3-13) illustrated the positive correlation between serum vitamin A level and age groups in healthy control subjects ($r=0.51$, $p<0.001$) and diabetes patients ($r=0.49$, $p < 0.001$).

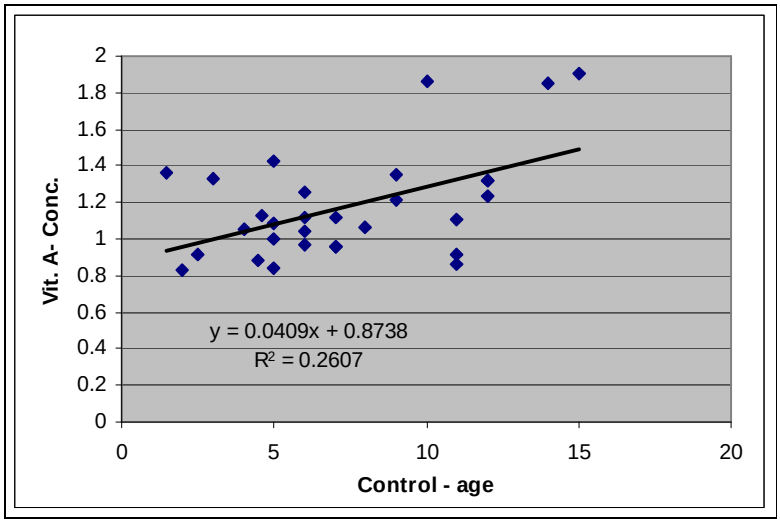


Fig.(3-12): Correlation between serum vitamin A level and age in healthy control subjects (n=30) $p<0.05$.

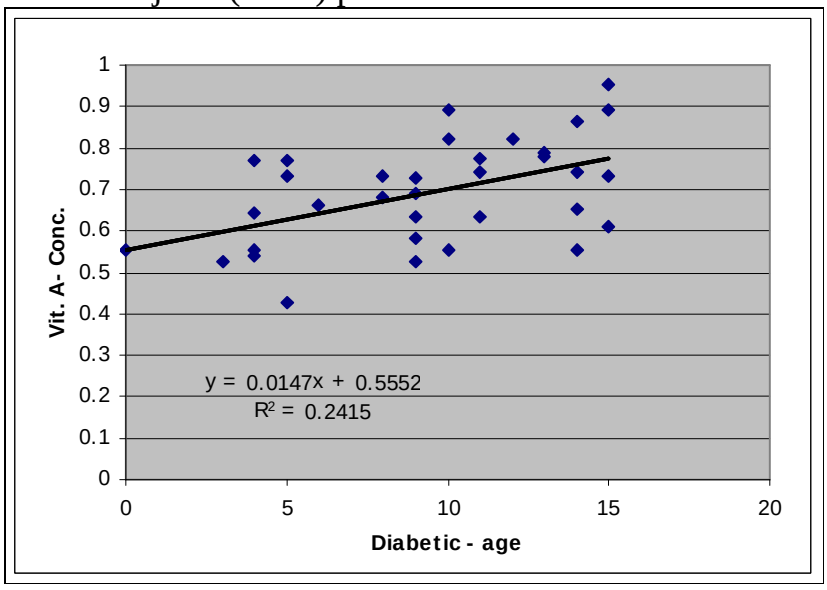


Fig.(3-13): Correlation between serum vitamin A level and age in diabetic subjects(n=34) $p<0.05$.

3:4:3- Age and serum vitamin C

There was positive correlation between serum vitamin C concentration and ages in healthy control subjects (r=0.68, p <0.001) and type 1 diabetes mellitus (r= 0.81, p < 0.001) as shown in figures(3-14 and 3-15respectively).

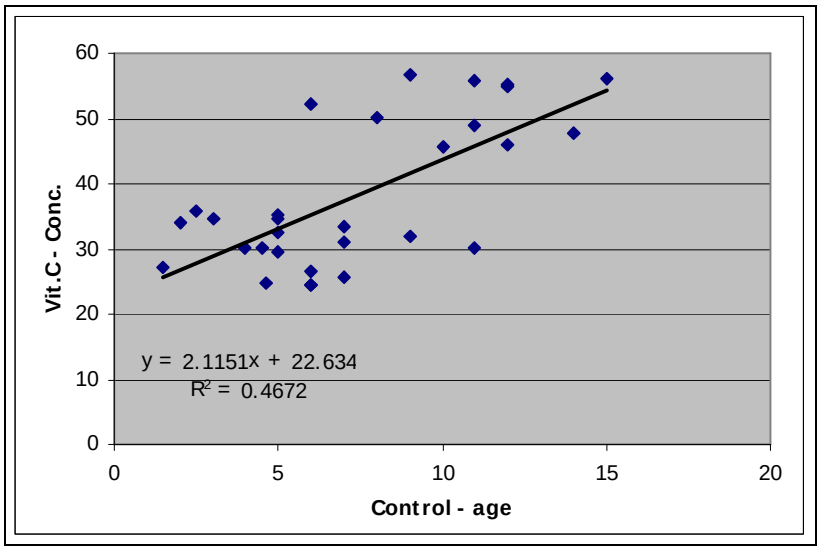


Fig.(3-14): Correlation between serum vitamin C level and age for healthy control subjects (n=30) p<0.05.

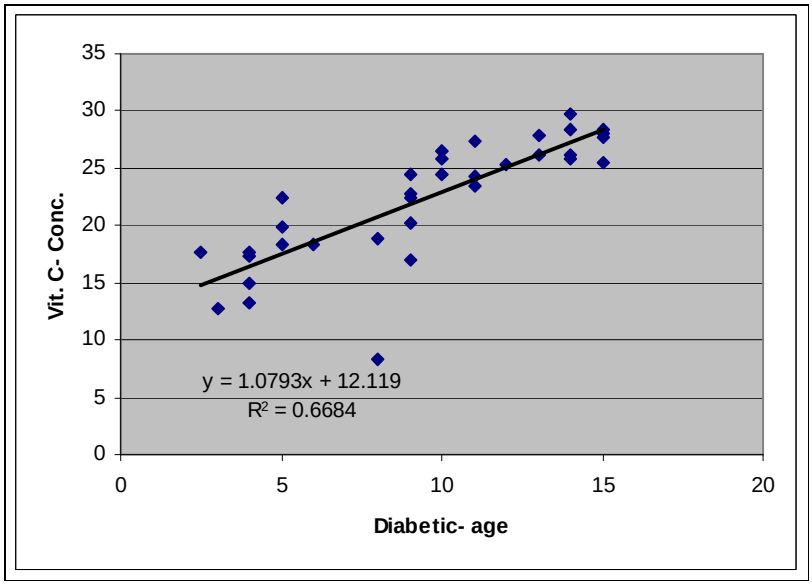


Fig.(3-15): Correlation between serum vitamin C level and age for diabetic subjects (n=34) p<0.05.

3:4:3- Age and serum vitamin E

The correlation between serum vitamin E level and age groups was positive correlation in healthy control subjects (r=0.81, p <0.001) and in diabetes patients (r=0.87, p < 0.001) as shown in figures (3-16 and 3-17 respectively).

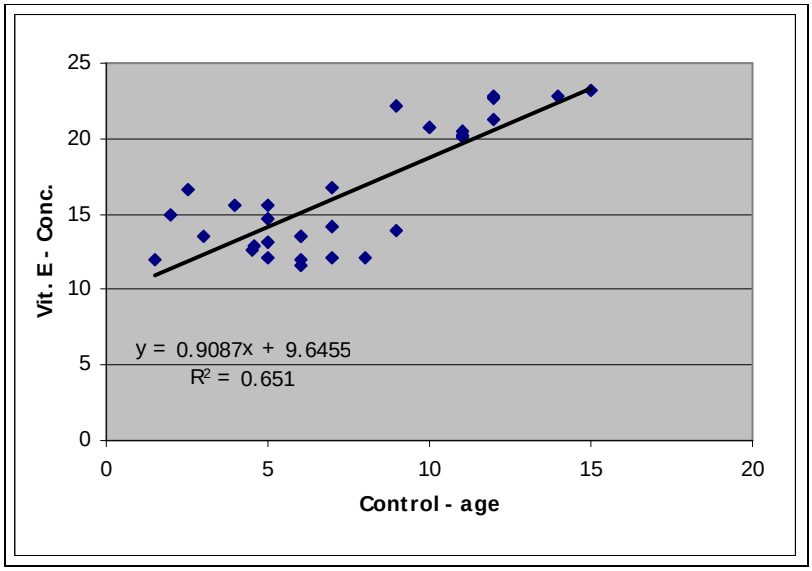


Fig.(3-16): Correlation between serum vitamin E level and age for healthy control subjects (n=30) p<0.05.

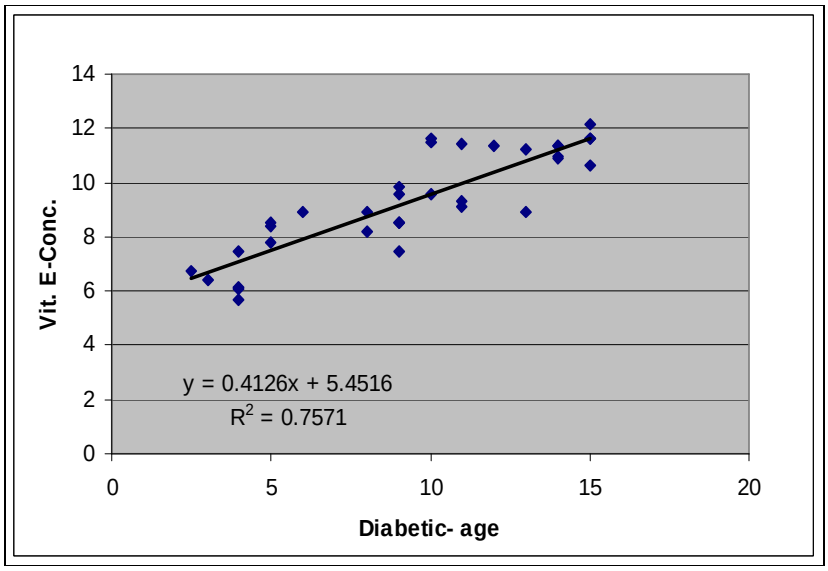


Fig.(3-17): Correlation between serum vitamin E level and age for diabetic subjects(n=34) p<0.05.

