JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2013. Vol. 7(4), p. 3177-3184

Chlamydia pneumoniae Infection in Diabetic Patients with Dyslipidemia

Sami A. Gabr[1,2]* and Ahmad H. Al-Ghadir[2,3]

[1]Department of Anatomy, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2]Rehabilitation Research Chair (RRC), King Saud University, Riyadh, KSA.
[3]Department of Rehabilitation Science, College of Applied Medical Sciences, King Saud University, Riyadh, KSA.

(Received: 06 April 2013; accepted: 27 May 2013)

***Chlamydia pneumoniae* is an obligate parasite capable of producing chronic infections. The severity of infection is closely related to hyperglycemia and changes in lipid profile. Since patients with *Diabetes mellitus* (DM) are at high risk of cardiovascular disease (CVD), the prevalence rate of specific *C. pneumonia* IgG / Ig A antibodies, its association to the change in diabetic control HbAc1 and lipid profile were estimated in 150 patients with DM and 50 healthy control subjects. *C. pneumoniae* (CPN) antibodies were estimated in 70% of DM patients using MIF and rDNA ELISA assays. 75 and 25% of patients were positive for CPN-IgG and IgA respectively using rDNA ELISA with prevelance rate 56.5 %, while 88 and 12% of patients were positive for CPN-IgG and IgA respectively using MIF assay which considered a gold standard method. There was significant (p=0.01) increase in the lipid profile except HDL-cholesterol (HDL-C) in diabetic patients with CPN-IgG antibodies compared to those having positive CPN-IgA antibodies. Significant positive correlations in BMI, Total cholesterol, triglyceride, LDL-C and negatively with HDL-C were reported in diabetic patients with varying *C. pneumoniae* antibodies titers. The data obtained suggested that, the change in lipid profile and HbA1c may play a pivotal role in the severity of *C. pneumoniae* infection. Consequently, the reduction of lipid profile may be a new therapeutic target against *C. pneumoniae* infection in diabetic patients.**

Key words: rDNA LPS ELISA, lipid profile, Diabetes, HbA1c, *C. pneumonia.*

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. The increased morbidity and mortality of diabetic patients is mostly attributed to complications of the disease[2] . Chronic hyperglycemia leads to several events that promote structural changes in tissues and are associated with impaired wound healing[3], higher susceptibility to infections[4], and micro and macro vascular dysfunctions[5].

* To whom all correspondence should be addressed.
Tel.: +966562060018; Fax: +96614698541;
E-mail: nadalab2009@hotmail.com

Infection with *Chlamydia pneumoniae* (CPN) occurs worldwide, resulting in a 40-90% prevalence of serum antibody to the species in various populations[6].

Indirect evidence suggests that increased blood glucose due to DM may help sustain persistent *C. pneumoniae* infections to compensate for the extra energy load on infected cells. Chlamydial infections increase glucose consumption, lactate production, glutamate synthesis, glycogen accumulation, and an associated increase expression of the glucose transporter, GLUT-1, *in vitro*[7]. Conversely, diabetics are assumed to be very susceptible to infections with C. pneumonia. There may be an association between chronic *C. pneumoniae* infections and diabetic nephropathy based upon

anti-*C. pneumoniae* serum IgG antibody titers in an ELISA[8].

Some other studies have described associations between Chlamydia pneumonia seropositivity and the metabolic syndrome and dyslipidemia[9,10].

Abnormal cholesterol and lipoproteins are major risk factors associated with atherosclerosis in diabetic and non diabetic patients. Atherosclerotic plaques contain foam cells and evidence of altered lipid composition[11]. Animal models show that *C. pneumoniae* can induce atheromas and mimic inflammatory processes present in human atherosclerosis[12].

Also, in previous cross-sectional study, a significant association between the presence of *C. pneumoniae*-specific IgG antibodies, elevated serum triglyceride, and lowered HDL cholesterol concentrations reported in a male population in Northern Finland[13].

Regarding the probable *C. pneumoniae* pathogenic severity in diabetic patients, the objective aim of this study is to investigate the possible role of serum lipids, BMI, HbA1c and their correlation to specific *C. pneumoniae* antibodies in diabetic patients.

