The level of Dehydroepiandrosterone sulfate hormone in Iraqi Diabetic patients with Nephropathy

Ekhlas Abdallah Hassan[1] , Alzahraa Ibrahim Abdulmajeed[2] and Shaymaa Ibrahim Khaleel[3]

[1,2,3] Department of chemistry- College of Science- University of Diyala- Diyala-Iraq
[1]ekhlaschem@yahoo.com

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Abstract

Diabetes Mellitus is a chronic and complex disease, requiring continued lifelong management aimed at reducing premature mortality and the high morbidity caused by chronic complications, one of the main causes of chronic renal failure is diabetic nephropathy. The responsive filtering system in the kidney becomes progressively destroyed after many years of diabetes. This study was conducted to establish relationship of Dehydroepiandrosterone sulfate (DHEA_S) level with increased risk factor of kidney damage in diabetic, diabetic nephropathy and control men. Eighty one subjects were divided in three groups were incorporated in this study, 32 were healthy control ,with mean age (54±0.34) years , 30 diabetic men with mean age (55±0.9) years, and 19 diabetic men with nephropathy with mean age (65±0.57) years. They were attended at Baquba Teaching Hospital. This investigation was carried out on serum sample to measure the concentrations of DHEA-S by using [Enzyme-Linked Immuno Sorbents by Assay [ELISA]] test using commercially available kits. In addition to that, serum creatinine, blood urea, albumin, fasting serum glucose (FSG), Glycohemoglobin (Hb_1AC), systolic blood pressure (SBP), body mass index (BMI), diastolic blood pressure (DBP) , and the duration of disease were identified in the control and patients. The experimental results revealed that the mean value of DHEA-S concentration was none significantly decreased in diabetic comparing to the control group ($p>0.005$), while the

level of DHEA-S concentration were decreased significantly in diabetic with nephropathy compared to healthy control subject ($p<0.005$). Also, low mean concentration of serum DHEA-S noticed in all groups of diabetic patients, and highly significant decrease in DHEA-S levels with advancing age in the studied groups. This finding had been proved by multiple regression model used in this study, which showed that the negative correlation found between age, and serum DHEA-S level. The concentration of the other parameters (blood urea and creatinine) increases in the serum of patient groups as compared to the control group, on other hands decreased albumin in patient groups as compared to the control group. In summary, there is a major association between DHEA-S levels and diabetic nephropathy. These conclusions promote that DHEA-S level inversely correlated with the progression of diabetic nephropathy in diabetic men. Also decrease in DHEA-S levels with advancing age in the studied groups suggest that apart from age being diabetic is related with lower mean value of this hormone.

Key words: Diabetes Mellitus, Dehydroepiandrosterone sulfate, Nephropathy.

مستوى هرمون الديهايدرو سلفيت عند المرضى العراقيين المصابين بالسكري مع اعتلال الكليتين

اخلاص عبد الله حسن [1] ، الزهراء ابراهيم عبد المجيد [2] و شيمـــاء ابراهيـــم خليـــل[3]

[1،2،3] قسم الكيمياء - كلية العلوم – جامعة ديالى

الخلاصة

داء السكرهو مرض مزمن و معقد، يتطلب متابعه مستمره مدى الحياه، بهدف الحد من ارتفاع معدلات الاعتلال والوفيات المبكرة الناجمة عن مضاعفات المزمنة. واحد من اسباب الرئيسيه للفشل الكلوي المزمن هو اعتلال الكلية السكري. استجابة نضام التصفية للكلية يتحطم تدريجيا بعد عدة سنوات من الاصابه بداء السكري . هذه ا لدراسه اجريت لتقدير علاقه مستوى هرمون الديهايدرو سلفيت مع زياده خطوره داء السكري بالنسبه للرجال مصابين بالسكري ومصابين بالسكري مع اعتلال كلوي والاصحاء. احدى وثمانون حاله قسمت الى ثلاث مجاميع ،اثنى وثلاثون من الاصحاء متوسط اعمارهم (54±0.34) سنه، وثلاثون من المصابين بالسكري متوسط اعمارهم (55±0.9) سنه، وتسعه عشر من المصابين بالسكري مع اعتلال الكليه سجلت متوسط اعمارهم 65± 0.57) سنه. تمت رعايتهم في مستشفى بعقوبه التعليمي . هذه التحقيقات نفذت على مصل النماذج لقياس تركيز هرمون الديهايدرو سلفيت بواسطه (ELISA) المتوفر تجاريا.

