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SERUM ANGIOPOEITIN LIKE-4 AS AN EARLY DETECTION MARKER OF DIABETIC KIDNEY DISEASE

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ABSTRACT

Diabetic kidney disease (DKD) is a long-term complication of diabetes mellitus (DM). The gold standard for diagnosis of DKD is kidney biopsy, but it is difficult to perform. Several clinical conditions have found an association between higher ANGPTL4 plasma levels in DKD. Angiopoeitin Like 4 (ANGPTL4) is a glycoprotein that regulates lipid and glucose metabolism, mediates proteinuria and is involved in glomerular disease. Studies have found ANGPTL4 levels to be elevated in diabetic conditions. Objective: To investigate the role of serum ANGPTL4 as a marker for early detection of DKD. Methods: A total of 87 patients with Type 2 DM were analysed using an observational analytic method with a cross-sectional design. The study subjects were divided into DKD and non DKD groups, then serum ANGPTL4 was measured using the sandwich ELISA method. Furthermore, ANGPTL index cutoff was determined. Several other variables including glycemic control, duration of DM, history of hypertension, and dyslipidemia were analysed for their association with DKD and non DKD groups. The study continued by conducting multivariate analysis with logistic regression. Results: A total of 23 (26.4%) subjects were classified as DKD and 64 (73.6%) subjects as non DKD. ANGPTL cutoff determination of 16.64 ng/mL had a sensitivity and specificity of 87% and 51.6%, respectively. Bivariate analysis showed glycaemic control, duration of DM, and serum ANGPTL4 (p=0.044; p=0.009; p=0.014) were associated with the incidence of DKD. Multivariate analysis showed that serum ANGPTL4 with a cutoff of >16.64 ng/mL could be used as an independent predictor of DKD incidence with an adjusted OR of 6.73 (95%CI= 1.79-25.30; p=0.005). Conclusion: serum ANGPTL4 is an independent predictor of DKD. Suggestion: Urine sampling for UACR examination twice, periodic examination of serum ANGPTL4 levels to assess the progressiveness of DKD.

KEYWORDS *ANGPTL4 serum, DKD, UACR, eGFR*

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INTRODUCTION

Diabetic kidney disease (DKD) is a common long-term complication in patients with Diabetes mellitus (DM). This condition is reported in 20%-50% of the diabetic population and is the leading cause of chronic kidney disease (CKD), which, if not properly managed, can progress to end-stage renal disease (ESRD). Diabetic kidney disease is defined by the presence of CKD along with a persistent (at least 3 months) increase in urinary albumin excretion (urinary albumin-to-creatinine ratio/UACR ≥ 30 mg/g and/or a decrease in estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m² in an individual with DM. The global prevalence of DM is rising sharply, with estimates from the International Diabetes Federation (IDF) predicting that 693 million people will be living with diabetes worldwide by 2045. This can impact the number of DKD patients if complications are not detected and managed early (Hoogeveen, 2022; WHO, 2020; IDF 2021; Saeedi et al., 2019).

Diabetic kidney disease is a multifactorial disease involving complex interactions between hemodynamic, inflammatory, fibrotic, and metabolic factors. Increased intraglomerular pressure and hyperfiltration occur in the early stages of DKD. Various risk factors influence the condition of DKD, including glycemic control, duration of DM, history of hypertension, and dyslipidemia. These factors are interconnected and contribute variably to the incidence of DKD. A series of complex molecules, receptors, enzymes, and transcription factors play roles in the early stages of kidney disease, leading to hypertrophy, expansion of the extracellular matrix, glomerulosclerosis, vascular hyalinosis, interstitial fibrosis, tubular atrophy, and loss of function, causing DKD and potentially progressing to ESRD (Agarwal, 2021; Farah et al., 2021).

Hyperglycemia contributes to the occurrence of DKD and causes progressive damage to the glomeruli and tubules, while glycemic control influences the onset of nephropathy. Glycemic control is a risk factor for DM associated with hyperglycemia, and tight glycemic control has been shown to reduce the incidence of albuminuria. Glycemia assessment using glycated hemoglobin (HbA1c) is used for monitoring and adjusting medications for glycemic control in DM patients with DKD. The recent Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study proved that strict glucose control significantly slows the decline in eGFR and progression to ESRD (Galindo et al., 2020; Heo et al., 2023).

The duration of DM is an unmodifiable risk factor for DM. The progression of DKD varies based on the pathophysiology of diabetes, age at diagnosis, and the development of DKD from microalbuminuria to macroalbuminuria within approximately 10 years after DM diagnosis. Some studies report that patients with a duration of DM less than 10 years have a lower risk of developing DKD compared to those with a duration of more than 10 years (Kebede et al., 2021; Siddiqui et al., 2022).

A history of hypertension as a risk factor for DKD causes a significant loss of nephron function over time, which becomes irreversible. The mechanism of

hypertension involves complex processes including the activation of the Renin-Angiotensin-Aldosterone System (RAAS), endothelial dysfunction, and increased oxidative stress (Banerjee et al., 2022).

Dyslipidemia is caused by imbalances in lipids such as cholesterol, triglycerides (TG), and lipoproteins. Dyslipidemia in the development of DKD causes podocyte apoptosis, macrophage infiltration, and increased extracellular matrix production. Chronic hyperglycemia and insulin resistance can exacerbate dyslipidemia in DKD (Hirano et al., 2022; Ishtiaq et al., 2019).

Albuminuria is an early clinical sign of DKD used for screening DKD. DM patients have twice the risk of CKD compared to non-diabetics. The prevalence of DKD with normoalbuminuria varies between 14.29% to 56.6%, and an autopsy study on 168 diabetic patients found that 63% had histological evidence of DKD, with almost 19% of these patients having no clinical evidence of DKD (Qazi et al., 2022). The unpredictability of kidney complications in DM patients necessitates additional biochemical markers to help detect and understand the pathophysiology of DKD, thereby minimizing kidney damage. Diabetes-related mortality is largely limited to those experiencing CKD, making timely prevention and diagnosis of DKD in diabetic patients crucial (Al Shawaf et al., 2019; Bano et al., 2023; Farah et al., 2021; Selby & Taal, 2020).