MATERIALS AND METHODS

Patients

A total of 200 individuals recruited from the clinic of diabetic patients, Mansoura University Hospital, Mansoura, Egypt. Out of these 50 healthy individuals (25 men and 15 women, between 18 and 65 years old) with a mean age of 30±7.12 served as controls who attended for routine health check up at the hospital. None of the healthy control was taking any medicine or dietary supplement; they were selected after detailed physical examination and laboratory tests. A total of 150 of type 2 diabetes mellitus (DM2) (110 men and 40 women, aged from 20 to 64 years old) with a mean age of 45.9±4.6 were included in this study. The diagnosis of DM was based on the American Diabetes Association criteria for DM2 (fasting plasma glucose level higher than 126mg/dl and/or glucose level exceeding 200 mg/dl at 2 hours in the 75 g oral glucose tolerance test) 1. Patients with type1 diabetes, smokers, anemia, overt complications of diabetes like nephropathy, neuropathy, retinopathy, obvious ischaemic heart disease (angina, myocardial infarction, and lead electrocardiogram abnormalities), HCV, HBV, chronic liver disease, hypothyroidism, overweight and obesity (BMI: ^{3} 25 and ^{3} 30 Kg/m2), and drugs (diuretics; oral contraceptives) were excluded from the study. All subjects completed a structured questionnaire with questions about demographic data, tobacco use and daily medication use. After 12 hrs fasting 5 ml of venous blood samples were collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 2000 rpm. Samples were given a coded study identification number and were shipped frozen at -80C until analysis. All serological and microbiological serum analysis was performed at clinical chemistry and microbiological laboratories, Mansoura University Hospital, Mansoura, Egypt. The demographics and baseline characteristics of patients and controls are presented in (Table 1). The study protocol was approved by ethical committee of Mansoura University Hospital, Mansoura, Egypt.

Serum samples were collected by standard procedures and were stored at 280°C prior to processing. From each patient with type 2 diabetes mellitus, nasopharyngeal specimens were collected with sterile cotton-tipped aluminum- shafted swabs and were suspended in 1.5 ml of Chlamydia transport medium (0.2 M sucrose phosphate [2SP]). Sputum samples were collected by standard procedures.

Laboratory assays for *C. pneumoniae*

ELISA. Chlamydia-specific IgG and IgA antibodies

ELISA. Chlamydia-specific IgG and IgA antibodies were detected by an rDNA LPS ELISA (Medac GmbH, Hamburg, Germany). The rDNA LPS ELISA may currently be the preferred tool for diagnosing acute respiratory Chlamydia infections in routine clinical practice. This ELISA includes a chemically pure structure of a recombinant LPS which contains a genusspecific epitope of the Chlamydia spp. pathogenic for humans 14-16. Initial serum dilutions for the detection of IgG, IgM, and IgA were 1:100, 1:50, and 1:50, respectively. Prior to the IgM determinations, IgG absorption was performed 17. Sera with optical density values exceeding 2.5 were retested with a 1:4 predilution. A twofold serially diluted standard serum sample

was used to calculate the log2 titer of the patients' samples. The IgG, IgM, and IgA cutoff values were calculated as prescribed by the manufacturer. The prevalence of Chlamydia IgG, IgM, and IgA antibodies was based on the following criteria: greater than or equal to the calculated cutoff 31.10, greater than or equal to the calculated cutoff 31.15, and greater than or equal to the calculated cutoff 31.10, respectively. Serological diagnosis of an acute Chlamydia infection was based on the ELISA results by using the following criteria: a threefold or greater increase in Chlamydia-specific IgG or IgA antibody titer, a twofold or greater change in the specific IgM titer, or a twofold increase in the specific IgG antibody titer in combination with a twofold increase in the specific IgA antibody titer.