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Ekhlas Abdallah Hassan, Alzahraa Ibrahim Abdulmajeed and Shaymaa Ibrahim Khaleel

بالاضافة الى عدة متغيرات مصلية لل (اليوريا، الكيراتنين.البروتين الكلي،الالبومين، السكر، ضغط الدم الانبساطي والانقباضي، مدة الاصابه،مؤشر كتله الجسم) بالنسبه للمرضى والاصحاء. النتائج التجريبيه اشارت الى انه لايوجد نقصان ملحوض في تركيز هرمون الديهايدرو سلفيت بالنسبه للمرضى السكري عند مقارنتهم بالاصحاء. ($p<0.005$)، نتائج الدراسه الحاليه اكدت وجود نقصان ملحوظ في تركيز الهرمون مابين الاصحاء عند مقارنتهم بالمصابين باعتلال الكليه السكري ($p<0.005$).ايضا تركيز هرون الديهتيدرو سلفيت ينخفض بشكل عام بالنسبه للمرضى السكري و هناك انخفاض ملحوض في قيم الهرمون بازدياد العمر في كل المجاميع التي اجريت عليها الاختبارات، ذلك يرجح انخفاض تركيز الهرمون بلنسبه للمرضى مع تقدم العمر. هذه النتيجة قد ثبت باستخدام نموذج الانحدار المتعدد التي بينت علاقه عكسيه مابين مؤشر العمر مع تركيز هرمون الديهايدروسلفيت.ازدياد تراكيز كل من اليوريا والكيراتنين في مصل المرضى عند مقارنتهم بمصل الاصحاء، او بتعبير اخر انخفاض نسبه البروتين والالبومين ملحوضه في المرضى عند مقارنتهم بالاصحاء. في الملخص يوجد ارتباط وثيق مابين مستويات هرمون الديهايدرو سلفيت مع المصابين بالسكري مع اعتلال الكليه. وهذا الاستنتاج يشجع الى وجود علاقه عكسيه بين نسبه الهرمون مع تطور السكري مع اعتلال الكلية. كذلك انخفاض هرمون الديهايدرو - سلفيت مع التقدم بالعمر في مجاميع الدراسه يقترح بغض النضر عن تقدم سن مرضى السكري فهو يرتبط مع انخفاض متوسط قيمة هذا الهرمون.

الكلمات المفتاحية : مرض السكري ، هرمون الديهايدرو سلفيت ، اعتلال الكلية

Introduction

Dehydroepiandrosterone sulfate: C19 is an important mammalian steroid hormone [1], is synthesized in the body from pregnenolone, which is synthesized from cholesterol DHEA is made primarily by adrenal glands which produce a greater quantity than any other glands [2]. DHEA is as a source for the production of the rest of the hormones that the body needs like estrogen and testosterone [3]. However, DHEA have distinctive and clear role in the function heart and blood vessels. DHEA is metabolized in the liver and combines with Silver to convert Dehydroepiandrosterone sulfate: DHEA-S [4]. DHEA-S gained interest antidepressant substance, its positive effects on autoimmune disease such as lupus, obesity, ulcerative colitis, cancer and diabetes [5]. Diabetes Mellitus is a metabolic disorder in which the body doesn't produce enough insulin or cells don't use insulin efficiently .This causes the levels of blood sugar to build up in the blood, which in the long term can lead to organ damage. Diabetics often have low levels of DHEA, which helps in regulating blood glucose levels by decreasing insulin resistance [6]. and also help decrease level triglyceride (TG), total

cholesterol(TC), LDL -C in blood stream. Indeed, recent research in Japan reveals that taking a daily of DHEA supplement help to regulating glucose in blood serum. [7]. There is no proof for a relation between DHEA-S and the diabetic nephropathy so we measured the level of DHEA-S in diabetic and diabetic nephropathy men.