The gold standard for diagnosing DKD is a kidney biopsy; however, this procedure cannot be routinely performed on all DM patients with indications of DKD due to its risks and complications such as infection, bleeding, or damage to surrounding organs. Currently, as an alternative, doctors conduct a series of blood and urine tests to assess kidney function and detect signs of DKD. Blood tests measure creatinine levels and eGFR to assess kidney function, and urinalysis tests measure albuminuria levels to determine kidney damage. The accuracy of these indicators is influenced by various factors, including muscle mass, diet patterns, and the calculation formula used, and there is evidence that diabetic patients may already have advanced glomerular lesions with normoalbuminuria. This highlights the uncertainty in using albuminuria as a marker for kidney disease progression and early diagnosis of DKD.

The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend assessing kidney conditions using a combination of eGFR and albuminuria tests to provide a low, medium, high, or very high-risk assessment of kidney function deterioration. However, no single marker can fully replace the diagnosis and monitoring of DKD (Siddiqui et al., 2022). Some studies indicate the involvement of ANGPTL4 in the occurrence of albuminuria and the progression of DKD. Advantages of serum ANGPTL4 as a DKD marker include: 1) ANGPTL4 is related to lipid and glucose metabolism, making it relevant in the context of diabetes (Li et al., 2020; Morelli et al., 2020), 2) Studies show increased ANGPTL4 in DKD patients, especially in the advanced stages, indicating its potential as an indicator for monitoring DKD progression (Shawaf et al., 2019), 3) ANGPTL4 is associated with inflammatory processes and oxidative stress that also contribute to kidney damage in DM, 4) The role of ANGPTL4 in lipid and glucose metabolism suggests its potential as a therapeutic target for DKD, 5) ANGPTL4's broad role is also found in early detection of renal and non-renal diseases (Morelli et al., 2020; Qin et al.,

2019; Shuff et al., 2021).

Research has explored the role of ANGPTL4 as a marker of DKD. For instance, Bano et al. found that podocyte dysfunction markers such as ANGPTL4 could serve as DKD markers, with increased urinary ANGPTL4 concentrations in patients with albuminuria (macroalbuminuria compared to normoalbuminuria) with sensitivity and specificity for microalbuminuria and macroalbuminuria at 77.8% and 100%, respectively (Bano et al., 2023).

Shawaf et al. analyzed the relationship between plasma ANGPTL4 in DKD patients and other DKD-related proteins such as IGFBP1 and IGFBP4. Plasma levels of ANGPTL4 and IGFBP increased in DKD, with ANGPTL4 levels rising in diabetic nephropathy (241.56±14.1 μg/ml) compared to the control group (178.43±24.09 μg/ml). Increased ANGPTL4 correlated with clinical parameters of DKD, including UACR (r=0.271, p=0.002), serum creatinine (r=0.381, p=0.0001), and eGFR (r=-0.349, p<0.0001) (Shawaf et al., 2019).

Based on the above background, this study aims to uncover the role of serum ANGPTL4 as an early detection marker for Diabetic Kidney Disease (DKD). An increase in ANGPTL4 levels is expected to influence kidney condition progression as a diabetes complication, making this biomarker potentially useful for detecting nephropathy in Diabetes Mellitus (DM) patients. Research questions include whether ANGPTL4 can function as an independent marker in early DKD detection, considering risk factors such as glycemic control, duration of Type II DM, history of hypertension, and dyslipidemia. This study is expected to provide academic benefits by contributing knowledge about the role of serum ANGPTL4 in early DKD detection and have practical implications as an alternative diagnostic examination for DKD patients.

RESEARCH METHOD

This study is an analytical observational study with a cross-sectional research design conducted on Type 2 DM patients in Surakarta from September 2023 to January 2024. The study involved 87 patients with Type 2 DM aged 18 years and older. Serum data ANGPTL4 obtained by ELISA examination using Elabscience's ELISA ANGPTL4 reagent kit (catalog number E-EL-H0337). The serum sampling method was carried out from December 2022 to January 2023, and the samples were stored at a temperature of -80°C until the analysis was carried out. The process of taking venous blood and urine samples and other laboratory tests, including creatinine and serum urea, was carried out according to the standard procedures described in this study (Reference: National Committee for Clinical Laboratory Standards, 2007; Sastroasmoro & Ismael, 2014; Ministry of Health of the Republic of Indonesia, 2013; Proline, 2020; Roche, 2014; Elabscience, 2023).

RESULT AND DISCUSSION

Validity of Analytical Tests

The results of the analytical performance test that have been carried out before conducting the research include precision tests and accuracy tests with results as a resultt:

Precision test.

A daily precision test was carried out to check serum ANGPTL4 parameters 8 times. The day-to-day precision test was carried out on the parameters of serum creatinine, urine albumin, HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Precision is expressed in the value of the coefficient of variation (KV). A smaller KV value (%) indicates the more thorough the system or method is, and vice versa.

Table 7. Within-Day Precision Test

Parameter	Average Level	SD	KV (%)	KV (%) maks
Serum ANGPTL4 (ng/mL)	19,05	0,81	4,81	<10
Urine Creatinine (mg/dL)	50,01	0,62	1,24	25,7

Note: SD = standard deviation; CV = coefficient of variation; Max = maximum; ANGPTL4 = Angiopoeitin Like-4; ng/mL = nanogram/milliliter; mg/dL = milligram/deciliter. A. Anonim, 2019; b. Westguard, 2014.