MIF assay

The MIF assay described before17, was used to measure *C. pneumoniae*-specific IgG, and IgA antibodies. Briefly, purified *C. pneumoniae* elementary antibodies (strain AR 39; Washington Research Foundation) were used to detect IgG, and IgA antibodies to *C. pneumoniae*. The prevalence of *C. pneumoniae*-specific IgG, and IgA antibodies was based on the presence of IgG at titers of ≥1:32, and IgA at titers of ≥1:32, respectively. Diagnosis of an acute *C. pneumoniae* infection was based on the following criteria: the presence of *C. pneumoniae*-specific IgM in either acute- or convalescent-phase serum or a fourfold or greater increase in the *C. pneumoniae*specific IgG and/or IgA antibody titer between the acute- and convalescent phase serum samples 17.

Analysis of Blood sugar and Glycated Hemoglobin (HbA1c):

Blood glucose was measured by Glucose Oxidase and Peroxidase (GOD-POD) colorimetric method (Quanti Chrom. TM Glucose Assay Kit, DIGL-100, BioAssay Systems, Hayward, CA 94545, USA). HbA1c was measured with the quantitative sandwich enzyme immunoassay technique (Human HbA1c ELISA kit,Cat. No.E0897h, eiaab.co).

Analysis of serum lipid profile

Serum total cholesterol levels was determined by enzymatic (CHOD-PAP) colorimetric method and triglycerides by enzymatic (GPO-PAP) method as previously described 18-19. HDL-cholesterol was estimated using precipitant method 20 , and LDL cholesterol by Friedewald formula 21.

Statistical analysis

Variables were summarized by standard descriptive statistics and expressed as mean ±SD. After entering the data to a masked database the analysis was performed by an unaware statistician, using the Statistical Package of Social Sciences (SPSS) version 11.5.0 (SPSS, Inc. Chicago, Illinois). The Student-test, chi-square, and ANOVA were used for analysis of quantitative and qualitative data, respectively. The significance level was set at 0.05.

RESULTS

One hundred and fifty patients with type 2 diabetes mellitus (110 men and 40 women), with a mean age of 45.9±4.6 and fifty healthy controls (25

Table 1. Clinical and demographic characteristics of the study population

Variables	Controls	DM2 Patients	*P-Value
No.	50	150	
Age (Year) (mean ± SD)	30 ±7.12	45.9±4.6	**0.001
Gender (Male/Female)	25/15	110/40	-
BMI (mean ± SD)	17.6±3.7	21.8±4.1	** 0.001
Time since the DM onset (n, %, mean ± SD)	-		
< 10 years		45 (30.0), 8.13± 4.1	
≥10 years		105 (70.0), 13.13±5.16	
Medication for control of DM (n, %)			
Hypoglycemic		97 (64.7)	
Insulin		35 (23.3)	
Hypoglycemic and Insulin		18 (12.0)	

*P for controls vs Diabetic patients; (Student- t test), * P<0.05; **P<0.01; SD: Standard deviation; DM2: type 2 diabetes mellitus; BMI: Body mass index

men and 15 women), with a mean age of 30±7.12 were studied. The demographic data of the patients and healthy controls were shown in table (1).

The prevalence of Chlamydia-specific antibodies in the healthy blood donor group and the diabetic patient group were 30 and 56.5%, respectively, as determined by the rDNA LPS ELISA (P , 0.0001) (Table 2). In diabetic group, only 75 % of patients were positive for CPN-IgG antibodies along with 25% positive CPN-IgA antibodies (Table 3). Significantly higher seroprevalences were observed if the *C. pneumoniae* MIF assay was used (Table 2). The seroprevalence by the MIF assay for healthy blood donors and diabetic patients was 42.3 and 72 % respectively. The prevalence rate of CPN- IgG and IgA antibodies was 88 and 12 % respectively among diabetic patient group (Table 3).

By using the MIF assay as the gold standard, the sensitivity and specificity of the

Table 2. Prevalence of Chlamydia-reactive antibody determined by MIF assay and rDNA LPS ELISA in the serum of 150 patients with type 2 diabetes and healthy blood donors.

Study population	No. of subjects	Prevalence (%) observed by the following		rDNA LPS ELISA[a]			
		MIF assay	rDNA LPS ELISA	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
DM2	150	72	56.5[b]	65.5	76	45	86.8
Control	50	42.3	30	33.5	82	32.6	81.8

[a]The MIF assay was used as the gold standard., b P , 0.0001 compared to blood donors.

