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Diagnostic Utility of Serum Angiopoietin-2 in Diabetic Nephropathy

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Abstract

***Background*: Diabetes mellitus (DM) is a major global cause of death. Diabetic nephropathy (DN) is the main microvascular complication of DM. Angiopoietin-2 (Angpt-2) enhances the progression of T2DM and accelerates vascular complications. *Patients and Methods:* 84 participants were recruited at Suez Canal University Hospital, Ismailia, Egypt, and divided into three equal groups: group I: patients with T2DM without albuminuria (n = 28), group II: patients with T2DM with albuminuria (n = 28), and group III: healthy volunteers (n = 28). For all participants. Laboratory work included serum creatinine, eGFR, UACR, glycemic markers, a lipid profile, and serum Angpt-2 by an ELISA-based method. *Results:* There was no statistically significant difference between patients with or without albuminuria regarding BMI, blood pressure, lipid profile, and glycemic control. Angpt-2 was significantly higher in patients with microalbuminuria and macroalbuminuria compared to normoalbuminuric diabetic patients and healthy controls, indeed, serum Angpt-2 progressively increases with the progression of albuminuria and renal impairment, Serum Angpt-2 showed a positive correlation with serum creatinine and a negative correlation with eGFR. *Conclusions:* Angpt-2 was significantly higher in albuminuric diabetic patients than the healthy controls, and it also progressively increased with the progression of UACR. the increased level of Angpt-2 may be associated with the development of CKD, and could be used as a diagnostic marker for diabetic nephropathy and its progression**

Keywords: Angiopoietin-2, Diabetic nephropathy, Egypt.

Introduction

Diabetic nephropathy (DN) is a main microvascular complication of DM, most cases are asymptomatic. Delayed pathological manifestation of glomerular disease in diabetic patients will possibly reverse and many patients with DN progress to CKD and ESRD[(1)]. Therefore, early diagnosis and intervention are crucial. Angiogenesis, endothelial dysfunction, abnormal vascular structure, and disruption of homeostasis are all causes of DN. In patients with DN, abnormal glomerular angiogenesis leads to glomerular hypertrophy, glomerular

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capillary damage, and excretes albumin in the urine (albuminuria) or reduced GFR. Albuminuria is indicative of glomerular damage in DN. Albuminuria has been established in numerous studies to be a reliable indicator of cardiovascular diseases among both CKD and non-CKD patients [2]. Angiopoietins are vascular growth factors that aid in blood vessel formation. Angpt-1 and Angpt-2 are the most studied angiopoietins. Angpt-1 is the major physiological ligand and activator (via phosphorylation) of the TIE-2 receptor. Angpt-1 is critical for early vascular development up to embryonic days 9.5–12.5 [3]. Angpt-1 signaling via TIE-2 is involved in capillary sprouting, endothelial cell survival, and vascular remodeling. Angpt-2 is a natural antagonist of Angpt-1[4]. Hyperglycemia raises Angpt-2, which then destabilizes vascular walls by competing with Angpt-1, and stimulates neovascularization when is bound to VEGF. Angpt-2 has been reported to be associated with indexes of endothelial damage/dysfunction [5]; which may affect podocytes in a paracrine fashion, leading to the decay of glomerular filtration barrier function [6]. Over-expression of Angpt-2 inhibits the binding of Angpt-1 to Tie-2 receptor, which contributes to the development of DN. In the pathophysiologic mechanism of DN, abnormal alterations in Angpt-1/Angpt-2 are responsible for excessive angiogenesis and inflammation. Increasing evidence suggests that the upregulation of Angpt-2 is harmful to kidney physiology and function. Podocyte-directed Angpt-2 transgenic over-expression has been reported to cause glomerular endothelial cell apoptosis, elevate albuminuria [20]. By growing the role of Angpt-2 as a future marker of vascular health status and diabetic complications, we were conducted to investigate the association of the serum levels of Angpt-2 in Egyptian diabetic adults and its relevance to diabetic nephropathy. As a diagnostic marker of diabetic enephropathy onset and progression especially in patients without an increase in albumin excretion. The study aimed of to assess serum Angpt-2 concentrations in patients with T2DM, for early diagnosis of diabetic nephropathy; to achieve a better quality of life for diabetic patients.