Precision is evaluated by calculating the average, SD, and CV. The within-day precision test results showed CV values of 4.81% for serum ANGPTL4 and 1.24% for urine creatinine, as shown in Table 7. These results are below the maximum CV parameters specified in the literature. Lower CV results indicate a more accurate method.

The day-to-day precision test was conducted with daily control materials. The CV values for each parameter were as follows: serum creatinine 4.62%; urine albumin 0.61%; HbA1c 3.03%; total cholesterol 2.04%; LDL cholesterol 7.16%; HDL cholesterol 2.04%; and triglycerides 2.81% (Table 8). All parameter results were below the maximum CV specified in the literature. Lower CV results indicate a more accurate method.

Tabel 8. Uji presisi hari ke hari (day to day)

Parameter	Average Level	SD	KV (%)	KV (%) maks
Urine Creatinine (mg/dL)	1,30	0,05	4,62	14,7a
Urine Albumin (mg/l)	196,2	1,20	0,61	55a
HbA1c (%)	10,9	0,33	3,03	5,7a
Cholesterol total (mg/dL)	178,4	3,64	2,04	15,3a
Cholesterol LDL	52,5	3,76	7,16	20,4a
Cholesterol HDL (mg/dL)	105,3	2,15	2,04	21,2a
Triglycerides (mg/dL)	212,1	5,97	2,81	32,7a

SD = standard deviation; CV = coefficient of variation; HbA1c = hemoglobin a1c; LDL = low-density lipoprotein; HDL = high-density lipoprotein; mg/l = milligram/liter; mg/dl = milligram/deciliter; max = maximum. a. Westguard, 2014.

Accuracy Test

The accuracy test was performed on serum creatinine, urine albumin, HbA1c, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. The results shown in Table 9 indicate that all parameters are within the acceptable range. Bias values can be positive, indicating a higher value than the true value, as seen in urine

albumin, HbA1c, total cholesterol, and LDL cholesterol. Negative bias values in HDL cholesterol and triglycerides indicate a lower value than the true value. A bias value of 0 for serum creatinine indicates that the value matches the true value. A smaller d (%) value indicates a more accurate method (Siregar, 2018). The serum ANGPTL4 parameter was not subjected to an accuracy test due to the lack of control materials showing true/target values according to the standard method.

Table 9. Accuracy Test

Parameter	Target Value (average ± 2 SD)	Average Measurement	Conclusion	d (%)
Urine Creatinine (mg/dL)	1,30 (1,10-1,50)	1,30	Within range	0
Urine Albumin (mg/l)	196 (101-300)	196,2	Within range	0,001
HbA1c (%)	10,7 (9,5-11,9)	10,9	Within range	0,02
Cholesterol total (mg/dL)	175 (157-193)	178,4	Within range	0,02
Cholesterol LDL	54,5 (45,7-63,3)	52,5	Within range	-0,04
Cholesterol HDL (mg/dL)	104 (88-120)	105,3	Within range	0,01
Triglycerides (mg/dL)	216 (194-238)	212,1	Within range	-0,02

Notes: HbA1c = hemoglobin a1c; LDL = low-density lipoprotein; HDL = high-density lipoprotein; SD = standard deviation; mg/dL = milligram/deciliter; mg/l = milligram/liter; ng/mL = nanogram/milliliter.

Determination of serum cutoff ANGPTL4 as a marker for early detection of PGD

The researcher calculated the cutoff value of serum ANGPTL4 levels as a marker for early detection of PGD in Type 2 DM patients using the ROC curve. The ROC curve and AUC area provide information in the form of images and numbers that show the performance of serum levels ANGPTL4 as markers for early detection of PGD (Figure 17).

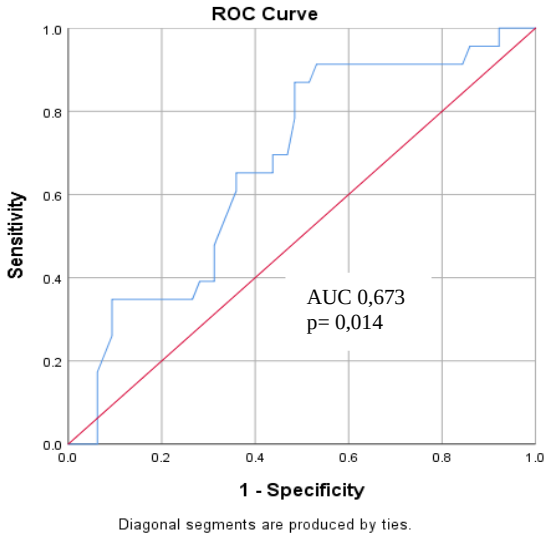

Figure 18. ROC curve and serum AUC area ANGPTL4 as early detection of PGD

in Type 2 DM

Comparison of serum ANGPTL4 level examination results against the incidence of PGD with an AUC value of 0.673 (95%CI= 0.550- 0.796; p=0.014). The results of the analysis with the ROC curve show that the cutoff value of the serum examination ANGPTL4 selected based on the highest Youden index. The results of the cutoff determination are seen in Tables 11 and 12.

Table 11. Serum cutoff table ANGPTL4 as early detection of PGD in DM Type 2 patients

Cutoff	Sencitivity	specificity	Youden Indeks
16,11	0,913	0,438	0,351
16,23	0,913	0,453	0,366
16,35	0,913	0,469	0,382
16,46	0,870	0,484	0,354
16,64	0,870	0,516	0,385
16,82	0,783	0,516	0,298
16,94	0,696	0,531	0,227
17,05	0,696	0,563	0,258
17,23	0,652	0,563	0,215
17,47	0,652	0,578	0,230
17,64	0,652	0,594	0,246
17,76	0,652	0,609	0,262

Serum levels of ANGPTL4 in PGD condition obtained an AUC of 0.673 which means that there is a 67.3% chance that PGD condition in Type 2 DM can be detected by serum ANGPTL4. The results of this study obtained a cutoff serum ANGPTL4 as a marker for early detection of PGD condition in Type 2 DM patients, which was ≥ 16.64 ng/mL with a sensitivity of 87.0% and a specificity of 51.6% as seen in Table 11 and Table 12.