3:5- Correlation between homocysteine and antioxidant vitamins

3:5:1- Homocysteine and vitamin A

There was positive correlation between homocysteine and vitamin A among the diabetic groups(r=0.44, p < 0.05) compared to control groups (r=0.48, p <0.05) as shown in figures (3-18& 3-19).

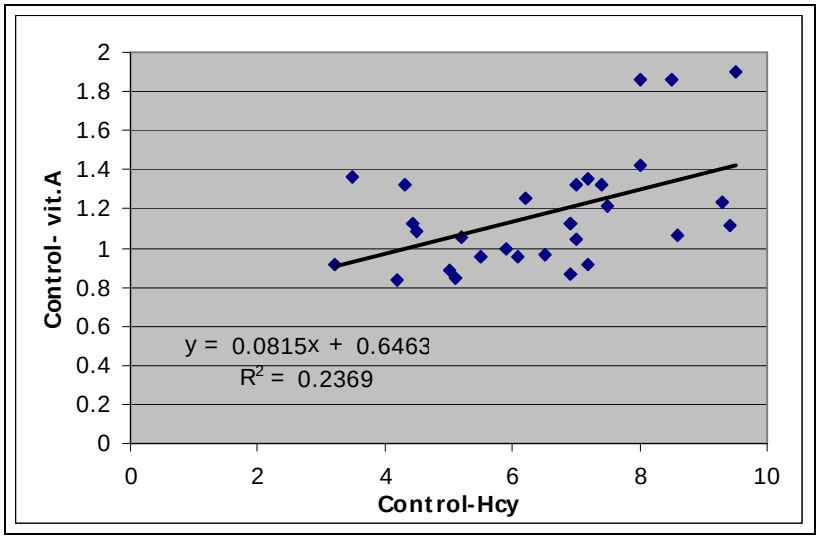


Fig.(3-18): Correlation between homocysteine and vitamin A for healthy control subjects(n=30) P<0.05.

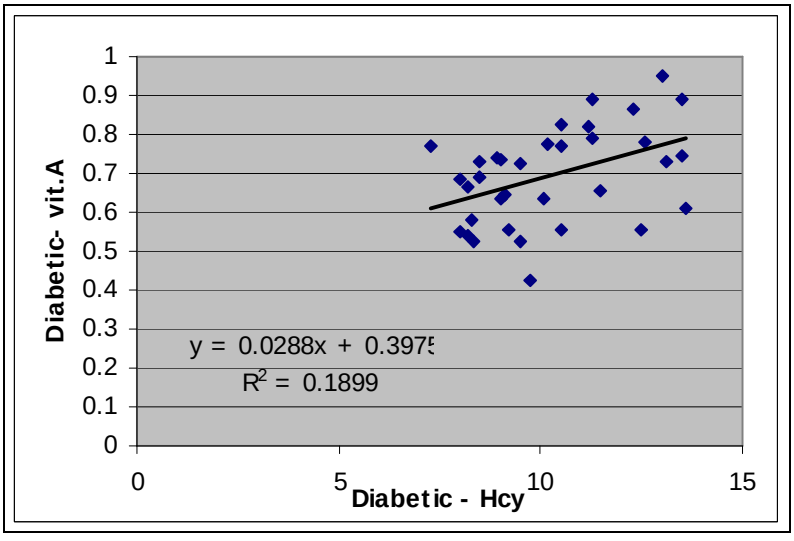


Fig.(3-19): Correlation between homocysteine and vitamin A for diabetic subjects(n=34) p < 0.05.

3:5:2- Homocysteine and vitamin C

There was positive correlation observed in figures(3-20 & 3-21) between homocysteine and vitamin C in healthy control (r=0.62, p <0.05)and diabetes patients (r=0.77, p < 0.05) respectively .

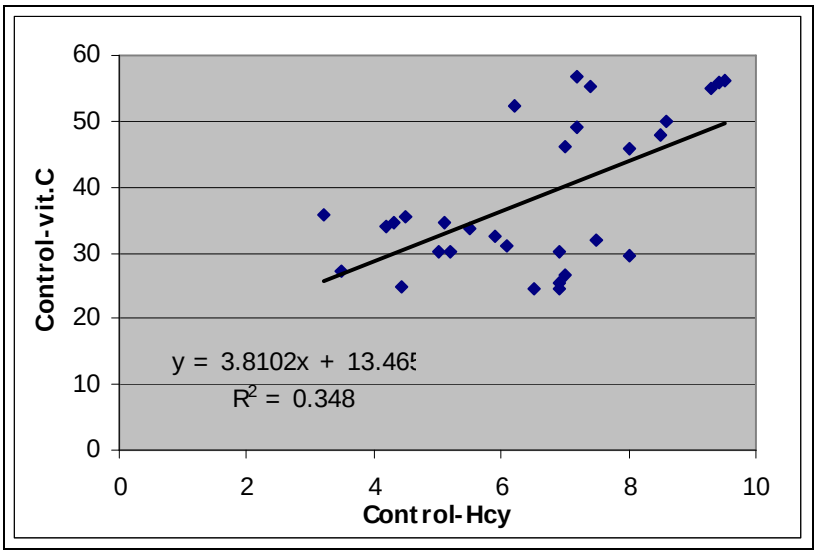


Fig.(3-20): Correlation between homocysteine and vitamin C for healthy control subjects(n=30) P<0.05.

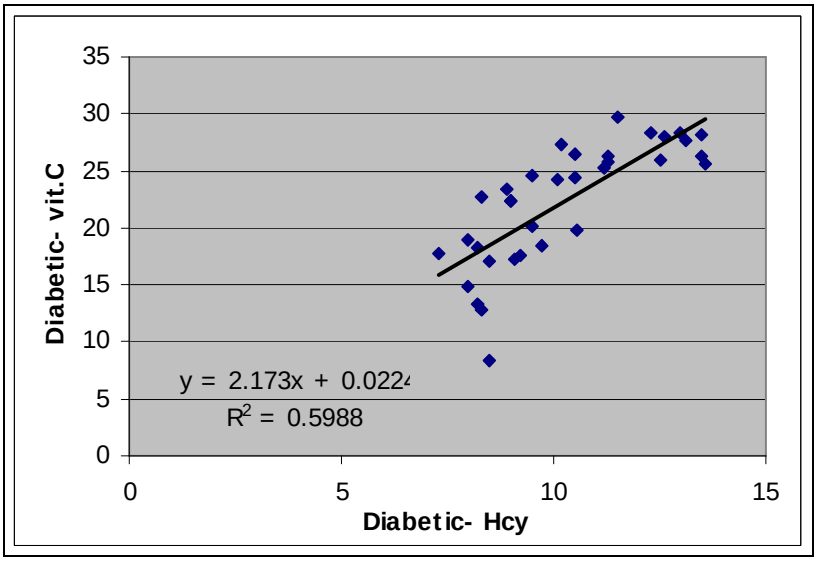


Fig.(3-21): Correlation between homocysteine and vitamin C for diabetic subjects(n=34) p < 0.05.

3:5:3- Homocysteine and vitamin E

In figure (3-22) there was positive correlation between homocysteine and vitamin E among healthy control subjects (r=0.49, p <0.05) , and there was also positive correlation between homocysteine and vitamin E among type 1 diabetes mellitus patients (r=0.79, p < 0.05)as shown in figure(3-23).

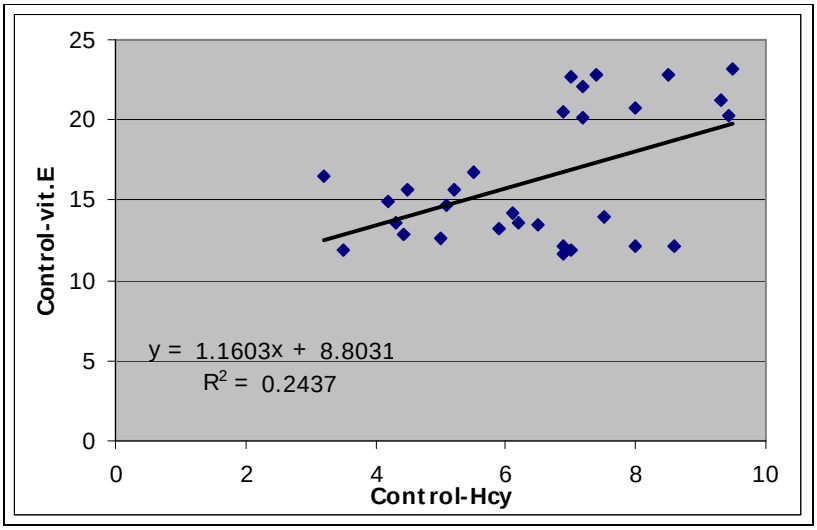


Fig.(3-22): Correlation between homocysteine and vitamin E for healthy control subjects(n=30) P<0.05.