Patients and Methods

This study was a cross-sectional study where 84 participants were recruited and further divided into three equal groups; Group I: Patients with T2DM without albuminuria (n=28), Group II: Patients with T2DM with albuminuria (n=28), and Group III: healthy volunteers (n=28). Patients were recruited from the outpatient diabetes and family clinic of the Suez Canal university hospital, Ismailia, Egypt. Laboratory tests were performed at the Clinical Pathology department of the Suez Canal University Hospital, Ismailia. Inclusion criteria:

include: Male and female aged 18: 65 years, Patients diagnosed with T2DM based on the American Diabetes Association [29], DN was diagnosed by the consensus reached by the American Diabetes Association and National Kidney Foundation [30]. eGFR <60 mL/min, or (UACR) >30 mg/g creatinine. Each last for more than 3 months. Pregnant women, patients with active infections, malignancy, CKD patients due to other causes than DM, type I diabetic patients, treatment with immunosuppressive drugs, systemic autoimmune disease, and kidney transplant recipients were excluded. The participants underwent history, clinical examination, and Laboratory work such as lipid profile, glycemic markers (FPG, 2-h PPG, and HbA1C), serum creatinine by cobas 6000, eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula, and Urinary Albumin Creatinine Ratio (UACR) were investigated. Serum Angpt-2 measured by ELISA based-method (SunRed Biological Technology Co). The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), Assay range: 20pg/ml-6000pg/ml, Sensitivity: 18.227 pg/ml, Diabetic kidney disease was diagnosed by the consensus reached by the American Diabetes Association and National Kidney Foundation[7]. eGFR <60 mL/min, or (UACR) >30 mg/g creatinine. Each last for more than 3 months. itten informed consent was obtained from all the participants before taking any data or doing any investigations.

Statistical Analysis

SPSS program (Statistical Package for Social Science) version 26 was used. The data's normal distribution was examined using the Shapiro Walk test. To calculate the difference between qualitative variables, the Chi-square test (χ^2) and Fisher exact were utilized. Kruskal Wallis test was used to calculate the difference between quantitative variables in three groups for non-parametric variables. Mann Whitney test was a tool to compute the difference between two groups of quantitative variables. *ROC* (receiver operating characteristic) curve analysis was established to assess how well predictors predict diabetic nephropathy. A level of P-value < 0.05 indicates significant difference.

Results

The mean age was 55.3± 8.9 years, 58.6± 6.9 years, and 51.9± 9.6 years among groups I, II, and III respectively with no significant difference. The gender was comparable between the three groups 21.4%, 39.3%, and 50% males among the three groups respectively.

Table1: Non-Laboratory Risk Factors for Diabetic Nephropathy

Variable		**Group I n= 28**	**Group II n= 28**	**Group III n= 28**	**P value**
Age (years)	Mean ± SD	55.3± 8.9	58.6± 6.9	51.9± 9.6	0.180 [a]
Gender	Male, n (%)	6 (21.4)	11 (39.3)	14 (50)	0.105[b]
	Female, n (%)	22 (78.6)	17 (60.7)	14 (50)	
Family history	Yes, n (%)	18 (64.3)	19 (67.9)	9 (32.1)	**0.017*[b]**
	No, n (%)	10 (35.7)	9 (32.1)	19 (67.9)	
BMI (Kg/m^2)	Mean ± SD	29.2± 4.3	29.6± 4.2	26.8± 4.3	**0.033*[a]**
SBP* (mmHg)	Mean ± SD	127.5± 13.2	130.7± 7.4	123.0± 10.3	**0.027***
DBP* (mmHg)	Mean ± SD	81.6± 7.8	84.9± 6.6	79.5± 7.1	**0.020***
Duration (years)	Mean ± SD		8.2± 3.9	10.9± 5.3	**0.036*[a]**

*a; Kruskal Wallis test, b; Chi square test; *p is significant at <0.05 ; c Mann Whitney U test*

The diabetics with albuminuria group II had a significantly higher duration than diabetics without albuminuria group I p=0.036. There was no statistically significant difference between diabetics with or without albuminuria groups regarding BMI, blood pressure, triglyceride, and glycemic markers. (Table 1). In this study, no significant correlation was found between serum Angpt-2 levels and glycemic markers, lipid profile, BMI, blood pressure (Table 2). There was a statistically significant negative correlation between serum Angpt-2 and eGFR (r=-0.414, p= 0.029), and a positive correlation with serum creatinine (r=0.398, p=0.036) (Table 2) (Figure 2).