Material and Method

This study has been carried out at Baquba Teaching Hospital , for the period from February to April, 2016. The study included (81) men with age range (30-70) years , (32) healthy individuals as control group and (49) patients with type 2 diabetes mellitus. The patients were divided into two groups (30) diabetic patients and (19) nephropathy diabetic patients. All groups were tested according to the following criteria: 1 all groups were males .2 men should have no history of heart disease, liver disease and not using medication on regular basis.3 non-Alcoholics. From each subject, 5 ml of blood were obtained by venipuncture, using a 10 ml disposable syringe between 9.00 and 11.00 A.M. in a plain tube the blood sample was dispensed and left for about an hour to clot at room temperature (22°C). Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum. The serum was divided into aliquots (250µl) in tubes and stored in the freezer (-20ºC) until use. Serum levels of DHEAS and were quantitatively determined in subjects by ELISA test using commercially available kit from DiaMetra-Italy. BMI is calculated by dividing the body weight (in Kg) by the square of the height (in m^2) Generally, healthy weight falls between BMI values of 18.5-24.9, overweight between 25-30 and obese above 30. Waist and hip circumferences were measured by using a tape measure, to assess the fat distribution. A waist circumference was measured at the level of umbilicus while hip circumferences was measured at the maximum protuberance of the buttocks (Ashwell et al, 1985)[8] .The waist hip ratio (WHR)was calculated by dividing waist circumference in centimeters by the hip circumference in centimeters (Frier et al 1999)[9] . The cutoff value for acceptable WHR that is used as an indicator of central obesity in male and female were 0.95 and 0.80 respectively (Truswell 1992; Jung 1997)[10] [11]. Regular physical activity was defined when it was repeated at least three times per week and last for more than 15 minutes each time. Individuals were registered to practice or not to practice

regular physical activity (Horton, 1986)[12]. Physical exercise which reported to be effective in diabetes mellitus was regular aerobic such as low to moderate intensity walking , cycling , swimming and jogging and resistance exercise such as use of weight , bands , or spring hydraulics (Elliot and Goldenberg , 1995)[13]. Smoking status /Individual registered to be currently smoker or not by asking the patient if he smoked or not. Cigarette smoking index estimated by multiplying cigarette smoked numeral per day by years of smoking.

Assessment of risk factor of nephropathy

One of the major causes of chronic renal failure is diabetic nephropathy. After many years of diabetes the responsive filtering system in the kidney becomes progressively destroyed, initially most blood proteins become leakage such as albumin which are then lost in the urine. Both serum creatinine and urea are widely used to estimate the kidney function. Creatinine, albumin and blood urea concentrations were measured by using enzymatic methods. Also two most important factors in the initiation and progression of nephropathy are blood glucose and blood pressure. Fasting serum glucose (FSG) was measured by using the method of glucose oxidase. In whole blood Glycohemoglobin (HbA1c) determination by kit for quantitative colorimetric was supplied by stanbio laboratory (USA). In all subjects, Blood Pressure was estimated by mercury sphygmomanometer in the right arm. The patients and control were asked to take rest for 5 minutes in a sitting position before measurement of his blood pressure. Hypertension was defined as systolic and diastolic blood pressure SDP and DBP of > 140/90 mmHg. (Rose et al 1982) [14]. The DBP was measured at the phase of final disappearance of the sound. The same instrument was used to measure the BP of the studied subjects.

Statistical Analysis

Data were analyzed using the computer facility with available statistical packages of SPSS 16. Data was presented in simple measure of number, mean, SD and the parametric statistical tests were used namely ANOVA to test the significance of difference in mean between more than two groups , while student t-test for independent samples was used to test the difference in mean between two groups. P value equal and less than 0.05 was used as the level of

significance. Multiple regression models were used to study the independent and net effect of a set of independent (explanatory) variables on the response (dependent or outcome) variable.