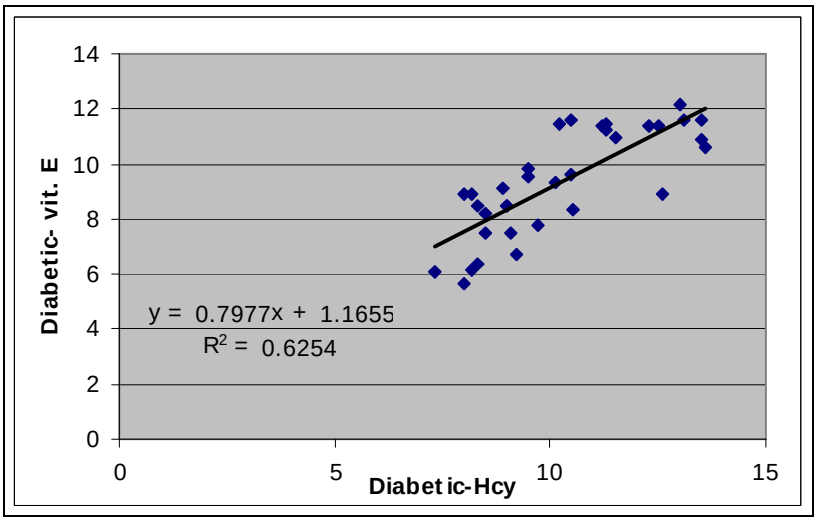


Fig.(3-23): Correlation between homocysteine and vitamin E for diabetic subjects(n=34) p < 0.05.

2-Experimental

2:1-Subjects

2:1: A- control

Thirty healthy control subjects (13females and 17 males) were taken, their age were ranged between 1.5-15 years. None of the subjects have a history of coronary heart diseases, renal failure, thyroid disease or any metabolic known diseases to have an influence on carbohydrate metabolism.

2:1: B - Patients

In this study thirty four diabetes mellitus patients are insulin dependent. None of those patients had liver, thyroid disease, renal failure or on lipid lowering drugs, steroid or other hormones. These patients consisted of 15 females and 19 males their age were ranged 1.5-15 years. None of these subjects have liver or thyroid disease, renal failure or on lipid lowering drugs, steroid or other hormones. All patients were undergoing insulin treatment, they were attended al-Kadhimiya teaching hospital and from national diabetes center. The characteristics of chosen subjects in this study are presented in table (2-1).

Table (2-1): Characteristics of healthy control and IDDM included in the study.

		Control(n=30)		Diabetic(n= 34)	
		n	%	n	%
Sex	Female	13	43.33	15	44.12
	Male	17	56.67	19	55.88
Age range/year		1.5- 15		1.5-15	

2:2-Sample collection

Five milliliters blood was collected from each patient. The blood samples were immediately transferred to a cleaned and sterilized tubes and allowed to stand at room temperature for 30 minutes to permit clot formation. The clots were separated from the walls of the tube using a wooden applicator stick after centrifuged for 15 min. at 3000(r.p.m). The serum was transferred to a second tube using a micropipette and stored in refrigerator from January -2005 until the day of analysis in March-2005.

2:3-Instruments

2:3:1-High Performance Liquid Chromatography (HPLC)[110]

2:3:1:1 -Pumping system

The chromatographic system consist of two pump model LC -10 AVP Shimadzu (Tokyo, Japan) deliver the mobile phase A and B from solvent reservoirs to the mixing cell to create the gradient system program for homocysteine analysis and controlled by Sil-6A system controller.

2:3:1:2- Column

Octa Decayl Silain C-18 column (ODS- Column) (250x4.6mm i.d) packed with 5µm particle size obtained from Fisher company (USA) was used. The column was thermo stating at 40 ºC using column's oven model CTO-6A (Shimadzu, Japan).

2:3:1:3- Detector

Homocysteine (Hcy) and antioxidant vitamins were detected by SPD-10AVP ultraviolet-visible detector with 8µl cuvette. The suitable wavelength was selected according to the sample absorption maximum required by individual samples.

2:3:1:4- System Control Unit

The controller solvent delivery units, column oven temperature, wave length, and flow rate are all controlled by Sil-6A system controller unit.

2:4- HPLC analysis

2:4:1- Reagents and solvents for HPLC analysis

Homocysteine standard, 5-sulphosalicylic acid (5-SSA), sodium dihydrogen phosphate& tetrahydrofuran(THF) (BDH, Pool, Dorset, U. K), Ascorbic acid (Merck Darmstadt), vitamin A and α-tocopherol(Supelco Park Belleonate USA), sodium acetate, acetonitrile, sodium hydroxide, ortho-phthaladhyde&2-mercaptoethanol (Aldrich Chemical Co., Mil-Waukee England) and Methanol for (HPLC grade). Deionized water was used for all preparations.

2:4:2 -Sample preparation

Two hundred microletter (200µl) of frozen serum after complete thawing at 4ºC was deprotenized by adding 25µl of (15%) 5-sulphosalicylic acid, mixed and centrifuged at 3000 (r.p.m)for 10 min at 4ºC.

2:4:3- Standards preparation

Stock solutions of homocysteine and antioxidant vitamins were prepared in deionized water at a concentration of 100 µg. Standard mixtures used for calibration during quantitative analysis were prepared by diluting the stock solution with deionized water to yield the final concentration of 10 µg for each individual component, and the mixture stored at (-20ºC to 80ºC) after being divided into 2 portions each portion contained 1ml.

2:4:4- Homocysteine analysis

2:4:4:1-Preparation of OPA /2-mercaptoethanol (2-MCE) reagent

Ortho phthaldhyde (OPA) reagent was prepared by dissolving 20 mg OPA in 0.5 ml of methanol (HPLC grade), adding 20µl of 2-mercaptoethanol &completing to 4ml with 0.4M sodium borate buffer adjusted to pH 9.5 with 4M sodium hydroxide. The solution was mixed and flushed with helium to displace dissolved oxygen, and stored in the dark for 24hr before use, this reagent was stable for at least two weeks [111]. A 1.6 µl of 2-mercaptoethanol was added each 3 days to maintain its potency.

2:4:4:2-Derivatization of Homocysteine

Aliquots of 25µl standard or 25µl deproteinized sera were added to 25µl OPA reagent. After 60 sec, 50µlof 0.02M sodium acetate (pH 5.9) was added. The solution mixed and after 1 min 20µl of this mixture was injected to be analyzed by HPLC and detected at 338 nm. Homocysteine concentration was determined by comparison of the peak area of the standard figure (2-3) with that of the sample under the same separation conditions which are given in table (2-2).

Table (2-2): HPLC conditions of gradient percentage for homocysteine analysis.

Chemical materials	Mobile phase A %	Mobile phase B %
Methanol	20	80
THF	2.5	2.5
0.02 M Sodium Acetate	77.5	17.5

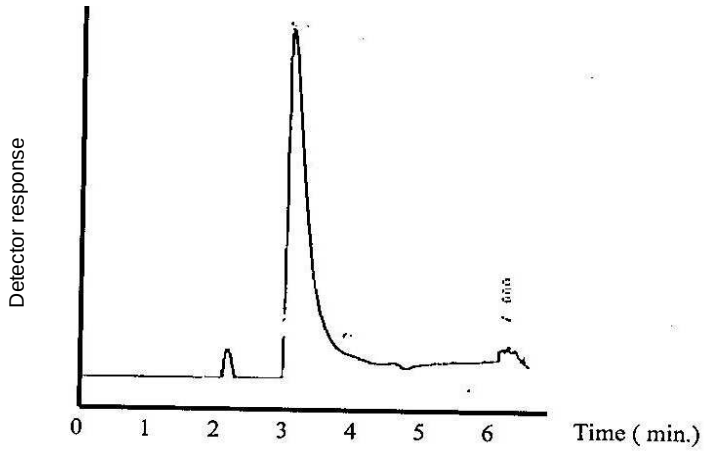


Fig.(2-1): Chromatogram of standard homocysteine on ODS column (25*0.46 cm i.d) flow rate 1 ml / min ,detection wavelength at 338 nm and using gradient elution conditions :
mobile phase A: Methanol, THF, 0.02 M Sodium Acetate pH 5.9 20:2.5:77.5 %
mobile phase B: Methanol, THF, 0.02 M Sodium Acetate pH 5.9 80:2.5:17.5 %

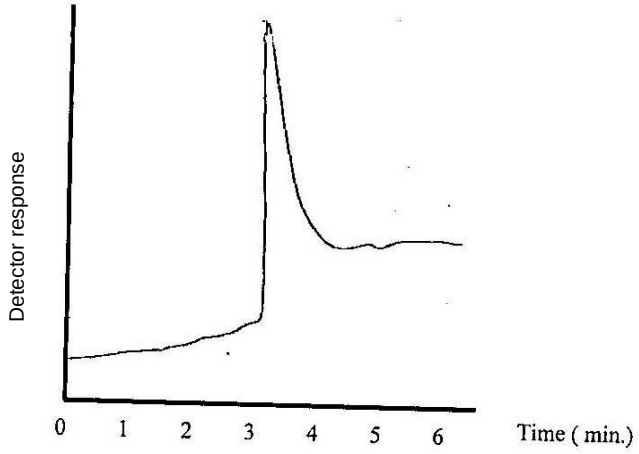


Fig.(2-2): Chromatogram of homocysteine in IDDM.