Table 2: Correlation Between Angpt-2 And Other Biomarkers Among the Protienuric Group.

Variable		r	P value
Kidney function markers	UACR	0.238	0.222
	Serum creatinine	0.398	**0.036***
	eGFR	-0.414	**0.029***
Glycemic markers	HbA1C	0.155	0.431
	FPG	0.136	0.491
	2-h PPG	0.249	0.201
Lipid profile	Cholesterol	-0.030	0.878
	Triglyceride	0.059	0.767
	HDL	0.004	0.983
	LDL	-0.113	0.566
Clinical markers	BMI	-0.150	0.446
	SBP	-0.136	0.489
	DBP	-0.304	0.116

*Spearman correlation, *p is significant at <0.05, eGFR; estmated glomerular filtration rate, UACR; Urinary Albumin creatinine ratio, FPG: fasting plasma glucose, 2-h PPG: 2 postprandial plasma glucose, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure.*

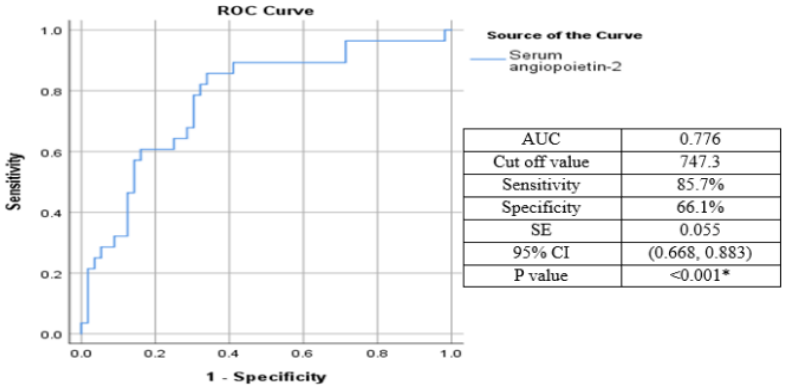

Figure 1. Angpt-2 has a cutoff point (747.3 pg/ml) for diagnosis of of DN with a sensitivity of 85.7% and specificity of 66.1% , PPV was 55.8%, NPV was 90.3% and the AUC was 77.6%.

This positive correlation with serum creatinine and the negative correlation with GFR suggest that increased Angpt-2 may be associated with the development

Table 3: Comparison between normo, micro, and macro UACR regarding serum Angpt-2					
Variable	**Normo n= 56**	**Micro n= 21**	**Macro n= 7**	**P value**	
S. Angiopoietin-2 (Pg/ml)	Mean ± SD	7.9± 3.7	11.2± 4.5	15.1± 4.2	**<0.001***
Post hoc test	Normo Vs. Micro	Micro vs. Macro	Normo vs. macro		
P value	**0.002***	**0.029***	**<0.001***		