Result and Discussion

Table (1) shows the distribution of the studied groups (number and proportion) according to their age, physical activity, family history of diabetes, body mass index, waist hip ratio and history of hypertension . As shown in the table, highest proportions of the healthy controls were found to be in the age group of less than 40 years. On the other hand, the table shows that highest proportion of diabetic nephropathy aged 50 years and more. There was a significant difference between mean age values of the studied groups. The majority of diabetic nephropathy were above the age of 50 years, and this is comparable with data reported by Vijay Viswanathan (2012)[15] , that the majority of diabetic nephropathy were in the age group (50+) years. Regarding BMI, the table shows that high proportion of healthy controls (46.8%) were overweighed, majority of diabetic patients were with normal weight, and high proportion of diabetic nephropathy cases were found to be obese. As shown in the table, higher proportion of diabetic nephropathy cases (78.9%) had a central obesity, and this is in agreement with Hui-Mei et al(2013) [16] finding that diabetic nephropathy was associated with obesity. The observed finding that high proportion of the studied control subjects were overweight is consistent with WHO report (1998)[17] that developing countries are increasingly facing the public health problem of overweight.

Table (1): Distribution of studied groups (number and %) by some epidemiological variables.

Variable		Control group (32)		Diabetic group (30)		Diabetic nephropathy Group (19)	
		N	%	N	%	N	%
Age (in years)	< 40	15	46.8	15	50	1	5.26
	40-49	13	40.6	3	10	6	31.57
	50 +	4	12.5	12	40	12	63.15
Physical activity	Negative	19	59.3	22	73	14	73.6
	Positive	13	40.6	8	27	5	26.4
Family history of DM	Negative	26	81	21	70	10	52.6
	Positive	6	19	9	30	9	47.4
BMI (Kg /m²)	Thin(<18.5)	1	3.1	4	13	0	0
	Normal (18.5-24.9)	10	31.2	15	50	4	21
	Over weight (25-29.9)	15	46.8	5	16	6	31.5
	Obese (30+)	6	18.7	6	20	9	47.3
WHR	Acceptable	19	59.3	17	56.6	4	21.1
	Central obesity	13	40.6	13	43.4	15	78.9
Personal history of hypertension	Negative	30	93.7	24	80	13	68.4
	Positive	2	6.3	6	20	6	31.5

Serum DHEA-S level ranged between (3.6151±0.1949µg/dl), (3.2964±0.583 µg/dl) (2,0306±0.49159 µg/dl)in healthy controls, diabetic and diabetic nephropathy cases respectively. As shown in the table (2), no statistically significant differences were found between mean serum DHEA-S level of diabetics and healthy controls and statistically significant differences were found between mean serum DHEA-S level of diabetic nephropathy and healthy controls. Mean serum DHEA-S level was lower in diabetic nephropathy compared to diabetics, however, no significant difference was found between mean values of the two groups. A large study of experimental and clinical evidence, especially in developed countries, supported alteration of DHEA-S level in case of diabetes mellitus (16). However, few studies had been held to examine the relationship between DHEA-S level and diabetic nephropathy in developing countries (Singh et al 1998) [18]. In other recently published studies, DHEA-S supplementation had been found to produce beneficial effects in individuals with diabetes mellitus (Rathna Kumari U1, Padma K2014)[19] . In vitro , DHEA-S may slow the progression of nephropathy in the obese