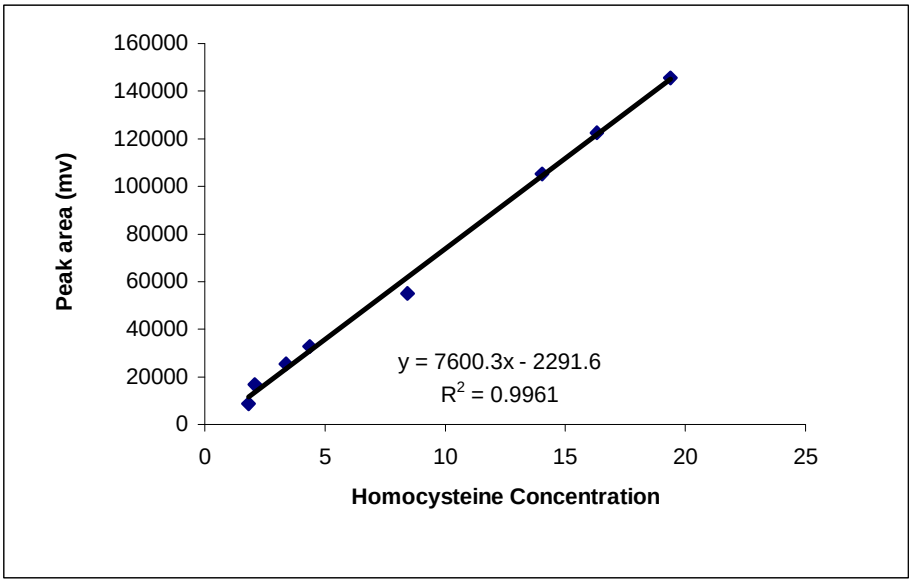


Fig.(2-3): Calibration curve between peak area &concentration of homocyst-eine separated on ODS column.

2:4:5-Vitamines analysis

A 0.25 mg ascorbic acid was dried at 80ºC for 2 hrs then cooled and stored over phosphorus pentoxide for 24 hrs. The weight required to prepare 25µg of vitamin was dissolved in 100ml deionized water containing 60% methanol. Diluted hydrochloric acid (0.1N) was added to bring the volume to 250 ml with deionized water [112].

Vitamins A&E (α-tocopherol) 100µg/ml were prepared. The concentrations of vitamens (A, C &E) were determined by comparison of the peak area of the standard with that of the sample under the same separation condition given as shown in figure (2-4).

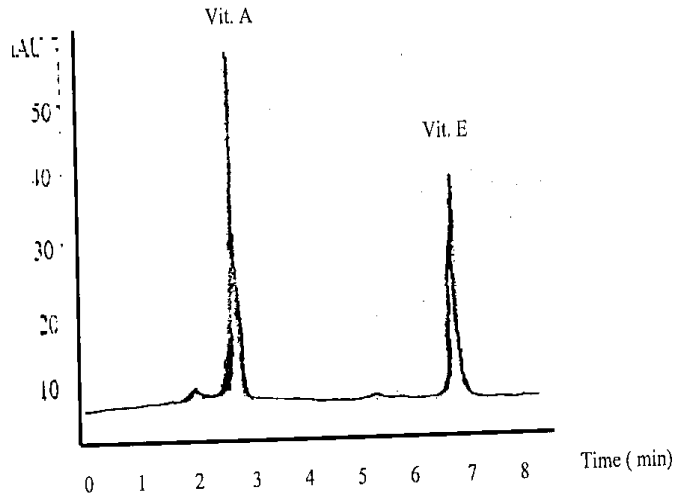


Fig. (2-4): Chromatogram of standard vit. A&E on ODS column (25*0.46 cm i.d), Mobile phase (100% acetonitrile), flow rate 1ml/min and detection wavelength at 290 nm.

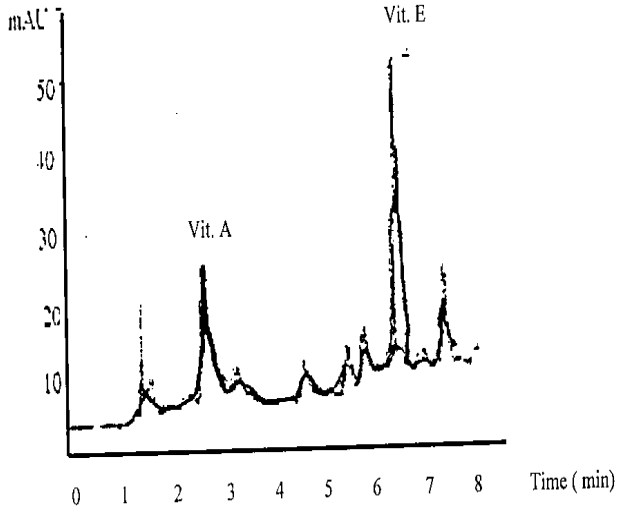


Fig. (2-5): Chromatogram of vit. A &E in IDDM.

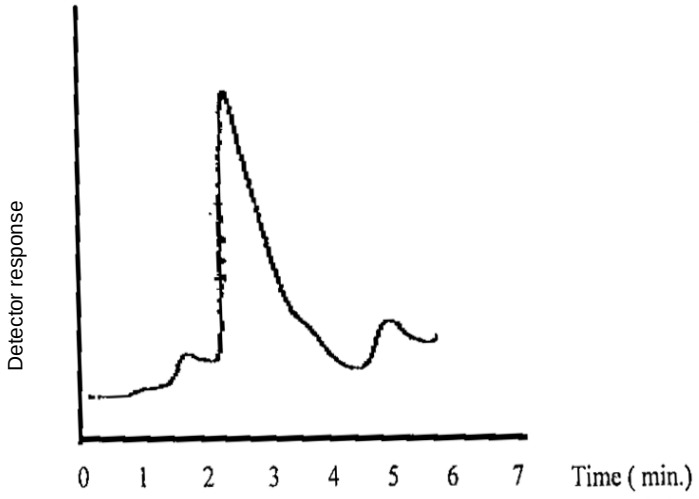


Fig.(2-6): Chromatogram of standard vit. C on ODS column (25x0.46 cm i.d), mobile phase (98:2%) methanol: water flow rate 1ml/min and detection wavelength 290nm.

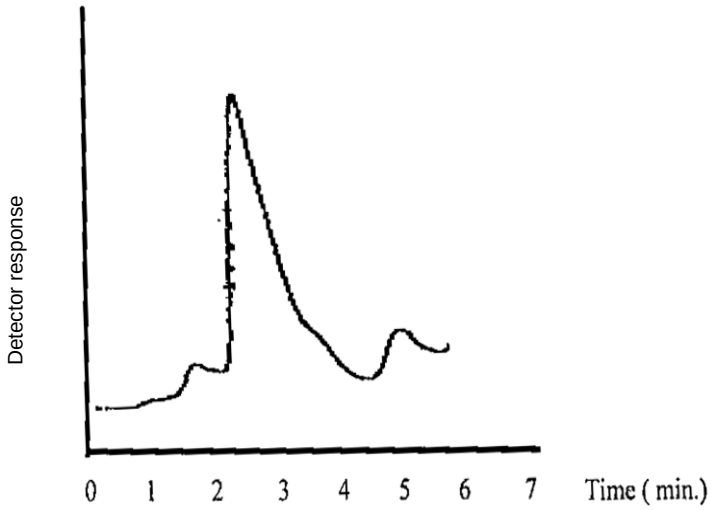


Fig.(2-7): Chromatogram of vit. C in IDDM.

2:6- Determination of Fasting Blood Sugar

Fasting blood sugar (FBS) measured using kits (Diamond, U.K), which were based on enzymatic determination of glucose[113,114].

2:6:1-Principle

Glucose present in the sample is determined according to the following reaction:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + 4H_2O$$

2:6:2-Reagent composition

Monoreagent: phosphate buffer 100 mmol/L pH7.5, glucose oxidase > 10 KU/L, 4-aminoantipyrine 0.5 mmol/L phenol 5 mmol/L.

2:7-Statistical analysis

Statistical analysis was performed with the SPSS 10.01 statistical package for social sciences. Data analysis was done using chi-square test. P-value of ≤ 0.05 was used as the level of significance.

Descriptive statistics for the biochemical features of patients and control was done using the range, mean and SD (standard deviation).

LIST OF ABBREVIATIONS

AGEs	Advance glycation end products
CAD	Coronary artery disease
CAT	Catalase
CI	Confidence interval
DM	Diabetes mellitus
DNA	Deoxy ribonucleic acid
FFA	Free fatty acid
GSH	Glutathione reductase
Hcy	Homocysteine
HPLC	High performance liquid chromatography
IDDM	Insulin dependant diabetes mellitus
LC	Liquid chromatography
LDL	low density lipoprotein
ODS	Octa decayl silain
OPA	Ortho phthaladhyde
PUFA	Poly unsaturated fatty acid
p-value	Probability factor
r	Correlation coefficient
RBP	retinol-binding protein
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Super oxide dismutase
THF	Tetra hydro furan
VLDL	Very low density lipoprotein
α-TH	Alpha- tocopherol

