Role of Galectin-9 (Gal-9) In Pathogenesis of Type 2 Diabetes Mellitus and Microvascular Complications

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Abstract: There has been a recent explosion of interest in the notion that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes. A major component of innate immunity is a series of sentinel cells including macrophages. M1 macrophages are polarized in adipose tissue under the effect of activated CD8+ve T-cells and are proinflammatory, secreting cytokines such as TNF-α and IL-1β. M1 macrophages express CD11-c +ve which is responsible for insulin resistance. Galectins are a family of carbohydrate binding proteins that have specific binding affinity for β-galactosides, and they exhibit evolutionary conservation from fungi to mammals and there is evidence that galectin-9 protein induces apoptosis, suggesting a role in negative selection of autoreactive T cells. Furthermore, Gal-9 induced apoptosis in activated CD-8+ve T cells. That is we investigated the relationship of galectin-9 to type 2 diabetes and diabetic nephropathy as an example to microvascular complications of diabetes. This study included 60 patients: 15 patients were diabetic without nephropathy (group I), 30 diabetic patients with nephropathy (group II) subdivided into 15 patients with microalbuminuria and 15 patients with macroalbuminuria and 15 as control healthy subjects (group III). All patients were subjected to thorough history taking, complete clinical examination, laboratory investigations including: Serum Galectin-9 level using ELISA technique, fasting serum glucose level and postprandial serum glucose level, HbA1c, serum creatinine level and BUN level, albumin/creatinine ratio in urine, estimated glomerular filtration rate. Imaging study of all subjects was done using ultrasonographic examination of kidney. We found that that there 's no significant difference between the three groups regarding age, weight and sex and studied laboratory parameters except galectin-9 serum level which showed a mean of 257.55 ± 205.98, 298.45 ± 136.54, and 2287.86 ± 295.52 in groups I, II, and III respectively. There was no significant difference between group I and II ($p > 0.05$), but there was significantly higher in control group than diabetic patients without nephropathy ($p < 0.01$), and was significantly higher in control group than diabetic patients with nephropathy ($p < 0.01$).
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Key words: Galectin-9, type 2 diabetes mellitus, Diabetic vascular complications.

1. Introduction

There has been a recent explosion of interest in the notion that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes **(Pickup *et al.*, 1997, Pickup *et al.*, 1998).** Circulating markers of inflammation, acute-phase reactants, or interleukin (IL)-6 (the major cytokine mediator of the acute-phase response) are strong predictors of the development of type 2 diabetes **(Pradhan *et al.*, 2001, Barzilay *et al.*, 2001, Vozarova *et al.*, 2002).**

The innate or natural immune system is the body's rapid first-line defense against environmental threats such as microbial infection and physical or chemical injury

(**Medzhitov *et al.*, 2000).** A major component of innate immunity is a series of sentinel cells (classically macrophages (M1and M2), antigen-presenting B-cells, and dendritic cells, but probably also intestinal epithelial cells, endothelium Kupffer cells in the liver, adipocytes, and others) that act as "trouble detectors." A number of germ line–encoded (i.e., nonclonal) pattern recognition receptors (PRRs) on and in these cells recognize conserved molecular structures (pathogen- associated molecular patterns) that are characteristic of a class of harmful agents. The most studied PRRs are probably the family of at least 10 toll-like receptors (TLRs) that are present at the cell surface as transmembrane receptors (**Medzhitov, 2001).** TLR-4, for example, recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, in conjunction with associated accessory molecules (CD14, MD-2). Other cell surface PRRs are macrophage scavenger receptors (**Pearson, 1996),** the mannose receptor (**Medzhitov *et al.*, 2000**), and the receptor for advanced glycation end products (AGEs) **(Li *et al.*, 1996).** There are also intracellular PRRs, e.g., for double-stranded RNA (present in viruses). Binding to PRR activates nuclear factor-$_k$B (NF-$_k$B) signaling pathways that induce immune response genes, especially those for inflammatory cytokines, which are the main mediators of inflammation and the acute-phase response. Secreted and circulating PRRs such as CRP and mannan-binding lectin function as

opsonins, binding to microbial cell components and flagging them for recognition by the complement system and phagocytes **(JOHN *et al.*, 2004).**

Macrophages have been segregated into two broad groups: M1s induced in vitro with granulocyte-macrophage colony-stimulating factor (GM-CSF) secrete proinflammatory cytokines; M2s induced in vitro with macrophage colony-stimulating factor (M-CSF) and IL-4 secrete anti-inflammatory cytokines. M2 macrophages have been associated with the repair of injured tissues and the resolu-tion of inflammation **(Gordon *et al.*, 2003).** In lean mice, M2-like macrophages are the predominant resident adipose tissue macrophages (ATMs). In the expanding adipose tissue of obesity, the local population shifts from M2-like macrophages to M1-like ATMs that express CD11c and are characterized by the accumulation of lipids. Greater than 90% of these M1-like cells originate from recruited monocytes that become CD11c+ **(Nguyen *et al.*, 2007, Prieur *et al.*, 2011).** Conditional ablation of CD11c+ cells in mice with diet-induced obesity significantly reduced adipose tissue and skeletal muscle macrophages, decreased proinflammatory cytokine levels, and increased insulin sensitivity **(Patsouris *et al.*, 2008).**

In lean adipose tissue, T-helper type 2 (TH2) cells produce anti-inflammatory cytokines such as interleukin (IL)-4, 10, and 13 which promote alternatively activated M2 macrophage polarization. M2 polarization is also induced by regulatory T cells (Tregs) and eosinophils via IL-4. M2 macrophages secrete other anti-inflammatory signals such as IL-10whichmaintain insulin sensitivity within lean adipose tissue. Conversely, T-helper type 1(TH1) cytokines such as interferon (IFN)- Ɣ stimulate

M1 macrophage polarization in obese adipose tissue. Other immune cells are also increased in obese adipose tissue which contribute to insulin resistance including mast cells, B cells, and immunoglobulins (Igs). CD8(+) T cells promote ATM accumulation and proinflammatory gene expression and are also increased as well. Macrophages are not homogenously distributed throughout obese adipose tissue but rather aggregated around dead adipocytes forming crown-like structures (CLS). M1 macrophages are proinflammatory, secreting cytokines such as TNF-α and IL-1β. Macrophages are bone-marrow-derived myeloid cells hence both M1 and M2 macrophages express the myeloid cell surface markers F4/80 and CD11b. However, only the M1 population expresses the marker CD11c **(Payal *et al.*, 2013).** Also; research works have demonstrated that local macrophage accumulation may herald the development of common diabetic complications such as atherosclerosis, nephropathy, neuropathy, and retinopathy. Glomerular infiltration of macrophages in diabetic kidneys has been fairly well established by examining diabetic kidney biopsies from mice and humans **(Mensah-Brown *et al.*, 2005, Sassy-Prigent *et al.*, 2000).**

Galectins are β-galactoside binding protein and involved in various biological processes such as development, organogenesis, oncogenesis, cell adhesion, cell cycle regulation and immunity **(Barondes *et al.*, 1994).**

Mouse and rat galectin-9 (Gal-9) was identified **(Wada *et al.*, 1997, Wada and Kanwar, 1997).**and its human homologue was independently cloned by using autoreactive antibodies in Hodgikin's disease **(Tureci *et al.*, 1997).** Galectin-9 exerted apoptotic potential against thymocytes (**Pickup *et al.*, 1998),** suggesting their important roles in the negative selection of thymocytes. Gal-9 lacking signal peptide is secreted out by non-classical pathway under inflammatory state and induced apoptosis in activated CD8+ T cells **(Tsuchiyama *et al.*, 2000, Wang *et al.*, 2007)** and activated T helper 1 (TH1) cells **(Zhu *et al.*, 2005),** suggesting a potential mechanism to eliminate the activated T cells at termination of the immune response in inflammatory tissues.