Zucker rat, and this appears to be due to reduced caloric intake under the conditions of this study [20]. fasting blood sugar values ranged between (86.2667±5.582mg/dL) (202.87±30.3mg/dL) (280.0±16.56715mg/dL) for healthy controls, diabetic and diabetic nephropathy group respectively. As shown in table (2), significant differences were found between mean (± SD) FSG of diabetics and healthy controls and that of diabetic nephropathy groups and healthy controls ($p< 0.001$). There was significant difference between mean (± SD) FBS of diabetic and diabetic nephropathy patients. For diabetic nephropathy only, HbA1c % value ranged between 7 -10.8. As shown in table (2), the mean (± SE) value for HbA1c% of diabetic nephropathy was 10.3±0.67. The mean value for HbA1c% of diabetic nephropathy with low serum DHEA-S level was higher than that for patients with normal serum DHEA-S level and there was a significant difference between mean HbA1c% of the 3 groups. Poorly controlled diabetic nephropathy patient were expected to have higher FSG than well controlled and impaired controlled diabetics, because HbA1c is formed at a rate proportional to the average glucose concentration, when there is hyperglycemia there will be an increase in glycosylated hemoglobin and it is become irreversible if insulin deficiency present (19). Significant differences were found between mean (± SE) values for blood urea and creatinine of diabetic and control groups. On the other hand Significant differences were found between mean (±SE) (1.43±0.9) (values for albumin of diabetic nephropathy and control groups (4.7±0.5). A significant increase in mean of blood urea in diabetic patients than matched healthy control , this could be attributed to protein metabolism impairment, kidney function impairment (glomerular function) and decrease vascular perfusion of kidney which leads to increase tissue necrosis as explained by Sugam Shresthain 2008(21). The previously mentioned authors explain the increase in the serum creatinine level in IDDM patients, results from a disorder of kidney function which reduce the creatinine excretion, resulting in increased serum creatinine level. As there was an increase HbA1c there will be more renal injury results in an increase mean of serum creatinine, urea level in poorly controlled diabetics more than impaired and well controlled (Michael, 2000)[22] . As shown in table (2), significant differences were found between mean (± SE) SBP of diabetic nephropathy and healthy controls ($p< 0.05$). There was no significant difference between

mean (± SE) SBP of diabetic and diabetic nephropathy patients. Also There was no significant difference between mean (± SE) SBP of diabetic and control groups. Hypertension has been found to be a main factor in the prediction and progression of diabetic nephropathy. It is mostly believed that the hypertension in diabetes is greatly due to arteriolar spasm of internal efferent or intraglomerular (Arieff, 2003)[23]. It may also due to alteration in cardiac structure and function, compared with age matched individuals without diabetes [24]. Statistically significant differences were found between mean serum albumin of diabetics nephropathy and healthy controls as shown in table (2) Schultz et al in 2001, found that the relation of blood pressure with decreased albumin remained significant for systolic BP ($P < 0.001$) and for diastolic BP ($p < 0.001$) in IDDM, that is agreed with the study results. They also stated that there was an association between increasing urinary albumin secretion in IDDM patients and hypertension [25]. As shown in table (2), significant differences were found between mean (± SE) duration of diabetic and diabetic patients with nephropathy ($p< 0.05$). One of a very important factor is duration of diabetes that established in many studies increased evolution of diabetic nephropathy as. Rudberg et al4, that found the duration of disease was an important factor in the overall severity of glomerulopathy [25a]. The diabetes control and complications trial detected that increased duration of diabetes lead to development of diabetic nephropathy.

Table (2) shows the mean (±SE) values for variable with their significance in study group

Variable	Control group	Diabetic group	Diabetic nephropathy	Significant p
Age(years)	54±0.34	55±0.9	65±0.57	NS
FSG(mg/dl)	86.26±5.58	202.87±30.3	280.0±16.56	P<0.001
HbA1c%	4.3 ±0.12	7.3±0.01	10.3±0.67	P<0.001
BMI(Kg/m^2)	26±1.2	27±0.98	29±1.34	NS
SBP (mmHg)	11±0.53	13±1.6	15±0.76	P<0.05
DPB(mmHg)	7.3±0.62	8±0.9	9±0.43	NS
Serum creatinine(µmol/L)	62±0.02	79±1.12	122.50±26	P<0.001
Blood urea(mmol/L)	4.6±0.92	19±2.32	35.77±4.74	P<0.001
Albumin(g/dl)	4.7±0.5	3.5±0.6	1.43±0.9	P<0.05
DHEA-S(µg/dl)	3.61±0.19	3.29±0.58	2,6306±0.49	P>0.05
Duration(years)	-	7±0.32	10±0.4	P>0.05

To show the net and independent effect of a set of explanatory variables on serum DHEA-S level, multiple linear regression models were used. All the possible explanatory variables were entered in the model in the first step and the resulting equation was shown in table (3). A negative correlation was observed between age, BMI and waist hip ratio) and the outcome variable (mean serum DHEA-S level).