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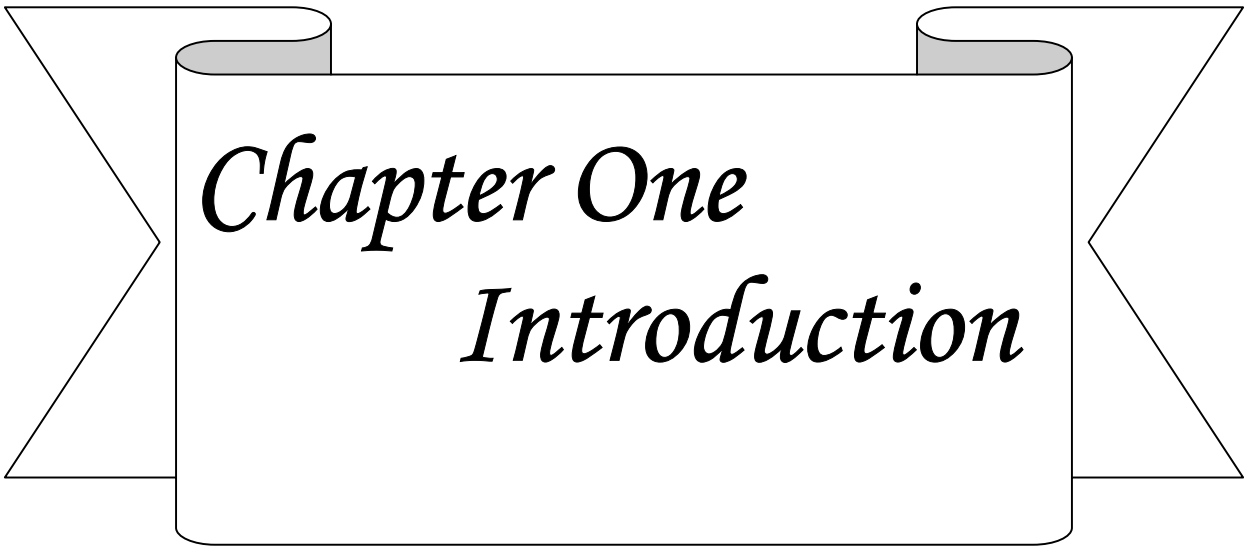
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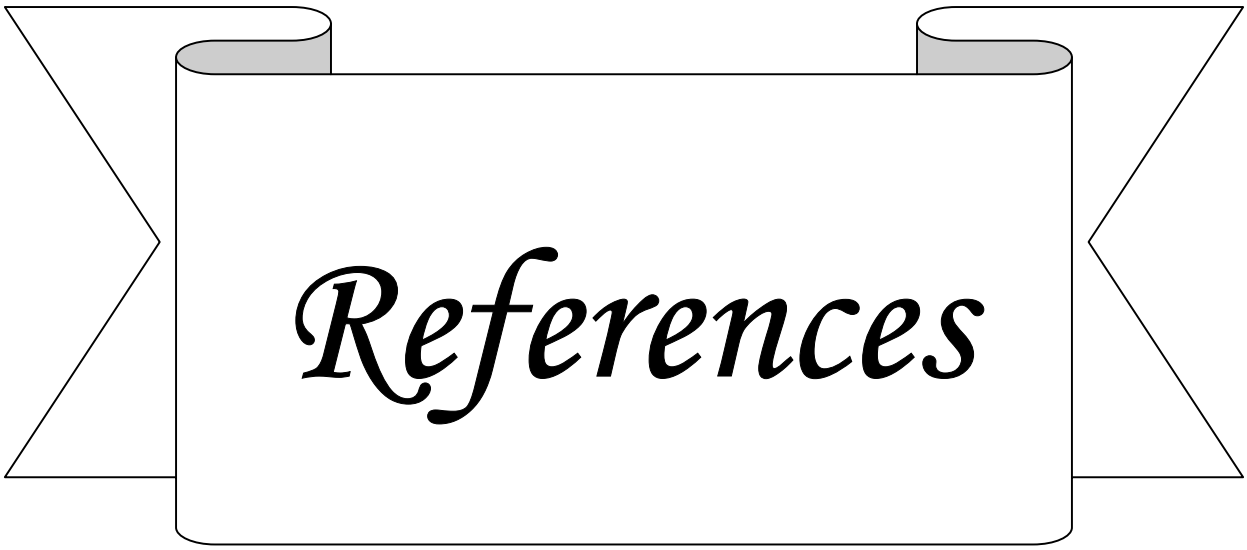
References

References:

1- Schmitt EW.; History in A principle in the treatment of diabetes mellitus Upjohn press 1:5 (1987).

2- Davidson JK.; Clinical Diabetes Mellitus. Thieme Inc. New York 3:26-27 (1986).

3- Whitby LG., Smith AF. & Beckett GJ.; Lecture Notes on Clin. Chem. 4th ed., 1:1(1988).

4- Alberti K.G.M. & Press C.M. Complications of diabetes, 2nd ed. Keen H.& Jarrett T. 231:70 (1982).

5- Canlish, JK.; Lecture Note on Biochem. Blackwell Scientific Publications Oxford, London (1984).

6- Romm F. T., Hulka S. Med. Care 17:84-96 (1979).

7- Backlund L.B., Alvere P.V.& Rosen vista S. Diabet. Med. 14:732-40 (1997).

8- Lipper P. Nephrol., Dial. Transplant 10:462-71 (1995).

9- Aviporath, M.G.F. Diabetes Care 23:495-99 (2000).

10- WHO Expert Committee on Diabetes Mellitus. Technical Report Series, 757; Geneva (1985).

11- National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance, Diabetes 28:1039 (1979).

12- Tattsall R.B.& Fajans S.S; A difference juvenile-onset type diabetes & maturity onset diabetes of young people, Diabetes 24:44 (1975).

13- Edwards C.R.W, Bouchie I.A, Haslett C.& Chilvers E. R; Davidson's principles & practice of medicine 17th ed. 12:724 (1996).

14- Gamble D. R, Taylor K. W & Brmed J.; Seasonal incidence of diabetes mellitus 3:631(1969).

15- Goldstein S. & Podolsky S.: The genetics of diabetes mellitus. Clin. Med. 62:639 (1978).

16- Anderson D.K, Svardsudd K.& Tibblin G.; Prevalence and Incidence of diabetes in a Swedish Community 1972-1987. Diabetes Med. 8:248-54 (1991).

17- Lillioja S., Mott D.M& Spraul M.; Insulin resistance & insulin secretary dysfunction as precursors of NIDDM. N Engl. Med 329:1988-92 (1993).

18- The world's Healthiest Food: Feeling Great 2004/12/13; 23:52:45.

19- Songer T., Zimmet P.Z; Epidemiology of Type II Diabetes: An international perspective , pharmacoenconomic 8: supp 1:1-11 (1995).

20- Hawthorne G, Robson S, Ryall EA, Sen D, Roberts SH & Ward Platt MP; Prospective population based survey of outcome of pregnancy in diabetic women: results of the Northern Diabetic Pregnancy Audit. BMJ 315: 279–81(1997).

21- O'sullivan J.B &Worshop Y.; Subsequent morbidity among gestational diabetes women. In Sutherland, HW,& stowers, meditors carbohydrate metabolism in pregnancy and the newborn, Edinburgh, (1984).

22- Philip D. Mayne & Zilva. P. Mayne; Clin. Chem. in Diagnosis& Treatment 6th ed. 10:207 (2000).

23- Shaw J., Courten M.& Boyko E.; Impact of new diagnostic criteria for diabetes on different populations. Diabetes Care 22:762-66 (2001).

24- Haffner S.M: Diabetes; Dyslipidemia & Coronary Artery Disease. Am. *J. Cardiol* **83**:17F – 21F (1999).

25- Foster D.W, Harrison's principles of Internal Medicine Churchill Livingston Publication, Oxford (1998).

26- Paolisso G. & Giuglatiano D.; Oxidative stress &insulin action: is there a relationship ? Diabetologia 39:357-63 (1996). [Abstract]

27- Ginstberg H.N: Lipoprotein physiology in non diabetic &diabetic states: Relation ship to atherogenesis Diabetes Care 14:839-55 (1991).

28- Moses G.P. Manual on cardio- diabetology introduction 1:1 (1990).

29- Arterioscl Thromb 12:647-59(1992). [Medline]

30- Tucker KL, Selhub J, Wilson PW& Rosenberg IH.; Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J. Nutr* **126**:3025-31(1996).

31- George N. Welcl, Joseph Loscaizo. New England *J. Medicine* **338**;15:1042-50 (1998).

32- Miller J.W& Russell R. M, Clin. Chem 32;48:228-30 (1990).

33- Boushey CJ. JAMA 125:1235-40 (1989).

34- Kruger WD. ; *J. Vitamins &Hormones* **60**:333-52 (2000).

35- Harpreet S. Sood, Matthew J. Hunt & Suresh C. Tyagl; "Peroxisome prolifrator ameliorates endothelial dysfunction in amuring model of hyperhomocysteinemia" *American J. Physiology*; **284**:333-41 (2003).

36- Ueland P.M, Refsum H., Stabler SP, Malinow M. R, Andersson A. & Allen R.H, *J. Clin. Chem.* **22**; 39:1764-79 (1993).

37- Finkestein J.D, Martin J.J & Harris B.J, *J. Biochem.* **263**; 417:50-4 (1998).

38- Ueland PM. Clin. Chim. 41:340-42(1995).

39- Christen H.; Plasma homocysteine &vascular diseases. *British J. Medicine* **316**:894-94(1998).

40- Elizabeth H.; Homocysteine &vascular disease *J. Clin. Biochem.* **50**:200-205 (2001).

41- Kang SS., Wong PW. & Malino MR. Ann. Rev. Nutr. 12:279-98(1992).

42- William R.H., Maggiore J.A., Reyndds R.D.& Helson C.M.; Novel approach for the determination of the redox status of homocysteine & other aminothiols in plasma for healthy subjects &patients with ischemic stroke. *J. Clin. Chem.* **47**:1031-39 (2001).

43- Mudd SH., Skovby F., Levy HL. Petligrew RD., Wilken B. & Pyeritz RE. Am. *J. Hum. Genet* **37**:4-12 (1985).

44- Neal Konecky, Rene Malinow, Robin S., Freedberg & Barry P. Rosenweig. *J. American heart* **133**:534-40 (1997).

45- Mudd S.H, Skovby F.& Levy H.L "The metabolic basis of inherited disease" 7th ed. 1279-1327 (1995).

46- Robinson K., Mayer E. & Jacobsen DW. *J. Clere. Clin.* **61**:438-50 (1994).

47- Kang SS. Zhou J., Wong Pwk, Kowalisym J. &Strokosh G. Am. *J. Hum. Genet* **43**:414-21 (1988).

48- Green R. & Jacobsen DW. New York, Marcel Dekker 75-122 (1995).