In this work we tried to explore the association of serum galectin-9 level, type 2 diabetes and diabetic nephropathy as a microvascular complication of diabetes.

2. Subjects and Methods

Subjects

This study was conducted on 60 patients with previously documented type 2 diabetes mellitus. Patients were divided into 3 groups:

Group I: (15) patients with type 2 diabetes mellitus without nephropathy

Group II: (30) patients with type 2 diabetes mellitus with recently discovered nephropathy, who were not on medical treatment for nephropathy and subdivided into:

A. Group II (a) (15) Microalbuminuria ACR 30-299 mg/gm.

B. GroupII (b) (15) Macroalbuminuria ACR ≥ 300 mg/gm.

Group III: (15) subjects of normal health as a control group.

The patients were selected from inpatient wards and outpatient clinic of Endocrinology Unit, Internal Medicine Department, Tanta University Hospital, in the period from June 2013 to November 2013.

Inclusion criteria:

Patients with type 2 diabetes mellitus under treatment with insulin therapy.

Exclusion criteria:

1. Patients with end stage renal disease.
2. Patients with any inflammatory or immunological diseases, excluded on clinical bases.

Methods

- **All studied groups included were subjected to:**

Thorough history taking, complete clinical examination, laboratory investigations including serum galectin-9 level, fasting serum glucose, postprandial serum glucose, blood urea, serum creatinine, urinary albumin/ creatinine excretion ratio, estimated glomerular filtration rate (eGFR), and estimation of serum galectin-9 level using ELISA kit for galectin-9 (Uscn Life Science Inc). Also; all subjects were subjected to ultrasonographic examination of kidneys to rule-out gross structural kidney abnormality.

3.Results

This study was conducted on 60 patients with previously documented type 2 diabetes mellitus. Patients were divided into 3 groups, group I included fifteen patients with type 2 diabetes mellitus without nephropathy, group II: included thirty patients with type 2 diabetes mellitus with recently discovered nephropathy, who were not on medical treatment for nephropathy and subdivided into fifteen patients with microalbuminuria ACR 30-299 mg/gm & fifteen patients with macroalbuminuria ACR ≥ 300 mg/gm. In group III there were fifteen healthy subjects as a control group.

Demographic data as shown in tables (1,2,3,4, 6), revealed that out of the fifteen patients in group I (type 2 diabetes mellitus without nephropathy), 4 of them (26.7 %) were males and 11 patients (73.3 %) were females with a mean age of 54.40 ±11.37 years and a mean weight 74.07 ±16.28 kg. Among the thirty patients in group II (type 2 diabetes mellitus with nephropathy), 10 (33.3 %) of them were males and 20 (66.7 %) patients were females with a mean age of 52.10 ±10.19 and a mean weight 78.43 ±17.96 kg. The subdivided groups from group II included: sub group (A) which included fifteen patients with type 2 diabetes mellitus with microalbuminuria, this subgroup showed that 4 (26.7 %) of them were males and 11 (73.3 %) patients were females with a mean age of 51.33 ± 12.94 and a mean weight 84.80 ± 19.89 kg. In sub group (B) fifteen patients with type 2 diabetes mellitus with macroalbuminuria showed that 6 (40 %) of them were males and 9 (60%) patients were females with a mean age of 52.87 ± 6.81 and a mean weight 72.07 ± 13.64 kg. The fifteen subjects of group III (control group) showed that 12 (80 %) subjects were male and 3 (20%) subjects were female with a mean age of 52.87 ± 6.81 and a mean weight 72.07 ± 5.74 kg. Comparison between all studied groups as regard age, sex and weight showed no statistically significant values in all analysis.(p>0.05).

As seen in table (5), our laboratory data regarding fasting blood glucose (FBG) in groups I, II, and III had a mean of 130.93 ± 19.01, 132.13 ± 15.18, and 82.60 ± 8.85 respectively. There was no significant difference between group I and group II ($p1$> 0.05), but there was significant difference between group I and group III ($p2$ < 0.01), and between group II and group III ($p3$ < 0.01).

Regarding two hour postprandial (2hr pp) in group I, II, and III the mean was 188.73 ± 19.82, 197.20 ± 13.89, and 109.67 ± 7.89 respectively. There was no significant difference between group I and II ($p1$ > 0.05) but there was significant difference between group I and group III (p 2 < 0.01), and between group II and group III (p 3< 0.01).

The mean of HbA1c was 9.07 ± 0.99, 9.08 ± 2.10, and 4.97 ± 0.50 in group I, II, and III respectively. There was no significant difference between group I and II (p 1 > 0.05) but there was significant difference between group I and group III (p 2 < 0.01), and group II and group III (p 3 < 0.01).

Albumin creatinine ratio (A/C) was shown to have a mean of 16.06 ± 7.38, 1135.36 ± 1896.98, and 15.20 ± 4.26 in group I, II, and III respectively . There was significant difference between group I and II (p 1 < 0.05), between group II and group III (p 3 < 0.01), but there was no significant difference between group I and group III (p 2 > 0.05).

For creatinine it was shown that the mean was 0.95 ± 0.26, 2.08 ± 1.65, and 0.68 ± 0.13 in groups I, II, and III respectively. There was significant difference between group I and II (p 1 < 0.05), between group II and group III (p 3 < 0.01), but there was no significant difference between group I and group III (p 2 > 0.05).

The mean of urea was 40.87 ± 15.06, 74.03 ± 53.74, and 29.33 ± 10.67 in groups I, II, and III respectively. There was significant difference between group I and II (p 1 < 0.05), between group II and group III (p 3 < 0.01), but there was no significant difference between group I and group III (p 2 > 0.05).

The mean of galectin-9 serum level was 257.55 ± 205.98, 298.45 ± 136.54, and 2287.86 ± 295.52 in groups I, II, and III respectively. There was no significant difference between group I and II (p 1 > 0.05), but there was significant difference between group I and group III (p 2 < 0.01), and between group II and group III (p 3< 0.01). (Fig. 1)

Regarding Estimated glomerular filtration rate (eGFR) it was revealed that the mean was 90.35 ±29.89, 61.18 ±37.69, and 127.65 ±16.34 in groups I, II, and III respectively. There was significant difference between group I and II (p 1 < 0.05), and between group II and group III (p 3 < 0.01), but there was no significant difference between group I and group III (p 2 > 0.05).

Table (6) shows no statistical significant difference between the 2 subgroups of group II regarding FPG, PPPG, galectin-9 serum level and HBA1C (> 0.05), while there was statistical significant difference between the 2 subgroups regarding Albumin/ Creatinine ratio with a mean of 145.86 ± 76.3, and 2124.87 ± 2313.04 in subgroup of microalbuminuria and macroalbuminuria respectively ($p < 0.0$). Also; the mean of serum creatinine level was 1.37 ± 0.80, and 2.79 ± 1.98 in subgroup of microalbuminuria and macroalbuminuria respectively, with statistical significant difference ($p < 0.0$). The mean of eGFR was 82.93 ± 36.07, and 39.43 ± 25.06 in subgroup of microalbuminuria and macroalbuminuria respectively, with statistical significant difference ($p < 0.0$).