*Kruskal Wallis test; *p is significant at <0.05*

Table 4. Laboratory parameters of the studied groups				
Variable	**Group I n= 28**	**Group II n= 28**	**Group III n= 28**	**P value**
UACR (mg/g creatinine)	11.1± 6.3	310.8± 434*#	8.5± 4.9	**<0.001**
Serum create (mg/dl)	0.9± 1.2	1.8± 1.9 *#	0.8± 0.2	**0.009**
eGFR (ml/min/1.73 m^2)	99.6± 31.2	53.8± 28.0 *#	96.0± 18.4	**<0.001**
FPG(mg/dl)	224.2± 88.8	197.3± 80.8 #	89.6± 7.6 *	**<0.001**
2-h PPG (mg/dl)	297.8± 120.7	251.7± 99.4 #	125.9± 15.8*	**<0.001**
HbA1C (%)	8.5± 1.9	8.3± 1.2 #	5.2± 0.4 *	**<0.001**
Cholesterol (mg/dl)	188.1± 50.7	212.7± 61.1	182.3± 42.3	0.073
Triglyceride (mg/dl)	131.8± 74.1	166.5± 105.4 #	95.6± 32.4	**0.004**
HDL (mg/dl)	46.3± 11.9	46.4± 13.5	52.4± 8.2	0.082
LDL (mg/dl)	115.5± 49.3	132.9± 52.5	110.8± 41.5	0.196

*Data are presented as Mean ± SD, Values are mean ± SD , analyzed by Kruskal Wallis test; Post hoc test ; Bonferroni test; Statistically significant p value < 0.05 * compared to group I ; $ compared to group II; # compared to group III, eGFR; estimated glomerular filtration rate , UACR; Urinary Albumin creatinine ratio*

of renal impairment. The Serum Angpt-2 was a higher in the diabetics with albuminuria group II than the diabetics without diabetics with albuminuria group I p <0.001. It was significantly higher in the diabetics with albuminuria group II than in the healthy control group p <0.001 (Figure 3). Angpt-2 was significantly higher in patients with microalbuminuria and macroalbuminuria compared to normoalbuminuric diabetic patients and healthy controls, indeed, serum Angpt-2 progressively increases with the progression of albuminuria and renal impairment (Table 3). This study showed that serum Angpt-2 is a diagnostic marker of DN with a sensitivity of 85.7% and specificity of 66.1% at a cut-off value of 747.3 pg/ml and the area under the curve was 77.6% (Figure 1).

Discussion

This study demonstrated that the decrease in total serum bilirubin level after different intervals of phototherapy (after 24 hours – after 48 hours or more than 48 hours) was statistically significant (P value < 0.001). This result is similar to findings in many studies such as: Shahriarpanah et al.

(2018) [23], Bezboruah & Majumder (2019) [14] and Amneenah (2022) [24] in addition to Abo_Hussein et al. (2022) [25] This effect is mostly due to bilirubin's structure changing as a result of phototherapy. These structural and configurational isomers of bilirubin become less lipophilic than normal bilirubin, so, can be easily excreted in urine and bile without being glucuronidated in the liver [11,12]. The present study showed that all changes that occurred in serum electrolytes (sodium, potassium, ionized calcium, magnesium) and creatinine levels after different intervals of phototherapy were statistically significant (P value < 0.001).

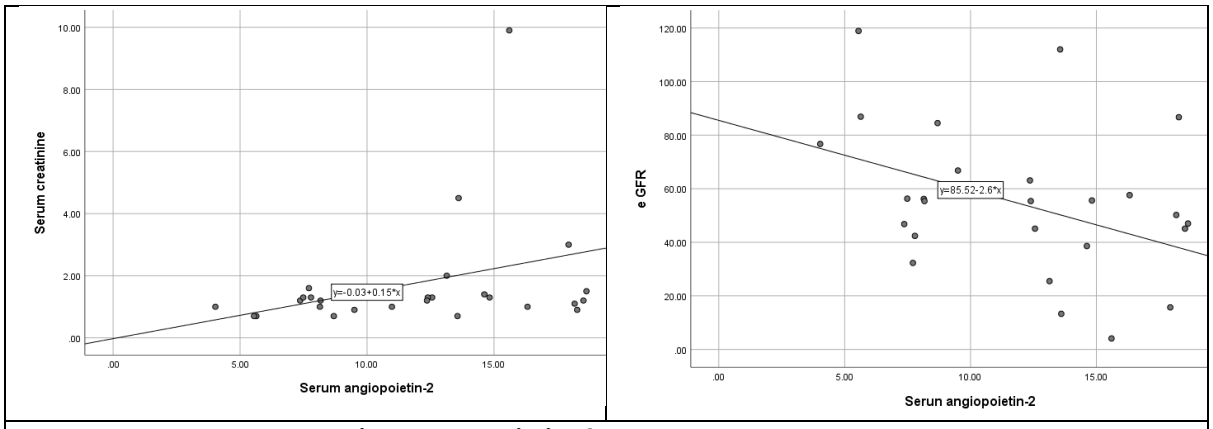

Figure 2. Association between serum Angpt-2, serum creatinine and eGFR among diabetic nephropathy group