49- Guttormsen AB., Ueland PM., Nesthus *J. Clin. Invest.* **98**:2174-83 (1996).

50- Lerchael Z. Stozenberg, Edgar R. Miller & Maureen G. Maguire, *J. Clin. Nutr.* **69**:467-75 (1999).

51- Velury S. & Howell SB. *J. Chromatogr*. **424**:141-46 (1988).

52- Welch G. N., Upchucch G. R.& Jr. Loscalzo , J. Ann. N.Y. Acad. Sci. 811:48-58(1997).

53- Misra H.P., J. Biochem. 249:2151-55(1974).

54- Rowley DA. & Hallwell B. *J. Clin. Invest.* **438**:33-36 (1982).

55- Loscalzo J., *J. Clin. Invest*. **98**:5-7 (1996).

56- Naruszewicz M., Mirkiewicz E. & Olszewski A., *J. Natrmetab Cardiovasc. Dis.* **4**:20-27 (1994).

57- Idem. Ann. Clin. Lab. Sci. 24:434-52 (1994).

58- Boushey CJ., Beresford SA., Omenn GS. &Motulsky AG. Jama 274:1049-57 (1995).

59- Den Heijer M., Kostor T. &Blom HJ. N Engld 334:759-62 (1996).

60- Ridker PM., Hennekens GH., Selhub J., Miletichd P. & Malinow MR. *Circulation* **95**:1777-82 (1997).

61- Celermajor DS., Sorensen K.& Ryalls M. *J. Am. Coll. Cardiol.* **22**:854-58 (1993).

62- Hallwell B.; Haemostasis 23(11):118-26(1993).

63- Hallwell B. Free radical, antioxidants & human disease:curiosity, causes or consequences? The Lancet 344(10):721-24 (1994).

64- Ardmore RD., free radicals &disease, Ransel Glutathione Peroxidase In Randox Anti-oxidant Products. Course Code: RIT 2-11, U.K. February (1997).

65- Westhuyzen J.; Ann *J. Clin. Biochem.* **27**; 1:1(1997).

66- Iunce, J. Ann. Clin. Biochem. 27:173-82 (1990).

67- Fus., Davies M.J, Stocker R. &Dean R.T, *J. Biochem.***333**;3:510(1998).

68- Bost A., Haenen RG. & Doleman CJ. Am. J. 91:12(1991).

69- Wolff SP. & Dean RT.: Glucose autoxidation & protein modification. The potential role of autoxidative glycosylation in diabetes: *J. Biochem.* **245;**1: 243-50 (1987).

70- JianZY., Hoollard AC. & Wolf SP.: Hydrogen peroxide production during experimental protein glycation. FEBS Lett. 268; 1: 69-71 (1990).

71- Tsia EC., Hilsch IB. & Chail A.; Reduced plasma peroxyl radical trapping capacity &increased susceptibility of LDL to oxidation in poorly controlled IDDM *J. Diabetes* **43**;8:1010-14 (1994).

72- Kawamura M., Heinecke JW. & Chial A.; Pathophsiological concentrations of glucose promote oxidative modification of LDL by superoxide dependant pathway. *J. Clin. Invest.* **94**; 2:771-78(1994).

73- Rosen P., Nawroth PP. & King G.: The role of oxidative stress in the onset & progression of diabetes and its complications; Asummary of a congress series sponsored by UNESCOMCBN, the American Diabetes Association & the German Diabetes Society. Diabetes Metab. Res. Rev. 17:189-212 (2001).

74- West IC.; Radicals & oxidative stress in diabetes. Diabetes Med. 17:171-80 (2000).

75- Evans JL., Goldfine ID., Maddux BA. & Grodsky GM.; Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endrocr Rev in press, (2002).

76- Activated signaling pathways; A unifying hypothesis of type II diabetes mellitus. Endrocr Rev. in press (2002).

77- Maxwells R. & Lip JY. ; *J. Clin Pharmacol.* **44**; 4:307 (1997).

78- Halliwall B. & Gutteridge J.M.C., "Free Radical in Biology & Medicine" 2nd ed. (1989).

79- AL-Khairi HY., Effects of antioxidants on hypertensive patients with different therapy M.Sc thesis, collage of pharmacy Baghdad University (2000).

80- Hayden MR. & Tyagi SC.; Islet redox stress; the manifold toxicities of insulin resistance, metderived islet amyloid in type II diabetes mellitus. JOP 2:86-108 (2002).[pubmed]

81- Sies H. Stahlw: Am. *J. Clin. Nutr*. **62**:1315s-21s (1995).

82- Senck, J. Nutr. Biochem. 8:660-72 (1997).

83- Robert Roskoski, JR.: β-carotene & its conversion to vitamin A derivatives *J. Biochem* **2**:29 (1996).

84- Chaber M.; Trigger & amplification mechanisms in visual phototransdud-ction. Ann. Rev. Biochem. 14:331 (1985).

85- Spom MB., &Roberts AB.; Role of retinoids in differentiation & carcinogenesis. Cancer Res. 43:3034 (1983).

86- International vitamin A consultative group. The symptoms &signs of vitamin A deficiency &their relationship to applied nutrition. Washington, DC: IVAGG, (1981).

87- Sommer A. & West KP.; Vitamin A deficiency: heath, survival & vision. New York: Oxford University press (1996).

88- Krinsky NI: Action of carotenoids in biological systems. Ann. Rev. Nutr. 13:561 (1993).

89- Burtan GW. & Ingold KU; retinol an unusual type of lipid antioxidant science 224:569 (1984).

90- Mapson IW. & Goddard DR.:*J. Biochem.* **49**:529-601 (1951).

91- Sauberlich HE.; Rev. Nutr. 14:371 (1994).

92- Lester Packer, Stefan U. Weber & Geraldrimbach; *J. Nutr.* **131**:369s-73s (2001).

93- Manohar LG., Robert JB. & Ron BH.; Am. *J. Nutr.* **133**:1060-68 (2003).

94- Inhibition of free radical chain oxidation b α-tocopherol & other plasmas antioxidants. Rev. Nutr. 46:206 (1988).

95- Erice, Conn, Paulk, Stumpf, George Brue&Roy H. Doi: Outlines of Biochem. 5:203-205 (1987).

96- Lorch V., Murphy D. & Hoesten LR. et al. Pediarics 75:598-602 (1985).

97- Niki E., Tsuchiya J., Tanimura R. & Kamiya Y.; Biochem. Acta. 27:689 (1982).

98- Altimimi DIJ. &Dormandy TL.; *J. Biochem.* **168**:284-88 (1977).

99- Prgor WA., vitamin E &heart disease; Basic Science to Clinical Intervention Trials. Free Radical Biol. Med. 28:141-64 (2000).

100- Hodge R., Hood J. & Canham JE.; Am. *J. Clin. Nutr.* **24**:432-43 (1971).

101- Devaraj S. & Jialal I.; Antioxidants and vitamins to reduce cardiovascular disease current atherosclerosis Rep. 2:342-51 (2000).

102- Soulhron P.& Pow G. Mayo, Clin. Proc. 36:381 (1986).

103- Fridovich I., *J. Biol. Chem.* **264**:7761 (1986).

104- Planka A. & Metodiewa D.; Biochem. Biophys. Res. 95:978 (1989).

105- Gutterige J., Halliwell B., Treffy A., Harrison PM. & Blake D.; *J.Biochem.* **209**:557(1983).

106- Stoyanarsky DA., Goldman R. & Darrow RM.; Endogenous ascorbate regenerates vitamin E in combination with exogenous dihydrolipoic acid. *J. Circulation* **14**;3:181-9 (1995).

107- Losted RA., INT.; *J. Biochem.* **13**:221 (1981).

108- National Academy of Sciences. Dietary Reference intakes for vitamin C, vitamin E, Selenium & carotenoids. National Academy press,186-283 (2000).

109- Geoffrey Zubay, Biochemist. 3rd ed.; 18:483 (1993).

110- Roks B.F., Sherwood R.A., Ann. Clin. Biochem.20:72-6 (1983).

111- Bird IM.; High performance liquid chromatography: principles & clinical applications. *J. sep.* **299**; 23:783 – 87 (1989).

112- Chromatography; products for analysis and purification. 543 (2001).

113- Bartham D. &Trinder P. Analyst, 97:142 (1972).

114- Teuscher A., Richterich P. & Schweiz Med. Wschr., 101:345 (1971).

115- Rutishauser W. & Lerch R.; Asymptomatic ischemia, an important part of the spectrum of coronary disease; Schweiz. Rundsch. Med Prax. 84(42):1181-5 (1995).

116- Grundy S.M., Benjamin I.J., Burke G.L., et al. Diabetes and cardiovascular disease. *J. Circ.* **100**:1134-46 (1999).

117- McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am *J. Pathol.* **56**:111–128 (1969).