It is clear from table (7) that there was no significant correlation between Galectin- 9 and standard variables including Age, weight, fasting blood glucose, 2hr postprandial, HbA1c, Albumin creatinine ratio (ACR), serum creatinine, serum urea and estimated glomerular filtration rate (eGFR) in diabetic patients without nephropathy. Fig(2)

Table (8) shows no significant correlation between Galectin- 9 and standard variables including Age, weight, fasting blood glucose, 2hrs postprandial, HbA1c, Albumin creatinine ratio (ACR), serum creatinine, serum urea and estimated glomerular filtration rate (eGFR) in diabetic patients with nephropathy. Fig(3)

In table (9) we can find that there was no significant correlation between Galectin- 9 and standard variables including Age, weight, fasting blood glucose, 2hr postprandial, HbA1c, Albumin creatinine ratio (ACR), serum creatinine, serum urea and estimated glomerular filtration rate (eGFR) in diabetic patients with nephropathy group with microalbuminurea.

Table (10) indicates that there was no significant correlation between Galectin- 9 and standard variables including Age, weight, fasting blood glucose, 2hr postprandial, HbA1c, Albumin creatinine ratio (ACR), serum creatinine, serum urea and estimated glomerular filtration rate (eGFR) in diabetic patients with nephropathy group with macroalbuminurea.

Table (1): Frequency of gender in each group:

Groups	Gender	Frequency	Percent
Type 2 diabetes mellitus without nephropathy	Male	4	26.7
	Female	11	73.3
	Total	15	100.0
Type 2 diabetes mellitus with nephropathy	Male	10	33.3
	Female	20	66.7
	Total	30	100.0
Control group	Male	12	80.0
	Female	3	20.0
	Total	15	100.0

Table (2): Frequency table of gender in each sub group:

Sub groups	Gender	Frequency	Percent
micro-albuminuria:	Male	4	26.7
	Female	11	73.3
	Total	15	100.0
Macro-albuminuria:	Male	6	40.0
	Female	9	60.0
	Total	15	100.0

Table (3): Differences between studied groups regarding age and weight:

		Mean ± SD	ANOV A test	*p*- value
Age	Type 2 diabetes mellitus without nephropathy	54.40 ±11.37		
	Type 2 diabetes mellitus with nephropathy	52.10 ±10.19	0.28	> 0.05
	Control group	52.87 ± 6.81		
Weight	Type 2 diabetes mellitus without nephropathy	74.07 ±16.28		
	Type 2 diabetes mellitus with nephropathy	78.43 ±17.96	0.97	> 0.05
	Control group	72.07 ± 5.74		

P VALUE > 0.05 non significant (NS)

Table (4): Differences between two subgroups in group 2:

Variables	Sub groups	Mean ± SD	t- test	*p*- value
Age	Micro-albuminuria:	51.33 ± 12.94	0.41	> 0.05
	Macro-albuminuria:	52.87 ± 6.81		
Weight	Micro-albuminuria:	84.80 ± 19.89	2.05	> 0.05
	Macro-albuminuria:	72.07 ± 13.64		

P VALUE > 0.05 non significant (NS).

Table (5): Differences between studied groups regarding all studied variables:

Variables	Groups	Mean ± SD	Kruskal Wallis test	*p*- value	Tamhane post Hoc p-value
FBS	Type 2 diabetes mellitus without nephropathy	130.93 ±19.01	60.33*	< 0.01	P1= > 0.05 P2= < 0.01 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	132.13 ±15.18			
	Control group	82.60 ±8.85			
2HPP	Type 2 diabetes mellitus without nephropathy	188.73 ±19.82	193.87*	< 0.01	P1= > 0.05 P2= < 0.01 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	197.20 ±13.89			
	Control group	109.67 ±7.89			
HBA1C	Type 2 diabetes mellitus without nephropathy	9.07 ±0.99	37.09*	< 0.01	P1= > 0.05 P2= < 0.01 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	9.08 ±2.10			
	Control group	4.97 ±0.50			
A/c ratio	Type 2 diabetes mellitus without nephropathy	16.06 ±7.38	44.41	< 0.01	P1= < 0.05 P2= > 0.05 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	1135.36 ±1896.98			
	Control group	15.20 ±4.26			
Creatinine	Type 2 diabetes mellitus without nephropathy	0.95 ±0.26	28.08	< 0.01	P1= < 0.05 P2= > 0.05 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	2.08 ±1.65			
	Control group	0.68 ±0.13			
Urea	Type 2 diabetes mellitus without nephropathy	40.87 ±15.06	18.65	< 0.01	P1= < 0.05 P2= > 0.05 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	74.03 ±53.74			
	Control group	29.33 ±10.67			
Galectin_9	Type 2 diabetes mellitus without nephropathy	257.55 ±205.98	36.01	< 0.01	P1= > 0.05 P2= < 0.01 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	298.45 ±136.54			
	Control group	2287.86 ±295.52			
eGFR	Type 2 diabetes mellitus without nephropathy	90.35 ±29.89	26.29	< 0.01	P1= < 0.05 P2= > 0.05 P3= < 0.01
	Type 2 diabetes mellitus with nephropathy	61.18 ±37.69			
	Control group	127.65 ±16.34			

P VALUE > 0.05 non significant (NS).

P1 between Type 2 diabetes mellitus without nephropathy and Type 2 diabetes mellitus with nephropathy.

P2 between Type 2 diabetes mellitus without nephropathy and control group.

P3 between Type 2 diabetes mellitus with nephropathy and control group.

As shown in table (11) we can find that there was no significant correlation between Galectin 9 and standard variables including Age, weight, fasting blood glucose, 2hr postprandial, HbA1c, serum creatinine, serum urea and estimated glomerular filtration rate (eGFR). There's positive correlation between galectin 9 and Albumin creatinine ratio (ACR) in normal healthy control subjects.

Table (6): Differences between two subgroups in group 2 (Diabetic nephropathy):

Variables	Sub groups	Mean ± SD	t- test	*p*- value
Age	Micro-albuminuria:	51.33 ± 12.94		> 0.05
	Macro-albuminuria:	52.87 ± 6.81	0.41	
Weight	Micro-albuminuria:	84.80 ± 19.89	2.05	> 0.05
	Macro-albuminuria:	72.07 ± 13.64		
FBS	Micro-albuminuria:	128.87 ± 18.72	1.19	> 0.05
	Macro-albuminuria:	135.40 ± 10.19		
2HPP	Micro-albuminuria:	199.40 ± 15.35	0.86	> 0.05
	Macro-albuminuria:	195.00 ± 12.42		
HBA1C	Micro-albuminuria:	9.50 ± 2.51	1.09	> 0.05
	Macro-albuminuria:	8.66 ± 1.57		
A/C ratio	Micro-albuminuria:	145.86 ± 76.31	4.67*	< 0.01
	Macro-albuminuria:	2124.87 ± 2313.04		
Creatinine	Micro-albuminuria:	1.37 ± 0.80	2.62*	< 0.01
	Macro-albuminuria:	2.79 ± 1.98		
Urea	Micro-albuminuria:	53.87 ± 32.83	2.34*	< 0.05
	Macro-albuminuria:	94.20 ± 63.51		
Galectin_9	Micro-albuminuria:	292.22 ± 115.80	0.33*	> 0.05
	Macro-albuminuria:	304.68 ± 158.51		
eGFR	Micro-albuminuria:	82.93 ± 36.07	3.13*	< 0.01
	Macro-albuminuria:	39.43 ± 25.06		

P VALUE > 0.05 non significant (NS).