Table 12. Determination of serum cutoff ANGPTL4 as early detection of PGD incidence in Type 2 DM patients

Variable	AUC	95% CI		Cutoff	Sencitivity	specificity	p
		Lower	Upper				
ANGPTL4 (ng/mL)	0,673	0,550	0,796	>16,64	87,0%	51,6%	0,014*

Notes: AUC= area under curve; CI= confident interval; ANGPTL4= Angiopoeitin Like 4; ng/mL= nanogram/mililiter, * Signifikan pada p<0.05.

Bivariate analysis of the relationship between independent variables and the occurrence of PGD

Bivariate analysis was carried out with a 2x2 cross-tabulation test to determine the value of the prevalence ratio (PR) then continued with the chi square/fisher exact test to determine the level of significance of the relationship test. The bivariate analysis of the relationship between the free variable and the

occurrence of PGD can be seen in Table 13.

Table 13. The relationship between the independent variable and the occurrence of PGD

Characteristic	PGD	Non PGD	PR	95% CI		p
				Lower	Upper	
Control glycemic			3,34	0,853	20,81	0,044*
Bad	21 (91,3%)	45 (70,3%)				
Good	2 (8,7%)	19 (29,7%)				
DM Duration			5,57	1,51	20,45	0,009*
≥ 10 year	6 (26,1%)	3 (4,7%)				
< 10 year	17 (73,9%)	61 (95,3%)				
HT History			1,34	0,85	2,12	0,237
Yes	13 (56,5%)	27 (42,2%)				
No	10 (43,5%)	37 (57,8%)				
Dyslipidemia			1,02	0,79	1,32	0,868
Yes	18 (78,3%)	49 (76,6%)				
No	5 (21,7%)	15 (23,4%)				
ANGPTL4 (ng/mL)			1,80	1,33	2,42	0,014*
≥ 16,64	20 (87%)	31 (48,4%)				
<16,64	3 (13%)	33 (51,6%)				

Notes: DM= Diabetes mellitus; PGD= Diabetic kidney disease; PR= Prevalence ratio; CI= Confident interval; HT= Hypertension; ANGPTL4= Angiopoeitin Like 4; * Chi Square test/Fisher exact test; * Significant at p<0.05.

The results of the analysis shown in Table 13 show that the history of hypertension (PR=1.34; p=0.237) and dyslipidemia (PR=1.02; p=0.868) has a p value of >0.05 which means that these variables do not have a significant relationship with PGD conditions in Type 2 DM patients. The results of the analysis of glycemic control variables (PR=3.34; p=0.044), duration of DM (PR= 5.57; p=0.009), and serum ANGPTL4 (PR=1.80; p= 0.014) had a p<0.05 value which means that glycemic control, duration of DM, and serum levels ANGPTL4 have a significant relationship with PGD conditions in Type 2 DM patients, so it can be interpreted that the duration of poor glycemic control, DM ≥ 10 years and serum levels ANGPTL4 ≥ 16.64 ng/mL were predictive factors for the incidence of PGD. The distribution of PGD and non-PGD patients is shown in the box plot diagram (Figure 18).

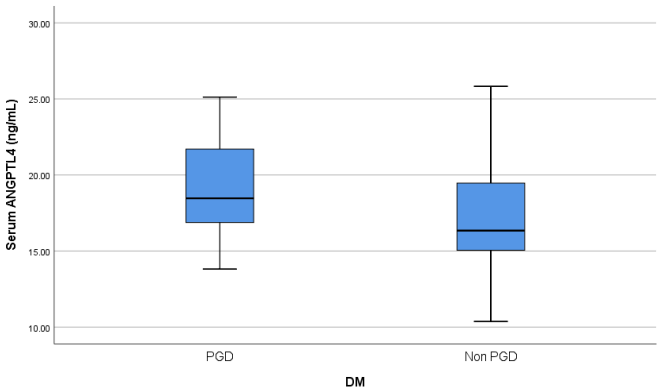

Figure 19. Serum plot box diagram ANGPTL4 based on PGD and non-PGD events

Multivariate analysis of the relationship between independent variables and the occurrence of PGD in Type 2 DM

The researcher continued the analysis with multivariate analysis using the backward stepwise logistic regression method, starting from the full model, then continuing with the next model if there were still variables that needed to be excluded from the analysis in the full model. Multivariate analysis results in several alternative models based on variable characteristics. Multivariate analysis was carried out by taking the characteristic variables of the research subjects and laboratory examinations that had indigo p< 0.25 in the bivariate analysis. The results of the multivariate analysis are shown in Table 14.

Table 14. Multivariate analysis with the bacward stepwise method

Model	Variable	B	S.E.	Z Score	p	OR	95%CI	
							Lower	Upper
Step 1	Control glycemic	1,08	0,83	1,31	0,191	2,96	0,58	15,03
	HT History	0,62	0,56	1,11	0,268	1,87	0,62	5,63
	DM Duration ≥10th	1,33	0,82	1,62	0,104	3,79	0,76	18,94
	ANGPTL4>16.64 ng/mL	1,61	0,69	2,30	0,021*	4,99	1,27	19,59
Step 2	Control glycemic	1,18	0,82	1,43	0,151	3,27	0,65	16,45
	DM Duration ≥10th	1,15	0,79	1,46	0,145	3,17	0,67	14,97
	ANGPTL4>16,64 ng/mL	1,68	0,69	2,43	0,015*	5,39	1,39	20,98
Step 3	Control glycemic	1,38	0,82	1,70	0,089	4,0	0,81	19,79
	ANGPTL4>16,64 ng/mL	1,91	0,67	2,82	0,005*	6,73	1,79	25,30

Note: OR= Odd ratio; DM= Diabetes mellitus; PGD= Diabetic kidney disease; CI= Confident interval; HT= Hypertension; ANGPTL4= Angiopoeitin Like 4
*significant at p<0.05

The results of the multivariate analysis shown in Table 14 show that serum levels of ANGPTL4 (OR=6.73; p=0.005) have a very large risk chance of PGD

incidence in Type 2 DM with p< 0.05, while glycemic control (OR=4.0; p= 0.089) has a lower risk than in serum ANGPTL4 levels. Based on this, serum ANGPTL4 with a cutoff of ≥ 16.64 ng/mL can be used as an independent predictor of the incidence of PGD in Type 2 DM patients.