118- Stehouwer CDA, Gall MA, Hougaard P, Jakobs C & Parving HH.; Plasma homocysteine concentration predicts mortality in non-insulin-dependent diabetic patients with and without albuminuria. Kidney Int. 55:308-314 (1999). [Medline]

119- Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV& Hennekens CH; A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA: 268:877-881 (1992). [Abstract]

120- Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC& Stampfer MJ; A prospective study of plasma homocysteine and risk of ischemic stroke, 25:1924-30 (1994). [Abstract]

121- Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM & Shaper AG; Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet. 346:1395-98(1995). [Medline]

122- Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH & Nordrehaug JE; Serum total homocysteine and coronary heart disease. Int. *J. Epidemiol.* **24**:704-9(1995). [Abstract]

123- Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J & Rosenberg IH; Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet. 348:1120-24 (1996).[Medline]

124- Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Fastad M & Vollset SE; Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 337:230-36 (1997). [Abstract]

125- Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobson DW, Robinson K & Dennis VW; Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *J. Circulation.* **97**:138-41 (1998). [Abstract]

126- Bostom AG, Silbershatz H, Rosenberg IH, Sehlhub JD, Agostino RB, Wolf PA, Jacques PF & Wilson PWF; Nonfasting plasma total homocysteine levels and all cause and cardiovascular disease mortality in elderly Framingham men and women. Arch Intern Med. 159:1077-80 (1999). [Abstract]

127- Kark JD, Selhub J, Bostom A, Adler B & Rosenberg IH; Plasma homocysteine and all cause mortality in diabetes. Lancet. 353:1936-37(1999). [Medline]

128- Van Beynum IM, Smeitink JAM, Den Heijer M, Poele Pothoff MTWB & Blom HJ; Hyperhomocysteineemia: a risk factor for ischemic strok in children. *J. Circulation* **99**:2070-72 (1999). [Abstract]

129- Folsom AR, Nieto FJ, Mcgovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL & Davis CE: Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and

B vitamins: the atherosclerosis risk in communities(ARIC) study. *J. Circulation.* **98**:204-10 (1998). [Abstract]

130- Evans RW, Shaten J, Hempel JD, Cutler JA & Kuller LH. For the MRFIT Research group. Homocystiene and risk of cardiovascular disease in the multiple risk factor intervention trial. Arterioscler Thromb Vasc Biol. 17:1947-53 (1997). [Abstract]

131- Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, Salonen JT & Ehnholm C; Relation of serum homocysteine and lipoprotein concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis. 106:9-19 (1994).[Medline]

132- Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, Terry P, TishlerPV, Willett W, Hennekens CH & Stampfer MJ; Aprospective study of folate and vitamin B6 and risk of myocardial infarction in US Physicians. JAM Coll Nutr. 15:136-43 (1996). [Abstract]

133- Hoogeveen EK, Kostense PJ, Jager A, Heine RJ, Jakobs C, Bouter LM, Donker AJM, & Stehouwer CDA; Serum homocysteine level and protein intake are related to risk of microalbuminuria: the Hoorn Study. Kidney Int. 54:203-9 (1998). [Medline]

134- Bellamy MF, McDowell IFW, Ramsey MW, Brownlee M, Bones C, Newcombe RG & Lewis MJ; Hyperhomocysteinemia after an oral methionine load acutely impairs endothelial function in healthy adults. *J. Circulation* **99**:1156-60 (1998). [Abstract]

135- Chambers JC, McGregor A, Jean-Marie J, Obeid OA & Kooner JS.; Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *J. Circulation.* **99**:1156-60 (1999) [Abstract]

136- Welch GN & Loscalzo J.; Mechanisms of disease: homocysteine and atherothrombosis. N Engl J Med. 338:1042-50 (1998). [Abstract]

137- Jakubowski H. Homocysteine is a protein amino acid in humans. Implications for homocysteine-linked disease. *J. Biol Chem.* 277:30425–48 (2002). [PubMed]

138- Misra HP. Generation of superoxide free radical during the autoxidation of thiols. *J. Biol Chem.* **249**:2151–2155(1994). [PubMed]

139- Blom HJ. Consequences of homocysteine export and oxidation in the vascular system. Semin Thromb Hemost. 26:227–32 (2000). [PubMed]

140- Melvin R Hayden & Suresh C Tyagi; Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: The pleiotropic effects of folate supplementation. *J. Nutr.* **3**:4(2004).

141- Hammerman C, Goldstein R & Kaplan M.; Clin. Chem. 44:1551-53 (1998).

142- Desco MC, Asensi M & Marquz R; Xanthine oxidase is involved in free radical production in type 1 diabetes: production by allopurinol. Diabetes 51:1118-24 (2002).

143- Boemi M, Leviev I, & Sirolla C.; Serum paraxonase is reduced in type 1 diabetic patients compared to non-diabetic, first degree relatives; influence on the ability of HDL to protect LDL from oxidation. Atherosclerosis 155:229-35 (2001).

144- Oberley LW; Free radicals and diabetes. Free Radical Biol Med 5: 113–124 (1988).[Medline]

145- Baynes JW; Role of oxidative stress in development of complications in diabetes. Diabetes 40: 405–412 (1991). [Abstract]

146- Maxwell SRJ, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GHG, Jones AF, & Barnett AH; Antioxidant status in patients with

uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur J. Clin Invest* **27**:484–90 (1997). [Medline]

147- Salonen JT, Nyyssonen K, Tuomainen TP, Maenpaa PH, Korpela H, Kaplan GA, Lynch J, Helmrich SP, & Salonen R; Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentrations: a four year follows up study in men. BMJ 311:1124–7(1995). [Abstract]

148- Opara EC, Abdel-Rahman E, Soliman S, Kamel WA, Souka S, Lowe JE, & Abdel-Aleem S; Depletion of total antioxidant capacity in type 2 diabetes. Metabolism 48:1414–17 (1999). [Medline]

149- Shigeta Y, Hiraizumi G, Wada M, Oji K, & Yoshida T; Study on the serum level of thioctic acid in patients with various diseases. *J. Vitaminol (Kyoto)* **7**:48–52(1961).

150- Bursell SE, Clermont AC, Aiello LP, Aiello LM, Schlossman DK, Feener EP, Laffel L, & King GL; High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. Diabetes Care 22:1245–51 (1999). [Abstract]

151- Bursell SE, & King GL; Can protein kinase C inhibition and vitamin E prevent the development of diabetic vascular complications? Diabetes Res Clin Pract 45:169–182 (1999). [Medline]

152- Baynes JW, & Thorpe SR; Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 48: 1–9(1999). [Abstract]

153- Eriksson UJ,& Borg LAH; Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations *in vitro*. Diabetologia 34: 325–331(1991). [Medline]

154- Eriksson UJ,& Borg LA; Diabetes and embryonic malformations: role of substrate-induced free-oxygen radical production for dysmorphogenesis in cultured rat embryos. Diabetes 42: 411–419 (1993).[Abstract]

155- Trocino RA, Akazawa S, Ishibashi M, Matsumoto K, Matsuo H, Yamamoto H, Goto S, Urata Y, Kondo T, & Nagataki S; Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. Diabetes 44: 992–998 (1995).[Abstract]

156- Sakamaki H, Akazawa S, Ishibashi M, Izumino K, Takino H, Yamasaki H, Yamaguchi Y, Goto S, Urata Y, Kondo T, & Nagataki S; Significance of glutathione-dependent antioxidant system in diabetes-induced embryonic malformations. Diabetes 48: 1138–1144 (1999).[Abstract]

157- Wentzel P, Welsh N, & Eriksson UJ; Developmental damage, increased lipid peroxidation, diminished cyclooxygenase-2 gene expression, and lowered PGE_2 levels in rat embryos exposed to a diabetic environment. Diabetes 48: 813–820(1999).[Abstract]

158- Jenkinson PC, Anderson D, & Gangolli SD; Malformations induced in cultured rat embryos by enzymically generated active oxygen species. Teratog Carcinog Mutagen 6: 547–554 (1986). [Medline]

159- Anderson D, & Francis AJ; The modulating effects of antioxidants in rat embryos and Sertoli cells in culture. Basic Life Sci 61: 189–200 (1993).[Medline]

160- Cederberg J, & Eriksson UJ; Decreased catalase activity in malformation-prone embryos of diabetic rats. Teratology 56: 350–357 (1997).[Medline]

161- Cederberg J, Galli J, Luthman H, & Eriksson UJ; Increased mRNA levels of Mn-SOD and catalase in embryos of diabetic rats from a malformation-resistant strain. Diabetes 49: 101–107 (2000).[Abstract]

162- Packer L, Rosen P, Tritschler H, King GL, & Azzi A; Antioxidants and diabetes management. 1st ed. New York: Marcel Dekker (2000).

163- Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, & Creager MA.; Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J. Clin Invest.* **97**:22–28 (1996). [Abstract]

164- Costagliola C, & Menzione M.; Effect of vitamin E on the oxidative state of glutathione in the plasma. Clin Physiol Biochem. 8:140–3 (1990).[Medline]

165- Stephen D, Skaper, Michele Fabris, Vanni Ferravi, Maurizio Dalle Carbonare & Alberta Leon; Quercetin Protects Cutaneous Tissue-Associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: Cooperative effects of ascorbic acid. FR *J. Biol. Medec.* **22**; 4:669-78 (1997).

166- Julie C. Will, Earl S. Ford & Barbara A. Bowman; Serum vitamin C concentrations and diabetes: findings from the third National Health and Nutrition Examination Survey. 70(1):49-52 (1999). [Abstract]

167- Pfleger R. & Scholl F.; Diabetes and vitamin C, Wien. Arch Im. Med 31:219 (1937).

168- Cia H. & Harrison DG.; Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87:840-4 (2000).

169- Laight DW, Carrier MJ,& Anggard EE; Antioxidant, diabetes and endothelial dysfunction. Cardiovasc Res 47:457- 64 (2000).

170- Bravenboer B, Kappelle AC, Hamers E.P.T, Van Buren D.W., Erketens D.W & Gispen W.H; Potential use of glutathione for the prevention and treatment o diabetic neuropathy in the streptozotocin-induced diabetic. *J. Diabetologia*. **35**:813-17 (1992).

171- Cameron.N.E, Cotter M.A, & Maxfield E.K, Antioxidant treatment prevents the evelopment of peripheral nerve dysfunction in streptozotocin- diabetic rats. Diabetologia 36:399-403(1993).

172- Olmedilla; Reference values from retinol, tocopherol and main carotenoids in serum of control and insulin dependent diabetic Spanish subjects. Clin Chem. 43:1066-71 (1997).