Table (7): Pearson Correlation between Galectin 9 and all studied variables in Type 2 diabetes mellitus without nephropathy group:

		Galectin_9
Age	R	- 0.271
	p- value	> 0.05
Weight	R	- 0.261
	p- value	> 0.05
FBS	R	0.196
	p- value	> 0.05
2HPP	R	0.226
	p- value	> 0.05
HBA1C	R	- 0.184
	p- value	> 0.05
A/C ratio	R	0.242
	p- value	> 0.05
Creatinine	R	- 0.131
	p- value	> 0.05
Urea	R	- 0.210
	p- value	> 0.05
eGFR	R	0.004
	p- value	> 0.05

P VALUE > 0.05 non significant (NS).

Table (8): Pearson Correlation between Galectin 9 and all studied variables in Type 2 diabetes mellitus with nephropathy group:

		Galectin 9
Age	R	0.080
	p- value	> 0.05
Weight	R	0.006
	p- value	> 0.05
FBS	R	0.068
	p- value	> 0.05
2HPP	R	- 0.052
	p- value	> 0.05
HBA1C	R	- 0.189
	p- value	> 0.05
A/C ratio	R	0.040
	p- value	> 0.05
Creatinine	R	- 0.020
	p- value	> 0.05
Urea	R	- 0.023
	p- value	> 0.05
eGFR	R	0.032
	p- value	> 0.05

P VALUE > 0.05 non significant (NS).

Table (9): Pearson Correlation between Galectin 9 and all studied variables in Type 2 diabetes mellitus with nephropathy group with microalbuminuria.

		Galectin_9
Age	R	- 0.030-
	p- value	> 0.05
Weight	R	- 0.258
	p- value	> 0.05
FBS	R	0.385
	p- value	> 0.05
2HPP	R	0.252
	p- value	> 0.05
HBA1C	R	- 0.046
	p- value	> 0.05
A/c ratio	R	- 0.004
	p- value	> 0.05
Creatinine	R	- 0.028
	p- value	> 0.05
Urea	R	0.147
	p- value	> 0.05
eGFR	R	.031
	p- value	> 0.05

***P* VALUE > 0.05 non significant (NS).**

4. Discussion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. In type 2 diabetes insulin resistance, or insufficient insulin secretion, or both are blamed for pathogenesis of diabetes **(The expert committee on the diagnosis and classification of diabetes mellitus, 2003)**.

Diabetic complications are of two types, short term complications (Acute) and long term complications (chronic). Short term complications are like diabetic ketoacidosis, hyperosmolar non ketotic coma and hypoglycemia. Long term complications include diabetic microvascular complications such as retinopathy, nephropathy and neuropathy, which are leading causes of blindness, end stage renal disease (ESRD) and various painful neuropathies, respectively. The other chronic complicatins include the macrovascular complications which involve atherosclerosis related diseases, such as coronary artery disease, peripheral vascular disease and cerebrovascular stroke **(Kitada *et al.*, 2010)** .

Diabetic vascular complications result from imbalances caused by increases in the toxic effects of systemic metabolic abnormalities such as hyperglycemia, dyslipidemia, and hypertension, and reductions in the regenerative effect of endogenous protective factors as insulin, vascular endothelial growth factor (VEGF), platelet derived growth factor (PEGF), nitric oxide (NO), and antioxidant enzymes.

Galectins are a family of carbohydrate binding proteins that have specific binding affinity for β-galactosides, and they exhibit evolutionary conservation from fungi to mammals **(Cooper, 2002)**.

Galectin-9 protein induces apoptosis, suggesting a role in negative selection of autoreactive T cells. Furthermore, Gal-9 induced apoptosis in activated CD8 T cells in Wistar Kyoto rats with glomerulonephritis **(Tsuchiyama *et al.*, 2000)**.

Our study showed that galectin-9 level was not significantly correlated to all standard variables including age, weight, fasting blood glucose, 2hr postprandial, HbA1c, Albumin creatinine ratio (ACR), serum creatinine, serum urea, estimated glomerular filtration rate (eGFR) in all studied groups except in the control group where it was significantly correlated to Albumin creatinine ratio.

Table (10): Pearson Correlation between Galectin 9 and all studied variables in Type 2 diabetes mellitus with nephropathy group with macroalbuminuria:

		Galectin_9
Age	R	0.247
	p- value	> 0.05
Weight	R	0.328
	p- value	> 0.05
FBS	R	- 0.364
	p- value	> 0.05
2HPP	R	- 0.317
	p- value	> 0.05
HBA1C	R	- 0.375
	p- value	> 0.05
A/C ratio	R	0.030
	p- value	> 0.05
Creatinine	R	- 0.052
	p- value	> 0.05
Urea	R	- 0.118
	p- value	> 0.05
eGFR	R	0.125
	p- value	> 0.05