Discussion

Rerata usia pada penelitian ini (59,51±8,4 tahun) dan pada klasifikasi PGD (61,91±9,02 tahun) dengan p=0,109. Hal ini sejalan dengan rerata usia pasien DM Tipe 2 dengan PGD pada penelitian Shawaf et al. (2019) yaitu 59±1,32 tahun (p=0,07). Penelitian Bano et al. (2023) melaporkan usia pasien PGD dengan rerata 61,9±12 tahun (p=0,0527). Hal ini sesuai dengan literatur yang dilaporkan oleh Morton et al. (2020) bahwa tidak diketahui dan dijelaskan dengan baik hubungan antara onset DM Tipe 2 dan risiko PGD sehingga pada beberapa penelitian kejadian PGD tidak bermakna bila dibandingkan dibandingkan terhadap usia.

Pengaruh usia dan jenis kelamin terhadap prevalensi PGD dipelajari dalam penelitian Pathyway study terhadap 4.839 subjek. Hasil penelitian menunjukkan bahwa secara keseluruhan wanita memiliki OR yang lebih rendah sekitar 28% (OR 0,72, 95% CI 0,62-0,83) dibandingkan pria, namun prevalensinya lebih tinggi terhadap kejadian PGD tahap lanjut. Wanita memiliki risiko yang lebih besar pada kejadian disfungsi ginjal lanjut dan prevalensi yang lebih tinggi tampak pada pasien yang lebih tua. Hal ini sejalan dengan penelitian ini dengan jumlah penderita DM pada penelitian ini didominasi oleh perempuan sebanyak 51 orang (58,6%) dibandingkan dengan laki- laki sebanyak 36 orang (41,4%) (Giandalia et al., 2021; Piani et al., 2022). Ciarambino et al. (2022) juga menjelaskan secara global prevalensi DM lebih banyak pada laki-laki, namun beberapa penelitian menyebutkan perempuan lebih sering menderita DM tipe 2 dibandingkan dengan laki-laki pada usia lanjut. Willer et al. (2023) menjelaskan perempuan mengalami transisi distribusi lemak menjadi android pada kondisi menopause, sehingga terjadi peningkatan akumulasi jaringan adiposa visceral yang meningkatkan risiko DM tipe 2.

Hormon seks memainkan peran penting dalam patofisiologi dan komplikasi diabetes, terutama pada wanita dengan diabetes mellitus (DM) yang kehilangan perlindungan estrogen pada sistem kardiovaskular meskipun sebelum menopause. Estrogen memiliki efek pleiotropik pada sistem reproduksi yang melibatkan reseptor spesifik pada vaskular dan endotel, seperti ER-α dan ER-β. Aktivitas estrogen berjalan melalui berbagai pathway seperti MAPK/ERK, PI3K, dan NF-KB, yang mempengaruhi proses apoptosis, pertumbuhan seluler, diferensiasi, dan inflamasi (Giandalia et al., 2021).

Penelitian terbaru menunjukkan bahwa obesitas memainkan peran krusial dalam pengembangan DM Tipe 2 dan komplikasinya. Studi menggunakan SNP sebagai variabel instrumental genetik menunjukkan bahwa BMI berhubungan dengan risiko peningkatan nefropati diabetik (PGD), dengan risiko meningkat sebesar 2,67 kali lipat pada peningkatan BMI (Lu et al., 2022). Meskipun demikian, penggunaan BMI sebagai indikator PGD pada pasien DM Tipe 2 masih menjadi perdebatan, mengingat hubungan yang kompleks antara obesitas, massa otot, dan penurunan fungsi ginjal (Zaman et al., 2018; Kim et al., 2021).

Prevalensi kondisi klinis seperti albuminuria dan penurunan laju filtrasi glomerulus (eLFG) <60 mL/min/1,73m2 signifikan pada pasien DM Tipe 2, dengan penelitian menunjukkan bahwa PGD terkait erat dengan stadium nefropati yang lebih parah (Nata et al., 2020; Farah et al., 2021). Faktor risiko seperti durasi DM yang lama juga berhubungan dengan risiko PGD meskipun bukan merupakan faktor risiko independen yang kuat (Shikata et al., 2020; Alicic et al., 2017). Selain itu, kontrol glikemik buruk dengan HbA1c ≥7% telah terbukti berhubungan signifikan dengan kondisi PGD pada pasien DM Tipe 2, menunjukkan bahwa hiperglikemia berperan dalam patofisiologi PGD melalui berbagai jalur biologis yang kompleks (Zaman et al., 2017; Su et al., 2016).

Studi terbaru juga menyoroti pentingnya pengelolaan komorbiditas seperti hipertensi pada pasien DM Tipe 2, meskipun hasil penelitian belum konsisten dalam menunjukkan hubungan yang signifikan dengan PGD (Verma et al., 2016). Penelitian ini menegaskan bahwa memahami faktor-faktor klinis terkait PGD, seperti obesitas, kontrol glikemik, dan komplikasi lainnya, penting dalam pengelolaan jangka panjang pasien DM Tipe 2. Studi lanjutan diperlukan untuk memahami lebih baik interaksi kompleks antara faktor-faktor ini dalam perkembangan PGD dan komplikasi DM lainnya.