Table 3. Correlation between Chlamydia-specific antibodies determined by rDNA LPS ELISA and MIF assays of 150 patients with type 2 diabetes.

No. of patients tested	Assay	Prevalence % 105 (150)	No. (%) of patients seropositive CPN antibodies IgGa , (%)	IgAb , (%)
150	rDNA LPS ELISA	56.5	75	25
	MIF	72	88	12

[a] Correlation coefficient, 0.902; P = 0.001; b Correlation coefficient, 0.975; P = 0.05 (by chi-square test).

Table 4. Biochemical characteristics of the study population

Biomarkers	controls	DM2 patients with CPN Negative antibodies	DM2 Patients with CPN Positive antibodies IgA	Ig G
No.	50	45	45	60
HbA1c, mmol/L	4.49±0.9	9.6±2.1	8.2±0.96	10.6 ±0.64
Sugar, mg/dL	82.14±24.8	231±63.6	187± 32.3	246± 21.6
T.Chol., mmol/L	3.9±0.81	8.17± 1.5	8.9 ± 0.95	10.7 ± 0.59
TG, mmol/L	2.42±0.66	4.3±0.94	6.9 ± 0.91	9.13 ± 0.58
HDL-C , mmol/L	2.46±1.2	1.9 ± 0.7	1.0 ± 0.45	0.9 ± 0.28
LDL- C mmol/L.	1.0± 0.14	2.65±0.49	3.0 ± 0.7	5.5 ± 044
P		**0.01	**0.01	**0.01
P-1			**0.001	**0.001
P-2				**0.001

P: control vs diabetic patients, CPN-IgG or CPN-IgA; P-1: diabetic patients vs diabetic patients with positive CPN-IgG or IgA antibodies; P-2: CPN-IgG patients vs CPN-Ig A; (Student- t test), * p<0.05; **p<0.01; SD: Standard deviation; DM2: type 2 diabetes mellitus; CPN: Chlamydia. Pneumoniae; HbA1c: Glycated Hemoglobin Ac1; T- chol.: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: Low Density Lipoprotein.

Table 5. Correlation coefficients of HbA1c and sugar compared to other variables in diabetic patients with or without *Chlamydia pneumoniae* IgG or IgA Antibodies.

Variables	DM2 patients with CPN Negative antibodies		DM2 Patients with CPN Positive antibodies			
			Ig A		Ig G	
No.	45		45		60	
	HbA1c	Sugar	HbA1c	Sugar	HbA1c	Sugar
	r	r	r	r	r	r
BMI	0.019*	0.795*	0.265*	0.37*	0.14**	0.58**
T.Chol., mmol/L	0.18*	0.956*	0.12*	0.163*	0.17**	0.517**
TG, mmol/L	0.258*	0.382*	0.231*	0.550*	0.78**	0.546**
HDL-C, mmol/L	- 0.26*	- 0.46**.	-0.10*	-0.449*	- 0.570**	-0.439**
LDL- C mmol/L.	0.203*	0.672*	0.144*	0.493*	0.303**	0.479**

*p HBA1c or sugar vs other variables in Diabetic patients with positive or negative CPN antibodies; * p<0.05; **p<0.01; SD: Standard deviation; DM2: type 2 diabetes mellitus; CPN: Chlamydia. Pneumoniae; HbA1c: Glycated Hemoglobin Ac1; T- chol.: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: Low Density Lipoprotein.

rDNA LPS ELISA for determination of serological evidence of a *C. pneumoniae* infection in the past thus depended on the population tested (Table 2). For the healthy blood donor group the sensitivity and specificity were 33.5 and 82%, respectively, while for the patients with DM2, the sensitivity and specificity were 65.5 and 76.0%, respectively (Table 2).

The data obtained showed a significant elevation in serum glucose, HbA1c and change in lipid profile of diabetic patients with or without CPN- antibodies. The data obtained showed a significant (P = 0.01) increase in the levels of T-Chol, TG, LDL-Chol, and decrease (P = 0.01) in the level of HDL-C. The change in lipid profile was higher in DM patients with CPN-IgG antibodies compared to those of CPN-IgA antibodies and healthy control group (Table 4).