***P* VALUE > 0.05 non significant (NS).**

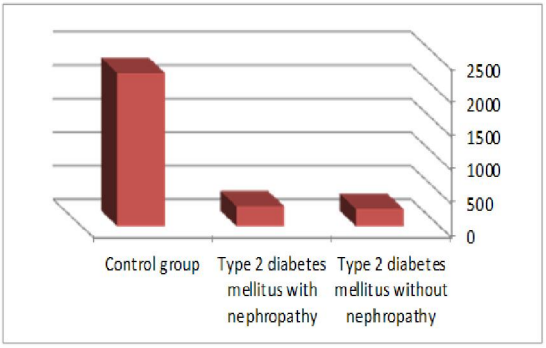

Fig (1): Comparison between the studied group regarding serum galectin-9 level

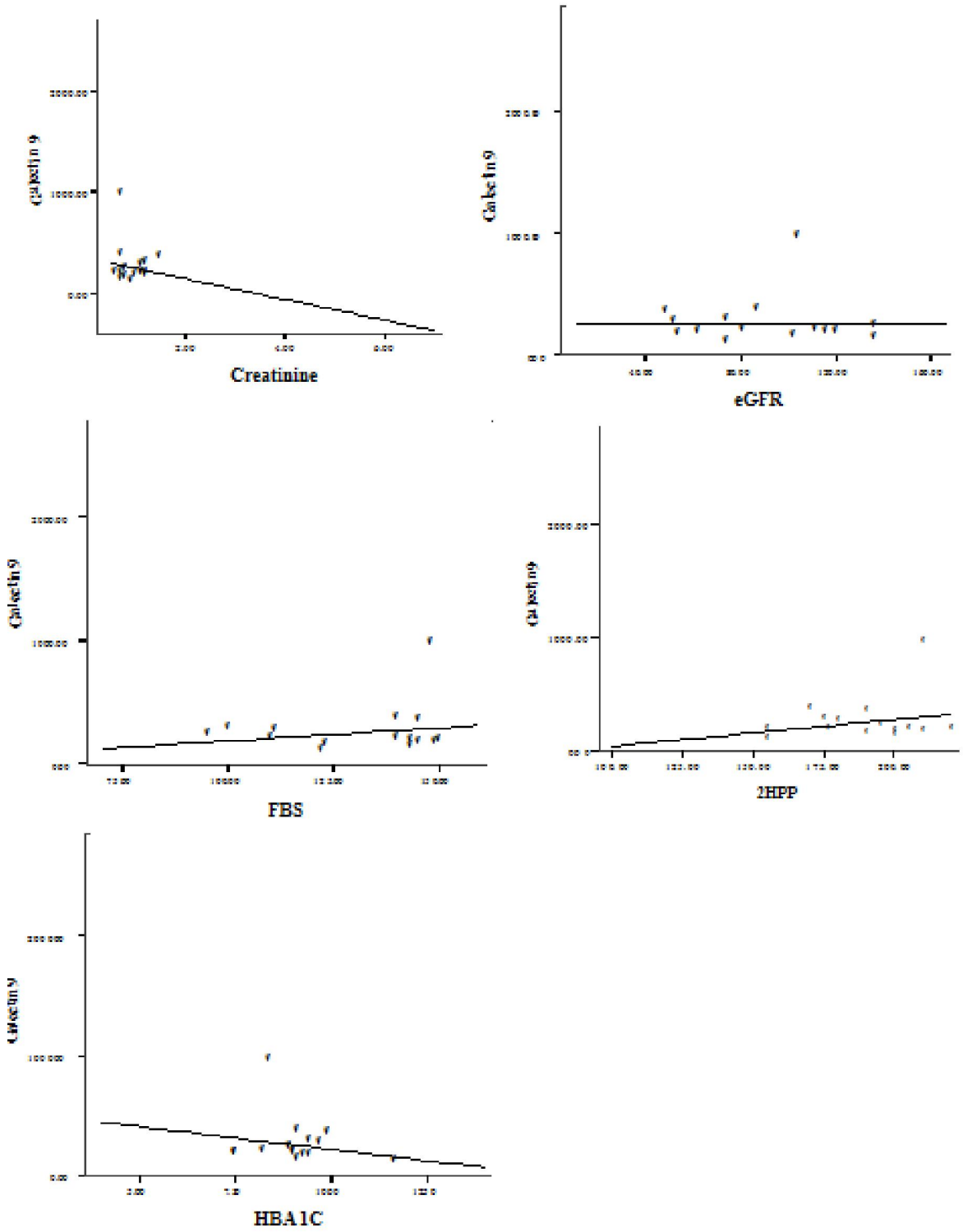

Fig (2): Correlation between Galectin-9 and all studied variables in Type 2 diabetes mellitus without nephropathy group:

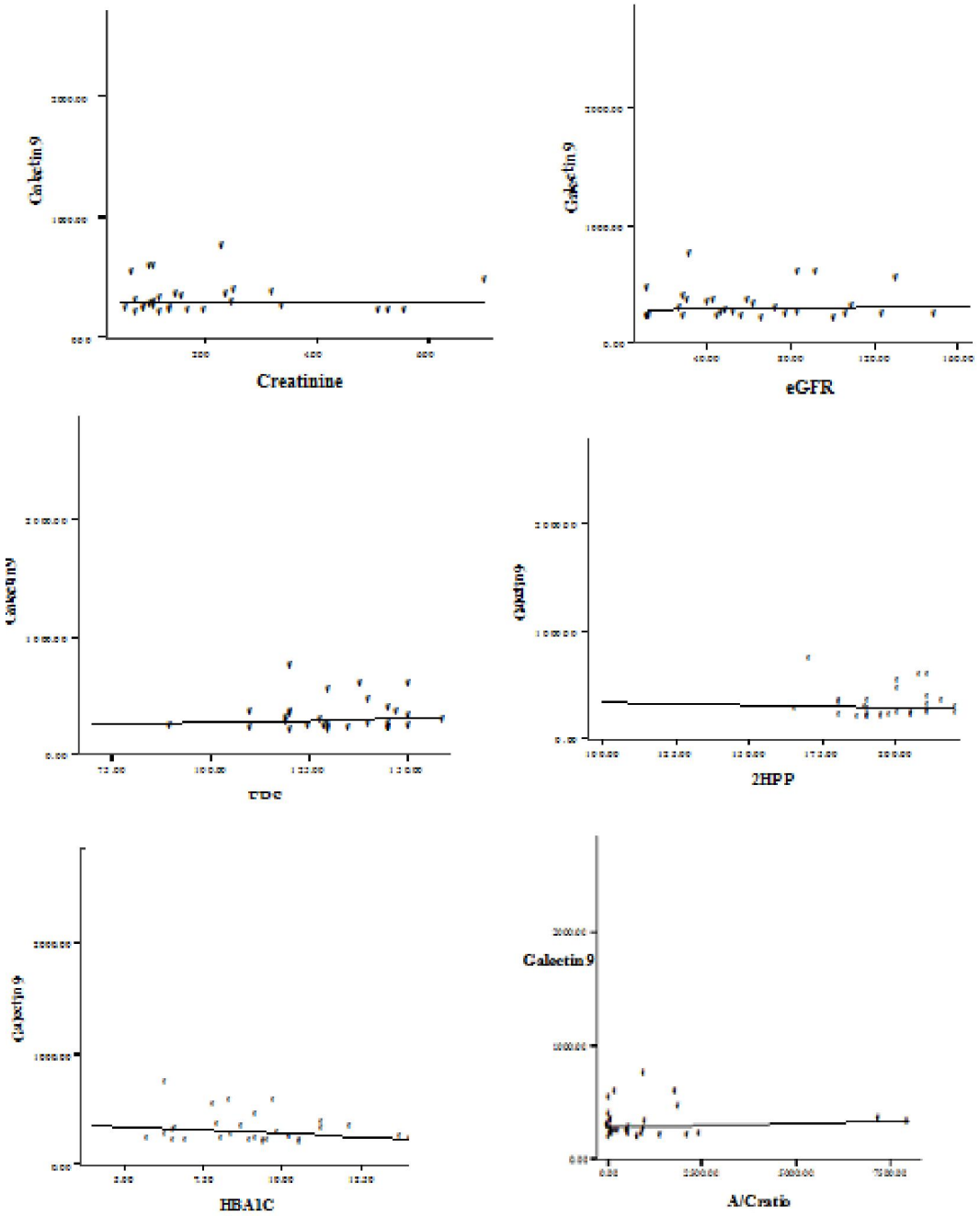

Fig (3): Correlation between Galectin 9 and all studied variables in Type 2 diabetes mellitus with nephropathy group:

Kurose ***et al.,*** 2013, had document similar result to ours regarding the correlation between galectin 9 and HbA1c and blood glucose, as it was documented that there's no significant correlation between galectin 9 and blood glucose and HbA1c. but, they were against our findings regarding other variables including: age, weight, Albumin creatinine ratio (ACR), serum creatinine, serum urea, Estimated glomerular filtration rate (eGFR). They found that Serum Gal-9 levels is significantly and positively correlated with age, creatinine, urea. and negatively correlated with eGFR **(Kurose *et al.*, 2013)**.