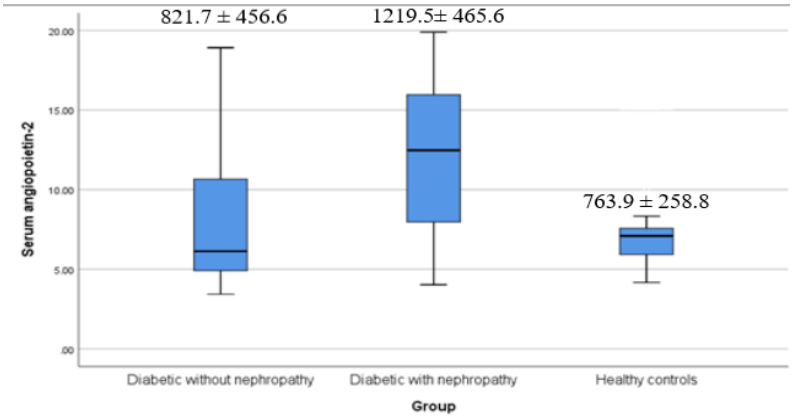

Figure 3. Serum Angpt-2 showed a statistically significant difference among the three groups p<0.001, significantly higher in the diabetics with albuminuria group II than in the diabetic without albuminuria group I P<0.001*, It was significantly higher in the diabetic with albuminuria group II than in the healthy control group P <0.001*, however, no statistical significance was found between the diabetics without albuminuria and the control group but it showed higher levels in diabetics without albuminuria p=0.595

Serum Sodium

All cases in this study had normal serum sodium levels on admission, 45% of them developed hyponatremia after 48 hours or more of phototherapy. This result is similar to other studies including Suneja et al. (2018) [26], Ghosh et al. (2020) [27] and Amneenah (2022) [24] in which significant decrease in serum sodium levels after phototherapy occurred. Unlike Abo_Hussein et al. (2022) [25] in which there was a decrease in mean (± SD) serum sodium level from 136.5 ± 12.85 before phototherapy to 135.8 ± 2.75 after phototherapy but

the difference was not statistically significant, which was explained by the small size of the studied group. Hyponatremia mostly occurs because phototherapy may result in diarrhea with transient impairments in water and sodium absorption [20,21].

Serum potassium

Regarding serum potassium in this study, all enrolled neonates had normal serum potassium levels on admission, 30% of them developed hypokalemia after 48 hours or more of phototherapy (a statistically significant decrease in serum potassium levels had occurred; P value < 0.001).This finding has similarity to some studies such as Abo_Hussein et al. (2022) [25] in which significant difference in serum potassium level after phototherapy occurred (p value < 0.001) where mean (± SD) level of serum potassium decreased from 4.48 ± 1.19 before phototherapy to 3.85 ± 0.53 after phototherapy. Also, a statistically significant decrease in serum potassium level occurred in other studies like Bezboruah & Majumder (2019) [14] and Jena et al. (2019) [28]. The decrease in serum potassium level most probably occurs in a way similar to that of hyponatremia as a consequence of impaired water and potassium absorption in neonates receiving phototherapy. [21]