The data obtained showed that, BMI, T-Cholesterol, triglycerides and LDL-C correlated positively and HDL-C negatively with HbA1c and glucose concentration in diabetic patients with or without anti-*C. pneumoniae* antibodies (Table 5).

DISCUSSION

Diabetes mellitus (DM) affects more than 100 million people worldwide and its incidence and prevalence are still rising with higher risk of cardiovascular disease [22]. Cross-sectional and longitudinal studies demonstrate significant positive correlations between measures of plasma glucose and corresponding plasma lipids [23-24].

Diabetic patients with accompanied dyslipidemia are soft targets of cardio vascular deaths. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular complications and mortality [25].

The purpose of this study was to determine the correlation between *C. pneumoniae* (CPN) infection and serum lipids in diabetic patients. The most important predictor of change in plasma lipids was the change in HbA1c and glucose levels.

In our study, significant higher levels of total cholesterol, TG, LDL-C levels and decrease in HDL-C were reported in diabetic patients compared to healthy volunteers. The data obtained matched with other studies which showed that elevated concentration of LDL-C in the blood are powerful risk factors for coronary heart disease in patients with diabetes [26-27].

In our study, BMI, T-Cholesterol, triglycerides and LDL correlated positively and HDL negatively with HbA1c and glucose level in diabetic patients. This matched with those who reported positive correlations between diabetes control parameters and the change in lipid profile which gives a new shed on the importance of lipids in insulin-dependent diabetes (IDDM) [28-29].

Diabetes mellitus (DM) is associated with a state of chronic inflammation and increase in

proinflammatory cytokines [30]. This condition with impaired immune- reactivity makes DM patients prone to microbial infections [31-32]. The development of atherosclerotic plaques in diabetic patients may be related to the presence of *C. pneumoniae* in blood [33- 34].

In our study, *C. pneumoniae* antibodies were estimated in 70 % of diabetic patients. Out of them, 40% of patients were positive for IgG and 30% with IgA antibodies. *C. pneumoniae* antibodies were significantly ($P < 0.001$) correlated with both serum glucose and HbA1C. It was reported that C. *pneumonia* infection is ubiquitous, with an antibody prevalence of 50% by age 20 and 70% to 80% at age 60 to 70 [35]. Recently, it was reported that *C. pneumoniae* antibody positivity was significantly more common in patients of poor metabolic control (HbA1c>9%) versus patients in good metabolic control (HbA1c<7%). These suggest an increased risk of *C. pneumoniae* infection in diabetic patients [36-37]. Thus, the development of diabetes might be a possible factor for promoting *C. pneumoniae* dissemination [38].

This study indicates that the severity of *C. pneumoniae* infection is associated with the change in lipid profile. in diabetic patients whereas, the atherogenic effects of Chlamydia are claimed to be dependent on serum cholesterol and change in lipid metabolism [39-44].

CONCLUSION

The change in lipid profile and HbA1c play a pivotal role in the severity of *C. pneumoniae* infection in diabetic patients. So, the reduction of plasma lipid profile may be a new therapeutic target against *C. pneumoniae* infection