Hasil analisis nilai GDP pada kelompok PGD dan non-PGD menunjukkan tidak adanya perbedaan signifikan dengan median 177,44 (114,02-312,05) dan 171,42 (114,02-403,08), serta p=0,256, sesuai dengan temuan Kim et al. (2020) yang menunjukkan bahwa glukosa puasa tidak terkait dengan risiko PGD. Penelitian Heo et al. (2023) menegaskan bahwa kontrol glikemik buruk pada DM Tipe 2 berhubungan dengan perburukan klinis GGK, dengan penelitian tambahan yang menunjukkan risiko ESRD meningkat pada pasien dengan HbA1c tinggi, terutama pada GGK stadium G3 (Hao et al., 2023). Analisis terhadap profil lipid menunjukkan bahwa tidak ada perbedaan signifikan dalam kolesterol total, LDL, dan TG antara kelompok PGD dan non-PGD (p=0,893; p=0,743; p=0,061), seperti hasil penelitian Gao et al. (2022) dan Lu et al. (2022). Namun, kolesterol HDL menunjukkan hubungan signifikan dengan PGD (p=0,004), yang mendukung temuan Hamzah (2019) tentang peran profil lipid dalam kondisi DM Tipe 2 dan fungsi ginjal. Analisis ureum serum dan kreatinin serum menunjukkan perbedaan signifikan antara kelompok PGD dan non-PGD (p<0,001), sejalan dengan penelitian Ullah et al. (2023) yang menunjukkan hubungan antara hiperglikemia dan disfungsi ginjal. Selain itu, nilai eLFG juga signifikan lebih rendah pada kelompok PGD (p<0,001), menurut penelitian Lu et al. (2022) dan Alici et al. (2017), yang menyoroti pentingnya eLFG dalam penilaian fungsi ginjal pada DM Tipe 2. Albuminuria, yang terdapat perbedaan signifikan antara PGD dan non-PGD (p<0,001), juga ditemukan sebagai parameter klinis penting dalam kondisi PGD, sesuai dengan penelitian yang menunjukkan kerusakan glomerulus terkait dengan hiperglikemia kronis (Raja et al., 2020). Secara keseluruhan, penelitian ini menggarisbawahi kompleksitas hubungan antara faktor-faktor risiko seperti kontrol glikemik, profil lipid, dan biomarker ginjal dengan perkembangan PGD pada pasien DM Tipe 2, menyoroti perlunya pemahaman yang lebih dalam untuk intervensi terapeutik yang efektif.

Pemeriksaan kreatinin urin pada subjek penelitian menunjukkan nilai

p=0,665 yang berarti tidak terdapat perbedaan yang signifikan antara kelompok PGD dan non PGD. Hasil analisis nilai kreatinin tidak sejalan dengan hasil analisis eLFG. Hal ini disebabkan adanya variabilitas yang signifikan antara individu dalam perkembangan PGD. Studi menunjukkan bahwa serum kreatinin tidak menujukkan korelasi dengan pasien DM Tipe 2, namun berkorelasi dengan pasien DM Tipe 1. Hal ini disebabkan oleh fakta bahwa durasi seseorang menderita DM Tipe 2 (2,47 ± 1,82) lebih singkat dibandingkan DM Tipe 1. Penyebab lainnya bahwa pada DM Tipe 2 hiperglikemia umumnya dimulai setelah usia 40 tahun ketika ginjal telah mengalami penurunan fungsi jangka panjang sebagai konsekuensi penuaan dan pemicu kelainan ginjal kronis lainnya seperti hipertensi, dislipidemia, atau obesitas (Chutani dan Pande, 2017).

Hasil pemeriksaan RAKU subjek penelitian menunjukkan median 28,83 mg/g (rentang 0,60- 571,70). Hasil pemeriksaan RAKU pada kelompok PGD dan pada kelompok non PGD menujukkan nilai p<0,001 yang berarti terdapat perbedaan yang signifikan pada pemeriksaan RAKU antara kelompok PGD dan non PGD. Diagnosis PGD saat ini dapat didasarkan pada hasil pemeriksaan laboratorium (RAKU dan eLFG) dan penilaian klinis pasien. Hasil analisis nilai RAKU pada pasien PGD di penelitian ini masuk kedalam kategori A2 (moderately increased albuminuria/RAKU 30-300 mg/g). Hal ini sejalan dengan penelitian Scultes et al. (2023) yang menemukan banyaknya modifikasi pengobatan pada kategori albuminuria A2 dan A3 (severely increased/RAKU >300 mg/g) dibandingkan kategori A1 (mild to moderate increased/RAKU <30 mg/g) dikarenakan terkait fakta bahwa pasien dengan kategori 1 kemungkinan tidak mengalami PGD (Schultes et al., 2023).

Kadar serum ANGPTL4 pada kelompok PGD memiliki nilai median 18,52 ng/mL (13,82- 25,12); hasil ini lebih tinggi dibandingkan dengan kelompok non PGD yang memiliki nilai median 16,52 ng/mL (10,37- 25,83). Hasil uji statistika didapatkan nilai p=0,014 yang berarti terdapat perbedaan yang signifikan antara kadar serum ANGPTL4 pada kelompok PGD dibandingkan non PGD. Hasil analisis bivariat menunjukkan adanya hubungan yang bermakna signifikan antara kadar serum ANGPTL4 ≥16,64 ng/mL terhadap kejadian PGD pada penderita DM Tipe 2 (p=0,014) dengan sensitivitas dan spesifisitas masing- masing 87% dan 51,6%. Spesifisitas hasil yang rendah dapat disebabkan karena pada penelitian ini sebagian besar populasi masuk dalam kriteria non PGD sebesar 73,6%. Hal ini sesuai dengan literatur bahwa kemampuan untuk membuat diagnosis suatu kondisi tergantung pada nilai diskriminatif dari tes dan tergantung pada prevalensi penyakit dalam populasi yang dianalisis. Subjek pada penelitian ini kebanyakan belum masuk dalam kondisi PGD, sehingga dapat menyebabkan spesifisitas yang kurang baik. Saran peneliti memberikan perhitungan nilai prediktif positif, nilai prediktif negatif, dan likelihood ratio apabila selanjutnya penelitian ini digunakan sebagai referensi marker skrining PGD bagi klinisi.