Serum Ionized calcium

All cases in this study had normal serum ionized calcium levels ranging from 4.2-5.58 mg/dl on admission, 65% of them were less than 4.2 mg/dl after 48 hours or more of phototherapy (P value <0.001). Similar statistically significant decrease in serum calcium levels also occurred in many studies, such as those by: Suneja et al. (2018) [26], Bezboruah & Majumder (2019) [14], Jena et al. (2019) [28], Amneenah (2022) [24] and Abo_Hussein et al. (2022) [25]. Asl et al. (2016) [29] reported a decrease in serum calcium levels, but the changes did not lead to hypocalcemia, and they recommended further complementary studies with larger sample size. Light entering the skull can have an inhibitory impact on pineal gland, causing a decrease in melatonin secretion and perhaps contributing to hypocalcaemia.[17,18] It may also occur as a result of the decrease in parathormone production that may occur in jaundiced newborns treated with phototherapy.[19,20] Regarding the occurrence of symptomatic hypocalcemia after phototherapy, 22% of this study group had symptomatic hypocalcemia (jitteriness- irritability), which has similarities to a study done by Jain et al. (1998) [30] in which 30% of full-term newborns and 55% of preterms had phototherapy-induced hypocalcaemia; 63.6% of the affected preterm infants with hypocalcemia became jittery, and 27.3% were irritable. In addition, 50% of the full-term newborns with hypocalcemia were jittery, and 16.7% developed irritability. Accordingly, they advised giving infants receiving phototherapy calcium supplements to prevent hypocalcemia.

Serum magnesium

All cases in the present study had normal serum magnesium levels on admission, 32.5% of them were less than 1.6 mg/dl after 48 hours or more of phototherapy. A significant decline occurred in mean serum magnesium level in this study, which is similar to findings in the studies by others[14,23,24]. Phototherapy affects serum magnesium in a way similar to that on serum calcium, through depression of pineal gland secretions. [23]

Serum creatinine

Concerning serum creatinine level, in the present study, 25% of cases had elevated

serum creatinine level more than 1.2 mg/dl (statistically significant), unlike the findings in other studies such as: Abo_Hussein et al. (2022) [25] and Suneja et al. (2018) [26] in which there were significant decrease in serum creatinine level after phototherapy.Elevated serum creatinine levels in this study may be explained by dehydration caused by phototherapy if fluid support was not adequate. This study considered the co-relation between the difference that occurred in serum electrolyte levels and the duration of phototherapy. All of them in the present study were insignificant except for the differences that occurred in serum creatinine level, which were statistically significant (P= 0.002). Other studies also demonstrated the significance of the duration of phototherapy on the changes that occurred in different serum electrolytes: such as Bezboruah & Majumder (2019) [14] and Rangaswamy et al. (2019) [21] in which the duration of phototherapy proved to have a highly significant negative correlation with the serum levels of sodium, potassium, ionized calcium and creatinine. These findings regarding the duration of phototherapy are unsimilar to those of Ghosh et al. (2020) [27], in which the changes that occurred in serum electrolytes were not statistically significant with the duration of phototherapy. Concerning the relation between gestational age and changes that occurred in serum electrolyte levels in this study, only changes that occurred in serum sodium were statistically significant (P 0.001), but other electrolyte changes were insignificant. This finding has similarities to a study by Bezboruah & Majumder (2019) [14] in which preterm neonates had higher rates of post-phototherapy hyponatremia and hypocalcemia (18.31% and 25.34%) than term (11.02% and 10.24%) and post-term (12.5% and 0%), respectively, the incidence of hypokalemia and hypomagnesemia did not correlate with gestational age. Jena et al. (2019) [28] also illustrated gestational age co-relation with electrolyte imbalances after phototherapy; preterm infants had more changes than full term in each of serum sodium (29.4% and 5% respectively), calcium (52.9% and 15% respectively) and potassium (all cases who developed hypokalemia were pre-term). Owing to their immature skin, which makes them more susceptible to insensible water loss, as well as their unstable acid-base balance and less developed renal system, which gets better with increasing gestational age, preterm neonates may be more susceptible to electrolyte imbalances than full-term neonates [5,10]. This study observed the relationship between different types of phototherapies used and changes that occurred in serum electrolytes. Contrary to our expectations, all changes were insignificant. We had assumed that the 360-dimensional phototherapy unit would result in more electrolyte changes than fluorescent tubes and LED phototherapy. So, further studies with a larger sample size are recommended. Findings are like those of others [27]. In the present study, no significant relationship was observed between birth weight and electrolyte changes, unlike others [14], in which the incidence of hyponatremia, hypokalemia and hypocalcemia was higher in low-birth-weight infants (18.75%, 10% and 26.25%, respectively) than in normal neonates (10.32%, 4.76% and 7.94%, respectively).