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2009; **32**: S13–61.
2. Ahmed N: Advanced glycation end products-role in pathology of diabetic complications. *Diabetes Res Clin Pract.* 2005; **67**:3–21.
3. lacopino AM: Diabetic periodontitis: possible lipid-induced defect in tissue repair through alteration of macrophage phenotype and function. *Oral Dis.,* 1995; **1**:214–29.
4. Hirsch T, Spielmann M, Zuhaili B, *et al:* Enhanced susceptibility to infections in a diabetic wound healing model. *BMC Surg*. 2008; **8**:5.
5. Collin HL, Niskanen L, Uusitupa M, et al: Oral symptoms and signs in elderly patients with type 2 diabetes mellitus A focus on diabetic neuropathy.*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; **90**:299–305.
6. Freidank HM and Brauer D : Prevalence of antibodies to *Chlamydia pneumoniae* TWAR in a group of German medical students. *J Infect* 1993; **27**: 89-93.
7. Ojcius DM, Degani H, Mispelter J, Dautry-Varsat A: Enhancement of ATP levels and glucose metabolism during infection by *Chlamydia*: NMR studies of living cells. *J Biol Chem* 1998; **273**:7052-7058.
8. Kanuchi M, Kawano T, Dohi K : Association of *C. pneumoniae* infection with diabetic nephropathy. *Diab Res Clin Pract* 2000; **47**: 45-48.
9. Leinonen M and Saikku P: Interaction of *Chlamydia pneumoniae* infection with other risk factors of atherosclerosis. *Am Heart* J 1999*;* **138**:S504–S506.
10. Muller J, Moller DS, Kjaer M, Nyvad O, Larsen NA, Pedersen EB: *Chlamydia pneumonia* DNA in peripheral blood mononuclear cells in healthy control subjects and patients with diabetes mellitus, acute coronary syndrome, stroke, and arterial hypertension. *Scand J Infect Dis* 2003*;* **35**:704–712.
11. Ross R : Atherosclerosis an in ammatory disease. *N Engl J Med* 1999; **340**:115-126.
12. Fong IW, Chiu B, Viira E, *et al*: Can an antibiotic (macrolide) prevent *Chlamydia pneumoniae*-induced atherosclerosis in a rabbit model. *Clin Diag Lab Immunol* 1999; **6**:891±894.
13. Laurila AL, Bloigu A, Nayha S, Hassi J, Leinonen M, Saikku P: *Chlamydia pneumonia* antibodies associated with altered serum lipid profile. *Int J Circumpolar Health* 1998; **57**: 329-332.
14. Brade, L., H. Brunnemann, M. Ernst, Y. Fu, O. Holst, P. Kosma, H. Naher, K. Persson, and H. Brade: Occurrence of antibodies against chlamydial lipopolysaccharide in human sera as measured by ELISA using an artificial glycoconjugate antigen. *FEMS Immunol. Med. Microbiol* 1994; **8**:27–41.
15. Brade, L., O. Holst, P. Kosma, Y. X. Zhang, H. Paulsen, R. Krausse, and H. Brade: Characterization of murine monoclonal and murine, rabbit, and human polyclonal antibodies against chlamydial lipopolysaccharide. *Infect.Immun* 1999; **58**:205–213.
16. Holst, O., L. Brade, P. Kosma, and H. Brade:

Structure, serologicalspecificity, and synthesis of artificial glycoconjugates representing the genus specific lipopolysaccharide epitope of Chlamydia spp. *J. Bacteriol*. 1991; **173:**1862–1866.