The average age in this study (59.51±8.4 years) and in the PGD classification (61.91±9.02 years) with p=0.109 aligns with the average age of Type 2 DM patients with PGD in Shawaf et al. (2019) at 59±1.32 years (p=0.07). Bano et al. (2023) reported the average age of PGD patients as 61.9±12 years (p=0.0527). According to Morton et al. (2020), the relationship between the onset of Type 2 DM and the risk of PGD is not well established, making the incidence of PGD not significantly different when compared to age

in several studies.

The impact of age and gender on the prevalence of PGD was studied in the Pathyway study involving 4,839 subjects. The results showed that overall, women had a 28% lower OR (OR 0.72, 95% CI 0.62-0.83) compared to men, but a higher prevalence of advanced PGD stages. Women had a greater risk of advanced kidney dysfunction, with a higher prevalence in older patients. This is consistent with this study, where the number of DM patients was predominantly female, 51 (58.6%), compared to males, 36 (41.4%) (Giandalia et al., 2021; Piani et al., 2022). Ciarambino et al. (2022) also noted that globally, DM prevalence is higher in men, but several studies indicate that women more frequently suffer from Type 2 DM compared to men in older age. Willer et al. (2023) explained that women experience a shift in fat distribution to android patterns during menopause, leading to increased visceral adipose tissue accumulation, raising the risk of Type 2 DM.

Sex hormones play a crucial role in the pathophysiology and complications of diabetes, particularly in women with diabetes mellitus (DM) who lose estrogen's protective effect on the cardiovascular system even before menopause. Estrogen has pleiotropic effects on the reproductive system involving specific receptors on vascular and endothelial tissues, such as ER-α and ER-β. Estrogen activity operates through various pathways like MAPK/ERK, PI3K, and NF-KB, influencing processes like apoptosis, cellular growth, differentiation, and inflammation (Giandalia et al., 2021).

Recent research indicates that obesity plays a critical role in the development of Type 2 DM and its complications. Studies using SNPs as genetic instrumental variables show that BMI is associated with an increased risk of diabetic nephropathy (PGD), with the risk rising 2.67 times with increased BMI (Lu et al., 2022). However, using BMI as an indicator of PGD in Type 2 DM patients remains debated due to the complex relationship between obesity, muscle mass, and declining kidney function (Zaman et al., 2018; Kim et al., 2021).

The prevalence of clinical conditions such as albuminuria and decreased estimated glomerular filtration rate (eLFG) <60 mL/min/1.73m2 is significant in Type 2 DM patients, with studies showing that PGD is closely linked to more severe nephropathy stages (Nata et al., 2020; Farah et al., 2021). Long-term DM duration is also associated with an increased risk of PGD, although not a strong independent risk factor (Shikata et al., 2020; Alicic et al., 2017). Furthermore, poor glycemic control with HbA1c ≥7% is significantly related to PGD conditions in Type 2 DM patients, indicating that hyperglycemia plays a role in PGD pathophysiology through various complex biological pathways (Zaman et al., 2017; Su et al., 2016).

Recent studies also highlight the importance of managing comorbidities such as hypertension in Type 2 DM patients, though research results have not consistently shown a significant relationship with PGD (Verma et al., 2016). This study emphasizes that understanding clinical factors related to PGD, such as obesity, glycemic control, and other complications, is crucial for the long-term management of Type 2 DM patients. Further research is needed to better understand the complex interactions between these factors in the development of PGD and other DM complications.

Analysis of GDP values in the PGD and non-PGD groups showed no

significant difference, with medians of 177.44 (114.02-312.05) and 171.42 (114.02-403.08), respectively, and p=0.256, consistent with Kim et al. (2020), who found that fasting glucose is not associated with PGD risk. Heo et al. (2023) confirmed that poor glycemic control in Type 2 DM is related to the worsening clinical condition of CKD, with additional studies indicating that high HbA1c increases the risk of ESRD, especially in CKD stage G3 (Hao et al., 2023). Lipid profile analysis showed no significant differences in total cholesterol, LDL, and TG between PGD and non-PGD groups (p=0.893; p=0.743; p=0.061), consistent with Gao et al. (2022) and Lu et al. (2022). However, HDL cholesterol showed a significant relationship with PGD (p=0.004), supporting Hamzah's (2019) findings on the role of lipid profiles in Type 2 DM and kidney function. Serum urea and creatinine analysis showed significant differences between PGD and non-PGD groups (p<0.001), consistent with Ullah et al. (2023), who found a link between hyperglycemia and kidney dysfunction. Moreover, eLFG values were significantly lower in the PGD group (p<0.001), according to Lu et al. (2022) and Alicic et al. (2017), highlighting the importance of eLFG in assessing kidney function in Type 2 DM. Albuminuria, with significant differences between PGD and non-PGD groups (p<0.001), was also found to be a crucial clinical parameter in PGD conditions, in line with studies showing glomerular damage associated with chronic hyperglycemia (Raja et al., 2020). Overall, this study underscores the complexity of the relationship between risk factors such as glycemic control, lipid profile, and kidney biomarkers in the development of PGD in Type 2 DM patients, emphasizing the need for deeper understanding for effective therapeutic interventions.