The resulting equation was statistically significant (P<0.05) and explains 89% of the observed variation in the dependent variable. In the present study, low mean concentration of serum DHEA-S noticed with increased age of diabetic patients with nephropathy; suggest that apart from age being diabetic is attached with lower mean value of this hormone. This finding had been proved by multiple linear regression model used in this study, which showed that the negative correlation found between age, and serum DHEA-S level. The results of the present research are in agreement with several previous studies that have proved lower levels of DHEA-S with advancing age (Moffat et al., 2000) [26] . With advancing age increases abdominal fat accumulation, and there is demonstrated that increases the risk for development of diabetes, insulin resistance, and atherosclerosis in abdominal obesity subjects [27] [28].In addition, changes of hormonal metabolic which occur with aging may cause to the increase in fat of abdominal that in any event occurs pending old and middle age[29]. One such change is the decline in production of the adrenal hormone DHEA [30]

Table (3): Multiple linear regression equation with serum DHEA-S level (µg/dl) as the dependent variable and the studied independent variables in diabetic with nephropathy

Independent variables	β	Standardized β	P-value
Age (in years)	-0.002	-0.269	P<0.05
Body mass index	-0.011	-0.924	P<0.05
Waist hip ratio	- 0.61	- 1.658	<0.05
Urban residence (Compared to rural)	- 3.87	- 0.13	0.02
Practicing regular physical activity	- 2.23	- 0.07	0.22
Cigarette smoking index	- 0.06	- 0.03	0.57

R^2=0.892
P<0.05

Conclusions

1. There is a major association between DHEA-S levels and diabetic nephropathy. These conclusions promote that DHEA-S level inversely correlated with the diabetic nephropathy progression.
2. Highly significant decrease in DHEA-S levels with advancing age in the studied groups suggest that apart from age being diabetic is related with lower mean value of this hormone. This finding had been proved by multiple regression model used in this study, which revealed that the negative correlation found between age, and level of serum DHEA-S

References

1. Takuya SUGAHARA †1, Yuh-Shyong YANG , Chau-Ching LIU‡, T. Govind PAI and Ming-Cheh LIU. 2003. Sulphonation of dehydroepiandrosterone and neurosteroids: molecular cloning, expression, and functional characterization of a novel zebrafish SULT2 cytosolic sulphotransferase. Biochem. J. 375: 785–791
2. Hu J1, Zhang Z, Shen WJ, Azhar S. 2010. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. . Nutrition & Metabolism J. 1186/1743-7075-7-47
3. Alan, R. and Gaby, M. D. 1996. Dehydroepiandrosterone: Biological Effects and Clinical Significance. Alternative Medicine Review, 1 (2): 60-69
4. Nathalie A. Compagnone†, Eduardo Salido, Larry J. Shapiro, and Synthia H. Mello .1997. Expression of Steroid Sulfatase during Embryogenesis*. Endocrinology. 138 (11): 4768-4773
5. Afsaneh Talaei1 , Masoud Amini2 , Mansour Siavash2 , Maryam Zare3.2010. The effect of Dehydroepiandrosterone on insulin resistance in patients with impaired glucose tolerance. Hormones .9(4):326-331

6. Ozougwu, J. C.1*, Obimba, K. C.2, Belonwu, C. D.3, and Unakalamba, C. B.4. 2013. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus Journal of Physiology and Pathophysiology. 4(4), pp. 46-57,
7. Asmaa, M. Salih; Isam, N. Salman and Rajaa M. Kaduhm. 2013. Study ABO/Rh system with Endothelial Inflammatory Factors in Iraqi Arab Female with Diabetes Mellitus Type II. Baghdad Science Journal, 11(1): 93-102
8. Ashwell M ,Cole TG , and Dixon AK . (1985) New insight into the anthropometric classification of fat distribution shown by computed tomography . BMJ . 290:1692-4
9. Frier B.M., Truswell A.S., Sheperd J., Delogy A., and Jung R. (1999). Diabetes mellitus and nutritional metabolic disorder. In: Davidson
10. Jung R. T. (1997). Obesity and nutritional factor in pathogenesis of non – insulin dependant diabetes mellitus, section 4 , chapter 19, In: Text book of diabetes ; pickup J.C. and William G. ;2nd ed. Vol. 1 . Blackwell science Itd. PP : 19.1.
11. Truswell S (1992). ABC of nutrition . 2nd edition. P.55.
12. Horton ES. (1986). Exercise and physical training effect on insulin sensetivity and glucose metabolism effect on insulin sensetivity and glucose metabolism . Diabetes Metab Rev.2:43-45.
13. Goldenberg RL , Tamura T , Neggers Y, Copper RL , Johnston KE et al . (1995). The effect of zinc supplementation on pregnancy outcome . JAMA . 274 :463 – 468 .
14. Rose G , Blackburn H , Gillum RF and Prineas RJ (1982) :Cardiovascular Survey Methods . Geneva :World Health Organization , 1982
15. Vijay Viswanathan, Priyanka Tilak & Satyavani Kumpatla (2012): Risk factors associated with the development of overt nephropathy in type 2 diabetes patients: A 12 years observational study. Indian J Med Res 136, July, pp 46-53