17. Verkooyen, R. P., M. A. Hazenberg, G. H. Van Haaren, J. M. Van Den Bosch,R. J. Snijder, H. P. Van Helden, and H. A. Verbrugh: Age-related interference with *Chlamydia pneumoniae* microimmunofluorescence serologydue to circulating rheumatoid factor. *J. Clin. Microbiol.1992*; **30**:1287–1290.
18. Allain C C, Poon I S, Chan C H G, Richmond W: Enzymatic determination of serum total cholesterol. *Clin. Chem.*1974; **20**: 470-471.
19. Jacobs N J, Van Denmark P J: Enzymatic determination of serum triglyceride. *Ch. Biochem. Biophys*. 960**; 88**: 250-255.
20. Gordon T, Gordon M: Enzymatic method to determine the serum HDL-cholesterol. *Am. J. Med.* 1977*;* **62**: 707-708.
21. Friedewald W T, Levy R I, Fredrickson D S: Estimation of the concentration of LDL-cholesterol. *Clin. Chem.*1977; **18 (**6): 499-515.
22. Amos AF, McCarty DJ, Zimmet P: The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 1997; **14**: S7–S85.
23. Gonen B, White N, Schonfeld G, Skor D, Miller P, Santiago J: Plasma levels of apolipoprotein B in patients with diabetes mellitus: the effect of glycemic control. *Metabolism* 1985; **34:**675-79.
24. Murray LJ, O'Reilly DP, Ong GM, O'Neill C, Evans AE, Bamford KB: *Chlamydia pneumoniae* antibodies are associated with an atherogenic lipid profile. *Heart* 1999; **8**:239– 244.
25. Sharma S, Kulkarni SK, Chopra K.: (2006) Curcumin the active principle of turmeric ameliorates diabetic nephropathy in rats. *Clin. Exp. Pharmacol. Physiol.*2006; **33**: 940-945.
26. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M : Mortality from coronary heart disease in subject with type 2 diabetes and in non diabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229-234.
27. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ: Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high density lipoprotein particles displaying impaired antioxidative activity. *J Clin Endocrinal Metabolism* 2004; **89**: 4963-4971.
28. Law MR: Lowering heart disease risk with cholesterol reduction: evidence from observational studies and clinical trials. *Eur. Heart J*. 1999; **1**: S3-S8.
29. Report of the National Cholesterol Education Program (RNCEP) :Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. *Arch Intern Med* 1998; **148:**36- 39.
30. Kumawat M, Singh I, Singh N, Singh V, Kharb S: Lipid Peroxidation and Lipid Profile in Type II Diabetes Mellitus. *Web med Central BIOCHEMISTRY* 2012; **3**:WMC003147.
31. Dondona P, Ajada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunnol*. 2004; **25**; 4-7.
32. Rajagopalan S: Serious infection in elderly patients with diabetes mellitus. Clin. Infect. Dis. 2005; **40:**990-996.
33. Thomsen RW, Hundborg HH, Lervang HH, Johnsen SP, Schonheyder HC, Sorensen HT: Diabetes mellitus as a risk factor for community aciquired bacteremia due to enterobacteria: a 10-year population based study among adults. *Clin. Infect. Dis*. 2005; **40:**628-631.
34. Iliescu EA, Fiebig MF, Morton AR, Sankar-Mistry P: *Chlamydia pneumoniae* DNA in peripheral blood mono nuclear cells in peritoneal dialysis patients. *Perit. Dial. Int*. 2000; **20**: 722–726.
35. Jha HC, Srivastava P, Divya A, et al: Prevelence of Chlamydiophila pneumoniae is higher in aorta and coronary artery than in carotid artery of coronary artery disease patients. *APMIS*.2009; **117**:905–11.
36. Campbell LA, Kuo CC, Grayston JT, *et al: Chlamydia pneumonia* and cardiovascular disease. *Emerg Infect Dis*1998; **4**:571- 579
37. Antonietta R, Rossella P, Dario I, Francesco P, Caterina RC: *Chlamydia pneumoniae* infection in adolescents with type 1 *Diabetes mellitus*. *J Med Microbiol* .2012; Doi: 10.1099/jmm.0.048512-0
38. Hoymans VY, Bosmans JM, Van Rentherghem L, et al: Importance of methodology in determination of *Chlamydia pneumoniae* seropositivity in healthy subjects and in patients with coronary atherosclerosis. *J. Clin. Microbiol.*2003; **41**:4049-4053.
39. Yamaguchi H, Oshio I, Osaki T, Kurata S, Yamamoto Y, Kamiya S: Development of diabetes in non-obese diabetic mice promotes *Chlamydia pneumoniae* dissemination from lung to peripheral blood. *Int. J. Exp. Path.*2006; **87**: 121–129.
40. Orrett F.A: (2000) Fatal *Bacillus cereus* bacteremia in a patient with diabetes. *J. Natl. Med. Assoc*.2000; **92**:206–208.

41. Kalayoglu MV, Hoerneman B, LaVerda D, Morrison SG, Morrison RP, Byrne GI:Cellular oxidation of low-density lipoprotein by *Chlamydia pneumoniae*. *J Infect Dis* 1999; **180**:780±790.
42. Laurila A, Bloigu A, Näyhä S, Hassi J, Leinonen M, Saikku P: *Chlamydia pneumoniae* antibodies and serum lipids in Finnish males. *BMJ.* 1997; **14**:1456-1457.
43. Gibbs RG, Carey N, Davies AH, *et al: Chlamydia pneumonia* and vascular disease. *Br J Surg* 1998; **85**:1191-1197.
44. Dosquet C, Weill D, Wautier JL, et al: Cytokines and thrombosis. *J Cardiovascular Pharmacol* 1998; **25**:13- 19.