Our results can be explained on the bases that there's no significant difference between the three groups regarding age, weight and sex. Also we found that there's no significant difference between group I

(diabetic without nephropathy) and group II (diabetic with nephropathy) regarding the following variables including fasting blood glucose, 2hrs postprandial, HbA1c and galectin-9. But there's significant difference between group III (control group) and groups I & II regarding fasting blood glucose, 2hr postprandial, HbA1c and galectin-9.

Our finding regarding Albumin creatinine ratio (ACR), serum creatinine, serum urea and estimated glomerular filtration rate (eGFR) showed that there's significant difference between group I and II, there's significant difference between group II and group III, but there's no significant difference between group I and group III.

Regarding the level of serum galectin-9 in the three groups we found that galectin-9 level was significantly increased in control group than other groups of diabetic patients with or without nephropathy.

Also, galectin-9 was higher in diabetic patients with nephropathy than those without nephropathy but it wasn't significant.

Gal-9 may act as an essential cell-cycle regulator. The cell cycle is dysregulated in the diabetic state, and G1-phase arrest is believed to be responsible for the high glucose induced cellular hypertrophy and increase in the de novo protein synthesis and consequential accumulation of extracellular matrix proteins, as typically seen in diabetic nephropathy **(Wolf, 2002, Wolf and Shankland, 2003)**.

There is a growing body of evidence that specific cyclin dependent kinase (CDK) inhibitors, p27Kip1 and p21Cip1, are critically involved in hypertrophy of mesangial cells that are exposed to high glucose ambience **(Abdel-Wahab *et al.*, 2002, Wolf *et al.*, 2003)** and in experimental types 1 and 2 diabetes and also in p27Kip1 knockout **(Awazu *et al.*, 2003).**

The inhibition of TGF (Transforming growth factor) mediated hypertrophy in cultured mesangial cells derived from p27Kip1 and p21Cip1 double null (-/-) mice also support the notion that CDK inhibitors are critical molecules in cellular hypertrophy **(Monkawa *et al.*, 2003).**

High glucose stimulates the expression of TGF-β1 **(Awazu *et al.*, 2003).**

Induction of expression of TGFβ1 stimulates the expression of the CDK inhibitors p21Cip1 and p27Kip1 through transcriptional and posttranslational mechanisms. Although high glucose mediated induction of p27Kip1 is to some extent independent of TGF-β1 pathway **(Wolf *et al.*, 2001).**

Thus, they concluded that high glucose mediated podocyte injury associated with G1 phase cell cycle arrest seems to be another major player in the pathogenesis of diabetic glomerulopathy.

Although mesangial cells can re-enter the cell cycle, they fail to progress through G1/S phase because of the induction of CDK inhibitors. Besides mesangial cells, recent studies have demonstrated that podocyte hypertrophy is observed in differentiated podocyte cell line and Zucker fatty rats (**Hoshi *et al.*, 2002)**.

Increase in p21Cip1 and p27Kip1 has been described in podocytes in Zucker fatty rats, which is also seen in db/db mice in their studies (**Mundel and Shankland 2002)**.

In type 2 diabetes, a decrease in podocyte number is well correlated with both microalbuminuria and progression of diabetic nephropathy (**Mundel and Shankland 2002)**.

Data from the work of Baba ***et al.,*** 2005, can be supportive to our studies as it reported that In db/db mice, chronic administration of Gal-9 reduced albuminuria and inhibited glomerular hypertrophy and accumulation of extracellular matrix **(Baba *et al.*, 2005)**.

Injection of Gal-9 inhibited both mRNA and protein expression of type IV collagen and glomerular expression of TGF-β1 protein **(Baba *et al.*, 2005)**.

In db/db mice, immune histochemistry clearly indicated that p27Kip and p21Cip1positive cells predominantly increased in podocytes compared with nondiabetic db/m mice **(Baba *et al.*, 2005)**.

Although the cell-cycle arrest and cellular hypertrophy in mesangial cells in diabetic nephropathy were well documented in the literature, they postulated that podocytes also are involved in such process **(Baba *et al.*, 2005)**.

The administration of Gal-9 reduced p27Kip1- and p21Cip1-positive cells in glomeruli in db/db mice. The in vivo data suggested that Gal-9 ameliorated early diabetic nephropathy via inhibition of TGF-β1 as well as cell-cycle–dependent pathways **(Baba *et al.*, 2005)**.

Gal-9 promotes and assists cell-cycle progression and successful replication in diabetic state, where cell-cycle progression is halted despite cell-cycle entry. Thus, Gal-9 exerts dual action on the cells and modulates the fate of cells, i.e., apoptosis subsequent to S-phase arrest or successful progression to G2 phase depending on the status or the nature of the cells **(Baba *et al.*, 2005)**.

In conclusion, galectin-9 has been found to be higher in non diabetic healthy subjects than those with diabetes. Although, galectin-9 was higher in diabetic patients with nephropathy, but it wasn't statistically significant.

This may be indicative of possible role of decreased galectin-9 to pathogenesis of diabetic nephropathy.

The findings in our work suggest that galectin-9 may play a role in the development of type 2 diabetes as reported before galectin 9 level was significantly higher in healthy control group rather than in those with type 2 diabetes with or without nephropathy.

So, the possibility of the link between low galectin-9 level and development of type 2 diabetes is highly suggested.

Data from previous research work showed collected evidence in humans and rodents has validated chronic inflammation as a promising target for prevention and therapy of insulin resistance, type 2 diabetes, and cardiovascular disease.

Macrophages are bone marrow derived myeloid cells; hence both M1 and M2 macrophages express the myeloid cell surface markers F4/80 and CD11b. However, only the M1 population expresses the marker CD11c, whereas M2 macrophages are CD11c(−) **(Lumeng *et al.*, 2007)**.

M1, CD11c(+) recruited macrophages account for the majority of the increase in adipose tissue macrophage observed in obese adipose tissue, where >90% of recruited monocytes become CD11c(+) adipose tissue macrophage **(Nguyen *et al.*, 2007)**.

Of worth it's to be clarified that M1 macrophages are proinflammatory, secreting cytokines such as TNF-□ and IL-1□, and have high phagocytic and bactericidal potential **(Goerdt *et al.*, 1999)**.

Also, M1 correlates with insulin resistance as demonstrated by Patsouris et al.[143] These investigators deleted CD11c(+) macrophages in mice using a genetic system in which the primate diphtheria toxin receptor (DTR) gene is driven by the CD11c promoter, the intention was to make CD11c(+) cells expressing DTR on their surface undergo apoptotic death when the animal was exposed to diphtheria toxin. In mice fed high fat diet (HFD) for 16 weeks, diphtheria toxin exposure ablated about half of all adipose tissue macropahges, while also reducing myeloid cell content in the liver and skeletal muscle. Remarkably, only 24–48 hours after diphtheria toxin administration, glucose tolerance tests completely normalized in these mice, associated with improved insulin sensitivity in both liver and skeletal muscle **(Patsouris *et al.*, 2008)**.

This study illustrated that CD11c(+) macrophage populations (M1) are responsible for insulin resistance in obese animals and demonstrated that their continued presence is required to maintain this state.

As it's known that type 2 diabetes is characterized by insulin resistance and impaired insulin secretion due to B-cell dysfunction. There are also several reports indicating that islets from patients with type 2 diabetes are infiltrated with macrophages, and human islets exposed to metabolic stress release increased levels of cytokines **(Elena, 2010)**.