173- Shoff SM, Klein R, & Klein BE.; Glycosylated haemoglobin concentrations and vitamin E, C and β-carotene intake in diabetic and non-diabetic old adults. Am Clin Nutr. 58:412-16 (1993).

174- Boeing H, Weisgeber UM, & Jeckel A.; Association between glycated haemoglobin and diet and other lifestyle factors in a non-diabetic population: cross-sectional evaluation of data from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition Study. *J. Clin Nutr*. **71**:1115-22 (2000).

175- Granado; Carotenoids, retinol and tocopherols in patients with insulin dependent diabetes mellitus and their immediate relatives. Clin Sci (Colch). 4:189-95 (1998).

176- The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. N Engl *J Med*. **342**:154-60 (2000).

177- Rapola JM, Virtamo J, & Ripatti S.; Randomised trial of α-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet. 349:1715-20 (1997).

178- Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomized placebo controlled trial. Lancet. 360:23-33 (2002).

179- Omenn GS, Goodman GE, & Thornquist MD.; Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficancy Trial. *J. Natl Cancer Inst.* **88**:1550-59 (1996).

180- Tesfamariam B.; Free radicals in diabetic endothelial cell dysfunction. Free Rad Biol Med. 16:383–91(1994). [Medline]

181- Hunt JV, Dean RT,& Wolff Sp.; Hydroxyl radical production and autooxidative glycosilation. Glucose outoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *J. Biochem* **256**:205–12(1988). [Medline]

182- Krieger-Brauer H, & Kather H.; Human fat cells possess a plasma membrane bound H_{2O2} generating system that is activated by insulin via a mechanism bypassing the receptor kinase. *J. Clin Invest.* **89**:1006–13 (1992).[Medline]

183- Habib MP, Dickerson FD, & Mooradian AD.; Effect of diabetes, insulin and glucose load on lipid peroxidation in the rat. Metabolism. 43:1442–45 (1994).[Medline]

184- Toborek M, & Henning B.; Fatty acid-mediated effects on the glutathione redox cycle in cultured endothelial cells. Am *J. Clin Nutr.* **59**:60–5 (1994).[Abstract]

185- Lowenstein CJ, Dinerman JL, & Snyder SH.; Nitric oxide: a physiologic messenger. Ann Intern Med. 120:227–37(1994). [Abstract]

186- Joannides R., Haefeli WE. & Lindner L.; Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries. *J. Circulation.* **91**:1314–19 (1995).[Abstract]

187- Cohen RA.; Dysfunction of vascular endothelium in diabetes mellitus. *J. Circulation.* **87**; 5:67–76 (1993).

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Measurement of homocysteine and antioxidant vitamins (A , C, &E) concentration in young patients with Type I Diabetes Mellitus

A

thesis

submitted to the

College of Sciences

Al-Nahraine University as a partial fulfillment

of the requirements for the Degree of M. Sc in

Chemistry

By:

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(B.SC. 2003)

2006 *1427*

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

قياس تركيز الهوموسيستين و الفيتامينات المضادة للأكسدة (فيتامين A، C وE) في الأحداث المصابين بداء السكري النوع الأول المعتمد على الأنسولين

رسالة
مقدمة إلى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
شيماء زهراو ندى
بكالوريوس ٢٠٠٣ (جامعة النهرين)

١٤٢٧ ٢٠٠٦

Abstract:

Diabetes Mellitus is a chronic and complex disease, requiring continued life long management aimed at reducing the high morbidity and premature mortality caused by chronic complications. In both type I and type II diabetes, late diabetic complications in nerve, vascular endothelial, and kidney, increased oxidative stress and it is consequences of either increased production of free radical, or reduced antioxidant defenses, arise from chronic elevation of glucose.

This work is concerned the main biochemical and statistical study on the sera of thirty healthy control subjects (13 female & 17 male), and thirty four children with insulin dependent diabetes mellitus (16 females & 18 males). They were attended at Al -Kahdmia Teaching Hospital & National Center of Diabetic.

This investigation was carried out on serum to measure the concentrations of homocysteine, vitamin A ,vitamin C & vitamin E by using High Performance Liquid Chromatography(HPLC-10 AVP) analysis. The experimental results showed that the mean value of homocysteine concentration was significantly higher in diabetic comparing to the healthy control subjects ($p<0.001$), while the mean level of serum vitamins (A, C& E) were significantly decreased in diabetic compared to healthy control subject ($p<0.001$). From the results of this study were found the age was effect groups on the level of both parameters (antioxidant vitamins and homocysteine) ($p<0.001$). There was no significant difference in levels of vitamins and homocysteine between females and males in both groups diabetic and healthy control.

قياس تراكيز الهوموسيستين و الفيتامينات المضادة للأكسدة (فيتامين A، C وE) في الأطفال المصابين بداء السكري النوع الأول المعتمد على الأنسولين

الخلاصة:

يعتبر مرض السكر احد الأمراض الشائعة الانتشار عالميا، و من المشاكل الصحية الرئيسة في معظم دول العالم يضم هذا المرض مجموعة من الاضطرابات الايضية المختلفة، اهمها ارتفاع مزمن في سكر الدم والذي يعود الى النقصان النسبي او المطلق في إفراز الأنسولين نتيجة فشل او عجز خلايا β البنكرياسية في إفراز الأنسولين.
ويؤثر الارتفاع المزمن لسكر الدم في مختلف اعضاء الجسم و بالأخص: العينين، الكليتين، الأعصاب و الأنسجة الوعائية.كما وتشير التفسيرات إلى حصول عملية التكسير للجزيئات الخلوية المهمة وخاصة البروتينات و زيادة الجذور الحرة وخاصة الجذور الأوكسجينية، التي تنتج عن بعض العمليات الايضية الاعتيادية في الخلية مثل الأكسدة الذاتية للكلوكوز و التي هي اكثر الأدلة أهمية في تطوير مرض السكر إلى مضاعفات خطيرة و مزمنة .

يتضمن هذا البحث دراسات كيمياحياتية و احصائية على مصل دم الاطفال المصابين بمرض السكري المعتمد على الانسولين و قد تم اخذ النماذج من المرضى الراقدين في مستشفى الكاظمية التعليمي و كذلك من المركز الوطني للسكري و ضم ٣٤ مريضا (١٦ من الاناث و ١٨ من الذكور) و ٣٠ نموذج من الاصحاء (١٣ من الاناث و١٧ من الذكور). و قد أجريت هذه الدراسة على نماذج مصل الدم لقياس تراكيز الهوموسيستين و الفيتامينات المضادة للأكسدة (فيتامين A، C وE).

وقد اظهرت هذه الدراسة ان معدل الهوموسيستين مرتفع بشكل معنوي لدى المرضى مقارنة بالاصحاء ($p<0.001$). كما لوحظ انخفاض متوسط تراكيز الفيتامينات (A، C وE) في مصل الأطفال المصابين بداء السكري بصورة ملحوظة بالمقارنة بتراكيزها في دم الاصحاء ($p<0.001$). وكان لاختلاف العمر بين المرضى و الاصحاء تاثيرا واضحا على مستوى كل من الفيتامينات و الهوموسيستين ($p<0.001$). ولم يكن هناك فرق ملحوظ عند الأطفال في مستوى الهوموسيستين و الفيتامينات بين الاناث والذكورالاصحاء و كذلك المصابين بداء السكري.

Supervisor Certification

I certify that this thesis was prepared under my supervision at the Department of Chemistry, College of Science, Al- Nahrain University as a partial requirements for the Degree of M. Sc in Chemistry.

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In view of the available recommendation, I forward this thesis for debate by the examining committee.

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Examining Committees Certification

We the Examining Committee, Certify that we have read this thesis and have examined the student **Shayma Zahraw Nada** in its contents, and that in our opinion it is adequate as a thesis for the degree M. Sc in Chemistry.

Chairman

Signature:
Name:
Date

Member

Signature:
Name:
Date:

Member

Signature:
Name:
Date:

Supervisor

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Date:

Approved for the Council of the College of Science.

Signature:
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Acknowledgments

Thanks Allah for all his blessing during the pursuit of my academic and career goals, more knowledge, praises and peace be upon the prophet Mohammad and His good Family and Companions.

I would like to express my deepest gratitude to my supervisors Dr. Abdul Wahab R. Hamad for his appreciable advice, patients, important comments support and encouragement.

Also, I would like to extend my acknowledgment to Dr. Shahbaz A. Maki for his advice & helps to my in post and undergraduate. Many thanks to Dr. Afaf Al- Derzi Head of the Department of Chemistry, Dr. Salman A. Ahmad for his assistance to my in Arabic part of this thesis.

Sincere thanks to the staff of Chemistry Department in both collages Chemistry and Medicine, Al –Nahrain University for their encouragement and efforts during my study.

Finally, I am deeply indebted to my family, Al- Hashemy Mr. Ali's family and my friends all of them, for their help, normal support, patience and for their encouragement.

Dedication

To the one whom my heart is beating with

his love

Ever and forever ..

My father

To the shining stars in my sky life ..

My mother

To the big heart& benignant friend

My brother

Nazar

With love and respect

To all my freinds

Shayma

بسم الله الرحمن الرحيم(١)

الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ(٢)الرَّحْمَنِ الرَّحِيمِ (٣)

مَالِكِ يَوْمِ الدِّينِ(٤)إِيَّاكَ نَعْبُدُ وَإِيَّاكَ نَسْتَعِينُ(٥)

اهْدِنَاالصِّرَاطَ الْمُسْتَقِيم(٦)صِرَاطَ الَّذِينَ أَنْعَمْتَ

عَلَيْهِمْ غَيْرِ الْمَغْضُوبِ عَلَيْهِمْ وَلا الضَّالِّينَ(٧)

(صدق الله العظيم)

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