Conclusion

Phototherapy results in a remarkable decline in serum bilirubin level together with different effects on serum electrolytes: sodium, potassium, ionized calcium, magnesium and creatinine (hyponatremia,

hypokalemia, hypocalcemia, hypomagnesemia and elevated serum creatinine level). All changes were statistically significant, but none of the cases showed any clinical manifestation since only marginal changes were observed. Such effects were mostly positively correlated with the duration of phototherapy used (especially regarding serum creatinine and serum magnesium).

Recommendations

1. Routine measurement of serum electrolytes (sodium, potassium, ionized calcium, magnesium and creatinine) before and after phototherapy with appropriate intervention (whenever needed).
2. Continuous follow-up and efforts to shorten / minimize the duration of phototherapy to be considered a high priority during management of neonatal hyperbilirubinemia.
3. Further well-designed clinical trials on a large geographical scale with a larger sample size and a longer period of follow-up to emphasize our conclusion.

References

1. Mok KY, Chan PF, Lai LKP, et al. Prevalence of diabetic nephropathy among Chinese patients with type 2 diabetes mellitus and different categories of their estimated glomerular filtration rate based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in primary care in Hong Kong: a cross-sectional study. J Diabetes Metab Disord. 2019 Nov 15;18(2):281-288.
2. Sagoo MK, Gnudi L. Diabetic nephropathy: an overview. Diabet Nephrop. Springer; 2020;3–7.
3. Heier JS, Singh RP, Wykoff CC, et al. THE Angiopoietin/Tie Pathway In Retinal Vascular Diseases: A Review. Retina. 2021 Jan 1;41(1):1-19.
4. Akwii RG, Sajib MS, Zahra FT, et al. Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology. Cells. 2019 May 17;8(5):471.
5. Sokolovska J, Stefanovics J, Gersone G, et al. Angiopoietin 2 and Neuropeptide Y are Associated with Diabetic Kidney Disease in Type 1 Diabetes Mellitus. Exp Clin Endocrinol Diabetes. 2020 Oct; 128(10) :654-662.
6. Aly MH, Arafat MA, Hussein OA, et al. Study of Angiopoietin-2 and vascular endothelial growth factor as markers of diabetic nephropathy onset in Egyptians diabetic patients with non-albuminuric state. Diabetes Metab Syndr. 2019 Mar-Apr;13(2):1623-1627.
7. de Boer IH, Khunti K, Sadusky T, et al. Diabetes management in chronic kidney disease: a consensus report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int [Internet]. Elsevier B.V.; 2022 Nov 1 [cited 2023 Jan 26];102(5):974–89.
8. Yasser N, M Saleh SA, Sabry R, et al. Evaluation of Endocan as a Novel Marker for Early Detection of Nephropathy in Patients with Type 2 Diabetes Milletus. Egypt J Med Microbiol. Egyptian Society for Medical Microbiology (ESMM); 2020;29(1):101–9.
9. Chen S, Li H, Zhang C, et al. Urinary angiopoietin-2 is associated with albuminuria in patients with type 2 diabetes mellitus. Int J Endocrinol. 2015;2015:163120.
10. Tsai YC, Lee CS, Chiu YW, et al. Angiopoietin-2, Renal Deterioration, Major Adverse Cardiovascular Events and All-Cause Mortality in Patients with Diabetic Nephropathy. Kidney Blood Press Res. 2018;43(2):545-554.
11. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, et al. Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep. 2017 Jun 2;7(1):2684.
12. Wu M, Lu J, Zhang L, et al. A non-