Thus, chronic innate inflammation due to local cytokine generation is a potentially important pathway mediating pancreatic B-cell damage in type 2 diabetes **(Elena, 2010)**.

It was demonstrated by Elena Galkina (2010) that there's distinct populations of lymphocytes and CD11c+CD11b+ cells within the islets and suggest an importance of the local immune response at the initial stages of type 2 diabetes development this data highlights that immune cells already reside within healthy human islets, and that type 2 diabetes induces an additional recruitment of various leukocytes into islets. This, in turn, further exacerbates the local immune response and islet dysfunction **(Galkina, 2010)**.

CD8+ effector T cells were shown to be involved in the recruitment and activation of adipose tissue macrophages and promoted a pro-inflammatory cascade associated with insulin resistance **(Nishimura *et al.*, 2009)**.

Depletion of CD8+ T cells results in reduced macrophage infiltration into visceral adipose tissue, decreased production of pro-inflammatory mediators, and increased insulin sensitivity **(Nishimura *et al.*, 2009)**.

Contrary to studies stating that macrophages are the first cells to infiltrate the visceral adipose tissue, Jenny Shu and his colleagues (2012) argued that infiltration of CD8+ T cells at 4 weeks of high fat diet (HFD) preceded the accumulation of macrophages **(Shu *et al.*, 2012)**.

These findings suggested that obesity promoted the activation of CD8+ T cells, which led to the recruitment, differentiation and activation of adipose tissue macrophages **(Shu *et al.*, 2012)**.

Because of its well established role in inflammation and insulin resistance in animal models, tumor necrosis factor-α seemed a rational target for new therapeutic intervention.

However, several approaches to antagonize tumor necrosis factor-α have had no effect on glucose levels in patients with type 2 diabetes **(Paquot *et al.*, 2000)** and only marginal impact on insulin sensitivity in non diabetic insulin-resistant patients **(Stanley *et al.*, 2011)**.

Goldfine ***et al.*** (2010) showed in their randomized trials with small sample size that the anti-inflammatory drug salsalate was found to curb insulin resistance and inflammatory parameters in obese individuals and to improve glucose control and triglyceride levels over a 3-month treatment period in patients with T2D **(Goldfine *et al.*, 2010)**.

The blockade of IL-1R1 by means of a specific binding protein, IL-1RA, improved insulin sensitivity and β-cell secretory profile though reducing markers of systemic inflammation **(Larsen *et al.*, 2009)**.

The beneficial effects on β-cell secretion persisted for >3 years after discontinuation of the IL-1RA.Subsequent clinical trials showed that this highly selective immune modulator lowered blood glucose, although degrees of HbA1c lowering were modest. Although the magnitude of glucose lowering may be less, the fact that both salsalate and IL-1β blockade do indeed lower blood glucose provides strong supporting evidence for roles of inflammation in obesity-induced insulin resistance **(Larsen *et al.*, 2009)**.

In previous studies galectin-9 induced apoptosis in various cells including thymocytes and activated CD4 & CD8 T cells.

This increased apoptosis of CD8 looks beneficial in down regulation of accumulation of macropahges in adipose tissue and islets of Langerhans hence; its possible role in the improvement of insulin resistance, protection against β cell decayand finally prevention of type 2 diabetes.

In conclusion

it is clear from this study that galectin-9 level which is lower in diabetic patients with or without nephropathy than in healthy individuals could be related to the development of type 2 diabetes and diabetic nephropathy as possibly as other micro and macro vascular complications of diabetes because of the exacerbated inflammatory process in patients with lower levels of galectin-9.

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References

1. Abdel-Wahab, N.; Weston, B. S.; Roberts, T. and Mason, R. M., (2002): Connective tissue growth factor and regulation of the mesangial cell cycle: Role in cellular hypertrophy. *J Am Soc Nephrol; 13: 2437–2445.*
2. Awazu, M.; Omori, S.; Ishikura, K.; Hida, M. and Fujita, H., (2003): The lack of cyclin kinase inhibitor p27(Kip1) ameliorates progression of diabetic nephropathy. *J Am Soc Nephrol; 14: 699–708.*
3. Baba, M.; Wada, J.; Eguchi, J.; Hashimoto, I.; Okada, T.; Yasuhara, A.; Shikata, K.; Kanwar, Y. S. and Makino, H., (2005): Galectin 9 inhibits glomerular hypertrophy in db/db Diabetic Mice via Cell-Cycle–Dependent Mechanisms. *J Am Soc Nephrol; 16: 3222–3234.*
4. Barondes, S. H.; Castronovo, V.; Cooper, D. N.; Cummings, R. D.; Drickamer, K.; Feizi, T.; Gitt, M. A.; Hirabayashi, J.; Hughes, C. and Kasai, K., (1994): Galectins: a family of animal beta-galactoside-binding lectins. Cell 1994; 76(4):597–598.
5. Barzilay, J. I.; Abraham, L.; Heckbert, S. R.; Cushman, M.; Kuller, L. H.; Resnick, H. E. and Tracey, R. P., (2001): The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. Diabetes; 50:2384–2389.
6. Cooper, D. N., (2002): Galectinomics: Finding themes in complexity. *Biochim Biophys Acta; 1572: 209–231.*
7. Elena, G., (2010): Type 2 diabetes and islets immune response. *Grant Winning Programme Abstract 1-2.*
8. Goldfine, A. B.; Fonseca, V.; Jablonski, K. A.; Pyle, L.; Staten. M. A. and Shoelson, S. E., (2010): TINSAL-T2D (Targeting Inflammation Using Salsalate in Type 2 Diabetes) Study Team. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann Intern Med.;152:346–357.*
9. Goerdt, S.; Politz, O.; Schledzewski, K.; Birk, R.; Gratchev, A.; Guillot, P.; Hakiy, N.; Klemke, C. D.; Dippel, E.; Kodelja, V. and Orfanos, C. E., (1999) "Alternative versus classical activation of macrophages. *Pathobiology; 67(5-6):222-6.*
10. Gordon, S., (2003): Alternative activation of macrophages. Nature Reviews Immunology; 3:23–35.
11. Hoshi, S; Shu, Y.; Yoshida, F.; Inagaki, T.; Sonoda, J.; Watanabe, T.; Nomoto, K. and Nagata, M., (2002): Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats. *Lab Invest; 82: 25–35.*
12. Kitada, M.; Zhang, Z. and Akira, A., (2010): Molecular mechanisms of diabetic vascular complications. *Journal of diabetes investigation; 1:(3),77-89.*
13. Kurose, Y.; Wada, J.; Kanzaki, M.; Teshigawara, S.; Nakatsuka, A.; Murakami, K.; Inoue, K.; Terami, T.; Katayama, A.; Watanabe, M.; Higuchi, C.; Eguchi, J.; Miyatake, N. and Makino, H., (2013): Serum galectin-9 levels are elevated in the patients with type 2 diabetes and chronic kidney disease. *BMC Nephrology; 14:23.*
14. Larsen, C. M.; Faulenbach, M.; Vaag, A.; Ehses, J. A.; Donath, M. Y. and Mandrup, P. T., (2009): Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care;32:1663–1668.*
15. Li, Y. M.; Mitsuhashi, T.; Wojciechowicz, D.; Shimizu, N.; Li, J.; Stitt, A.; He, C.; Banerjee, D. and Vlassara, H., (1996): Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST 48 and p90–80K-H membrane proteins. Proc Natl Acad Sci U S A; 93:11047–11052.
16. Lumeng, C. N.; DeYoung, S. M.; Bodzin, J. L. and Saltiel, A. R., (2007): "Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes; 56(1): 16–23.*
17. Medzhitov, R., (2001): Toll-like receptors and innate-immunity. Nat Rev Immunol; 1:135–145.
18. Mensah-Brown, E. P.; Obineche, E. N.; Galadari, S.; Chandranath, E.; Shahin, A.; Ahmed, I.; Patel, S. M. and Adem, A., (2005): Streptozotocin-induced diabetic nephropathy in rats: the role of inflammatory cytokines. Cytokine; 31: 180-190.
19. Monkawa, T.; Hiromura, K.; Wolf, G. and Shankland, S. J., (2002): The hypertrophic effect of transforming growth factor-beta is reduced in the absence of cyclin dependent kinase inhibitors p21 and p27. *J Am Soc Nephrol; 13: 1172–1178.*
20. Mundel, P. and Shankland, S. J., (2002): Podocyte biology and response to injury. *J Am Soc Nephrol; 13: 3005–3015.*
21. Nguyen, M. T.; Favelyukis, S.; Nguyen, A. K.; Reichart, D.; Scott, P. A.; Jenn, A.; Liu-Bryan, R.; Glass, C. K.; Neels. J. G. and Olefsky, J. M., (2007): A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2