16. Hui-Mei etal (2013); The relationship between obisty and diabetic nephropathy in china . chen etal . BMC nephrology . 2013,14-69

17. World Health Organization (1999). Definition, diagnosis and classification of diabetes mellitus and its complication. Report of WHO consultation. NCD/NCS/99.2.Geneva.

18. Singh R. B. , Mohammad MD., Niaz A. , Rastogi S.S. , Bajaji S. et al . (1998). Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations in North India . J Am Coll Nutr. 17 (6) ;564-570.

19. Rathna Kumari U1, Padma K2 (2014): Dehydroepiandrosterone level in type 2 Diabetes. Int J Med Res Health Sci ;3(2): 411-415

20. Robert J. Richards, Johnny R. Porter, Felipe inserra, Leo ´ N F. Ferder,ine ` S Stella, Efrain reisin, and Frank svec. (Effects of dehydroepiandrosterone and quinapril on nephropathy in obese Zucker rats) . Kidney International, Vol. 59 (2001), pp. 37–43

21. Sugam Shrestha1, Prajwal Gyawali2, Rojeet Shrestha2, Bibek Poudel2, Manoj Sigdel2, Prashant Regmi2, Manoranjan Shrestha2, Binod Kumar yadav2(2008); Serum Urea and Creatinine in Diabetic and non-diabetic Subjects. Journal of Nepal Association for Medical Laboratory Sciences V:9,N:1 P. 11-12

22. Michael L, Bishop M, Janet L, Duben–Engel K. Clinical chemistry, principles, procedures, correlations. 4th edition, Lippincolt, Williams and Wilkins 2000, pp 166.

23. Arieff MD, Allen I. Endotext. 1st edition. W.B. Saunders Company. 2003, pp. 341-50.

24. Lawrence H, Young M. Diastolic function and type I diabetes. Diabetes Care 2004, 27, 2081-3

25. Schultz C, Dalton R. Neil H, Konopelska B, Dunger D. Blood pressure does not raise before the onset of microalbuminuria in children followed from diagnosis of type I diabetes Oxford Regional Prospective Study Group. Diabetes Care 2001, 24(3), 555-60

26. Tung P, Levin SR. Nephropathy in NIDDM. American Journal of Medical sciences. 1988; 85: 131.

27. Moffat, S. D.; Zonderman, A. B.; Mitchell Harman, S.; Blackman, M.R.; Kawas, C. and Resnick, S. M. (2000). The relationship between longitudinal declines in dehydroepiandrosterone sulfate concentrations and cognitive performance in older men. Arch. Intern. Med. 160: 2193-2198.

28. Kopelman, P. G. (2000). Obesity as a medical problem. Nature, 404: 635-643. Kopelman, P. G. (2000). Obesity as a medical problem. Nature, 404: 635-643.

29. Ferroni P, Basili S, Falco A, and Davi G. (2004). Inflammation, insulin resistance, and obesity. Curr. Atheroscler. Rep., 6: 424-431.

30. Heiat A, Vaccarino V, Krumholz HM. An evidence-based assessment of federal guidelines for overweight and obesity as they apply to elderly persons. Arch Intern Med 2001; 161: 1194–203.

31. Dennis T. Villareal, John O. Holloszy. Effect of DHEA on Abdominal Fat and Insulin Action in Elderly Women and Men.2004. American Medical Association, November 10,Vol 292, No. 18