laboratory-based risk score for predicting diabetic kidney disease in Chinese patients with type 2 diabetes. Oncotarget. 2017 Oct 9;8(60):102550-102558.
13. Salem M, Sallam A, Amer E, et al. The potential use of Endostatin and Angiopoietin-2 as valuable biomarkers for the prediction of Diabetic Nephropathy in Type 2 Diabetes Mellitus. Arch Pharm Sci Ain Shams Univ. Ain Shams University, Faculty of Pharmacy; 2019;3(2):277–84.
14. Shurraw S, Hemmelgarn B, Lin M, et al. Alberta Kidney Disease Network. Association between glycemic control and adverse outcomes in people with diabetes mellitus and chronic kidney disease: a population-based cohort study. Arch Intern Med. 2011 Nov 28;171(21):1920-7.
15. Tziomalos K, Athyros VG. Diabetic Nephropathy: New Risk Factors and Improvements in Diagnosis. Rev Diabet Stud. 2015 Spring-Summer;12(1-2):110-8.
16. De Boer IH, Afkarian M, Rue TC, et al. Renal outcomes in patients with type 1 diabetes and macroalbuminuria. J Am Soc Nephrol [Internet]. J Am Soc Nephrol; 2014 Oct 1 [cited 2023 Feb 18];25(10):2342–50.
17. Tapp RJ, Shaw JE, Zimmet PZ, et al. Albuminuria is evident in the early stages of diabetes onset: results from the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). Am J Kidney Dis. 2004 Nov;44(5):792-8.
18. Jenkins D, Wolever T, Rao A V., et al. The New England Journal of Medicine Downloaded from nejm.org on March 29, 2011. For personal use only. No other uses without permission. N Engl J Med [Internet]. 1993;329(1):21–6.
19. Sokolovska J, Stefanovics J, Gersone G, et al. Angiopoietin 2 and Neuropeptide Y are Associated with Diabetic Kidney Disease in Type 1 Diabetes Mellitus. Exp Clin Endocrinol Diabetes. 2020 Oct;128(10):654-662.
20. Siddiqui K, Joy SS, Nawaz SS. Serum Angiopoietin-2 levels as a marker in type 2 diabetes mellitus complications. International Journal of Diabetes in Developing Countries; 2019;39 (June): 387–93.
21. Salem M, Sallam AM, Abdel-Aleem E, et al. Effect of Lisinopril and Verapamil on Angiopoietin 2 and Endostatin in Hypertensive Diabetic Patients with Nephropathy: A Randomized Trial. Horm Metab Res. 2021 Jul;53(7):470-477.
22. Aly MH, Arafat MA, Hussein OA, et al. Evaluation of Angiopoietin-2 As an Early Marker For Diabetic Nephropathy in Zagazig University Hospitals. Zagazig Univ Med J. Zagazig University, Faculty of Medicine; 2018;24(2):93–101.
23. Martynov SA, Shestakova MV, Kutyrina IM, et al. [Role of circulating angiogenic factors in diabetic kidney disease]. Vestn Ross Akad Med Nauk. 2013;(2):35-42. Russian.
24. Chang FC, Lai TS, Chiang CK, et al. Angiopoietin-2 is associated with albuminuria and microinflammation in chronic kidney disease. PLoS One. 2013;8(3):e54668.
25. Lip PL, Chatterjee S, Caine GJ, et al. Plasma vascular endothelial growth factor, angiopoietin-2, and soluble angiopoietin receptor tie-2 in diabetic retinopathy: effects of laser photo coagulation and angiotensin receptor blockade. Br J Ophthalmol. 2004 Dec; 88(12):1543-6.
26. Lim HS, Lip GY, Blann AD. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. Atherosclerosis. 2005 May;180(1):113-8.
27. Shroff RC, Price KL, Kolatsi-Joannou M, et al. Circulating angiopoietin-2 is a marker for early cardiovascular disease in children on chronic dialysis. PLoS One. 2013;8(2):e56273.
28. David S, John SG, Jefferies HJ, et al. Angiopoietin-2 levels predict mortality in

CKD patients. Nephrol Dial Transplant. 2012 May;27(5):1867-72.

29. Committee, A. D. A. P. P. (2022a) '2. Classification and Diagnosis of Diabetes:Standards of Medical Care in Diabetes—2022', Diabetes Care, 45 (Supplement_1), pp.S17–S38.
30. de Boer, I. H., Khunti, K., Sadusky, T., et al. 'Diabetes management in chronic kidney disease: a consensus report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO).', Kidney international,102(5), (2022) pp. 974–989.