and 4 and JNK-dependent pathways. J Biol Chem; 282:35279–92.
22. Nishimura, S.; Manabe, I.; Nagasaki, M.; Eto, K.; Yamashita, H.; Ohsugi, M.; Otsu, M.; Hara, K.; Ueki, K.; Sugiura, S.; Yoshimura, K.; Kadowaki, T. and Nagai, R., (2009): CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Medicine;15(8):914–20.*
23. Paquot, N.; Castillo, M. J.; Lefèbvre, P. J. and Scheen, A. J., (2000): No increased insulin sensitivity after a single intravenous administration of a recombinant human tumor necrosis factor receptor: Fc fusion protein in obese insulin resistant patients. J Clin Endocrinol Metab; 85:1316–1319.
24. Patsouris, D.; Li, P. P.; Thapar, D.; Chapman, J.; Olefsky, J. M. and Neels, J. G., (2008): Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metabolism; 8:301–9.
25. Payal, S. P.; Eric, D. B. and Ashok B., (2013): The Role of the Immune System in Obesity and Insulin Resistance. Journal of Obesity; 2013: 1- 9.
26. Pearson, A. M., (1996): Scavenger receptors in innate immunity.
27. Curr Opin Immunol; 8:20–28.
28. Pickup, J. C. and Crook, M. A., (1998): Is type II diabetes mellitus a disease of the innate immune system? Diabetologia; 41:1241–1248.
29. Pickup, J. C. and Frcpath, D., (2004): Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes. Diabetes Care; 27:813–823.
30. Pickup, J. C.; Mattock, M. B.; Chusney, G. D. and Burt, D., (1997): NIDDM as a disease of the innate immune system: association of acute phase reactants and interleukin-6 withmetabolic syndrome X. Diabetologia; 40:1286–1292.
31. Pradhan, A. D.; Manson, J. E.; Rifai, N.; Buring. J. E. and Ridker, P. M., (2001): C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA; 286:327–334.
32. Prieur, X.; Mok, C. Y.; Velagapudi, V. R.; Núñez, V.; Fuentes, L.; Montaner, D.; Ishikawa, K.; Camacho, A.; Barbarroja, N.; O'Rahilly, S.; Sethi, J. K.; Dopazo, J.; Orešič, M.; Ricote, M. and Vidal-Puig, A., (2001): Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. Diabetes; 60:797–809.
33. Sassy-Prigent, C.; Heudes, D.; Mandet, C.; Bélair, M. F.; Michel, O.; Perdereau, B.; Bariéty, J. and Bruneva,l P., (2000): Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. Diabetes; 49: 466-475.
34. Shu, C.J.; Benoist, C. and Mathis, D., 2012: The immune system's involvement in obesity-driven type 2 diabetes. Semin Immunol; 24(6):436–442.
35. Stanley, T. L.; Zanni, M. V.; Johnsen, S.; Rasheed, S.; Makimura, H.; Lee, H.; Khor, V. K.; Ahima, R. S. and Grinspoon, S. K., (2011): TNF-alpha antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. J Clin Endocrinol Metab; 2096:E146–E150.
36. The expert committee on the diagnosis and classification of diabetes mellitus (2003) : Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care*; *26: S5-S20.*
37. Tsuchiyama, Y.; Wada, J.; Zhang, H.; Morita, Y.; Hiragushi, K.; Hida, K.; Shikata, K.; Yamamura, M.; Kanwar, Y. S. and Makino, H., (2000): Efficacy of galectins in the amelioration of nephrotoxicserum nephritis in Wistar Kyoto rats. *Kidney Int; 58: 1941–1952*.
38. Tureci, O.; Schmitt, H.; Fadle, N.; Pfreundschuh, M. and Sahin, U., (1997): Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease. J Biol Chem; 272(10):6416–6422.
39. Vozarova, B.; Weyer, C.; Lindsay, R. S.; Pratley, R. E.; Bogardus, C. and Tataranni, P. A., (2002): High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes; 51:455–461.
40. Wada, J. and Kanwar, Y. S., (1997): Identification and characterization of galectin-9, a novel beta-galactoside-binding mammalian lectin. J Biol Chem; 272(9):6078–6086.
41. Wada, J.; Ota, K.; Kumar. A.; Wallner, E. I. and Kanwar, Y. S., (1997): Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin. J Clin Invest; 99(10):2452–2461.
42. Wang, F.; He, W.; Zhou, H.; Yuan, J.; Wu, K.; Xu, L.and Chen, Z. K., (2007): The Tim-3 ligand galectin-9 negatively regulates CD8+ alloreactive T cell and prolongs survival of skin graft. Cell Immunol; 250(1–2):68–74.
43. Wolf, G., (2002): Molecular mechanisms of diabetic mesangial cell hypertrophy: A proliferation of novel factors. *J Am Soc Nephrol; 13: 2611–2613.*
44. Wolf, G.; Reinking, R.; Zahner, G.; Stahl, R. A. and Shankland, S. J., (2003): Erk1,2 phosphorylates p27(Kip1): Functional evidence for a role in high glucose-induced hypertrophy of mesangial cells. Diabetologia; 46: 1090–1099.
45. Wolf, G.; Schroeder, R.; Zahner, G.; Stahl, R. A. and Shankland, S. J., (2001): High glucose-induced hypertrophy of mesangial cells requires p27(Kip1), an inhibitor of cyclin-dependent kinases. *Am J Pathol;158: 1091–1100*.
46. Wolf, G. and Shankland, S. J., (2003): P27Kip1: The "rosebud" of diabetic nephropathy? *J Am Soc Nephrol; 14: 819–822.*
47. Zhu, C.; Anderson, A. C.; Schubart, A.; Xiong, H.; Imitola, J.; Khoury, S. J.; Zheng, X. X.; Strom, T. B. and Kuchroo, V. K., (2005): The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol; 6(12):1245–1252.