Urine creatinine analysis in the study subjects showed a p-value of 0.665, indicating no significant difference between the PGD and non-PGD groups. The creatinine analysis results were inconsistent with the eLFG analysis results. This discrepancy is due to significant individual variability in PGD progression. Studies show that serum creatinine does not correlate with Type 2 DM patients but does correlate with Type 1 DM patients. This is because the duration of Type 2 DM (2.47 ± 1.82) is shorter compared to Type 1 DM. Additionally, in Type 2 DM, hyperglycemia generally starts after age 40, when the kidneys have already experienced long-term functional decline due to aging and other chronic kidney disorder triggers such as hypertension, dyslipidemia, or obesity (Chutani and Pande, 2017).

The RAKU examination of study subjects showed a median of 28.83 mg/g (range 0.60-571.70). The RAKU examination results in the PGD and non-PGD groups showed p<0.001, indicating a significant difference in RAKU examination between the PGD and non-PGD groups. PGD diagnosis can currently be based on laboratory examination results (RAKU and eLFG) and clinical assessment of the patient. The RAKU analysis results in PGD patients in this study fall into category A2 (moderately increased albuminuria/RAKU 30-300 mg/g). This is consistent with Scultes et al. (2023), who found many medication modifications in A2 and A3 albuminuria categories (severely increased/RAKU >300 mg/g) compared to category A1 (mild to moderate increased/RAKU <30 mg/g) because category 1 patients are less likely to have PGD (Schultes et al., 2023).

Serum ANGPTL4 levels in the PGD group had a median of 18.52 ng/mL

(13.82-25.12); this was higher than the non-PGD group with a median of 16.52 ng/mL (10.37-25.83). Statistical testing showed p=0.014, indicating a significant difference in serum ANGPTL4 levels between the PGD and non-PGD groups. Bivariate analysis showed a significant relationship between serum ANGPTL4 levels ≥16.64 ng/mL and PGD occurrence in Type 2 DM patients (p=0.014) with sensitivity and specificity of 87% and 51.6%, respectively. The low specificity may be due to most of the population in this study falling into the non-PGD criteria at 73.6%. According to the literature, the ability to diagnose a condition depends on the discriminatory value of the test and the prevalence of the disease in the analyzed population. Most subjects in this study had not reached PGD status, potentially resulting in poor specificity. The researchers suggest calculating the positive predictive value, negative predictive value, and likelihood ratio if this study is used as a reference for PGD screening markers for clinicians.

The analysis then proceeded to a multivariate model, with serum ANGPTL4 levels ≥16.64 ng/mL (OR=5.50; p=0.005) showing a significant high-risk chance for PGD occurrence. Therefore, serum ANGPTL4 with a cutoff of ≥16.64 ng/mL becomes a significant predictor of PGD occurrence. The researchers searched various literature sources using keywords like biomarkers, Type 2 DM, and Diabetic Nephropathy (DN) or PGD. Most studies were conducted with a limited number of subjects or focused on specific subjects (eLFG 30-60 mL/min/1.73 m2, having normoalbuminuria and/or microalbuminuria, or subjects undergoing hemodialysis).

A study involving 1207 Type 2 DM subjects conducted by Yu et al. (2019) classified the PGD index based on eLFG, stages of kidney dysfunction, and albuminuria. They then analyzed serum ANGPTL4 against each index with an Odds ratio in CKD vs. non-CKD, normal to mild albuminuria vs. moderate, and moderate vs. severe albuminuria at 1.12 (p=0.0003); 1.05 (p=0.071); 1.18 (p=0.0008) respectively. The study reported that serum ANGPTL4 levels correlate highly with the PGD index. Further research on rat kidneys using immunostaining, western blot, and cell culture found that ANGPTL4 increased in the glomerulus and tubular cells under DM conditions, showing hyperglycemia induces ANGPTL4 production in tubular cells. This reinforces the role of ANGPTL4 in PGD progression (Yu et al., 2019).

Recent studies found that podocytes produce two types of ANGPTL4: hyposialylated and normosialylated forms, based on electron microscopy examinations. Normosialylated ANGPTL4 can protect glomerular endothelial cells from oxidative injury and maintain glomerular structure, while hyposialylated forms can increase endothelial cell injury, damage glomerular structure, and trigger albuminuria. The production of hyposialylated ANGPTL4 in kidney tubular cells due to hyperglycemia still requires further research (Yu et al., 2019).

The strengths of this study include the use of community-based patient subjects from PPK 1 who routinely undergo medical check-ups, making it suitable for early detection of PGD patients. The study also examines several risk factors related to PGD pathogenesis, thus identifying the most significant risk factors for PGD occurrence. Additionally, this study shows that the cutoff value of serum ANGPTL4 has clinical significance in PGD conditions.

However, there are some limitations to consider. First, this study only performed a one-time RAKU sample examination without evaluating PGD progression over time. Second, the researchers did not collect a complete history and effects of medications that might affect kidney function, potentially causing bias in the study results. Third, although several risk factors related to PGD pathogenesis were examined, other risk factors such as obesity/BMI, duration of hypertension, and duration of dyslipidemia were not considered. Lastly, this study is cross-sectional and cannot evaluate the long-term progression of PGD through serum ANGPTL4 levels.

CONCLUSION

This study concluded that serum levels of ANGPTL4 (PR=1.80; 95%CI=1.33-2.42), glycemic control (PR=1.26; 95%CI=1.01-1.59), and duration of DM (PR=5.57; 95%CI=1.51-20.45) were significantly associated with the incidence of PGD in Type 2 DM patients. Serum levels ANGPTL4 ≥ 16.64 ng/mL showed a 6.73-fold increased risk of PGD (OR=6.73; 95%CI=1.79-25.30; p=0.005), and could be used as an independent marker for early detection of PGD. For further research, it is recommended to conduct periodic urine sampling, collect data on medication history, and consider additional risk factors such as obesity, duration of hypertension, and dyslipidemia to explore the progression of PGD.

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