

## Original Article

## Serum low density lipoprotein receptor related protein-6 level is a discriminator of occluded coronary artery assessed by coronary angiogram

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### Abstract

**Background:** Human lipoprotein receptor-related protein -6 (LRP6) plays a role in the development of coronary artery disease. This study aimed to determine the serum level of the LRP6 in patients referred to the coronary angiogram taking into considerations the findings of coronary angiography, evidence of dyslipidemia, obesity and co-existed diabetes mellitus.

**Methods:** This cross-sectional study included 96 patients who referred to coronary angiogram as an interventional diagnostic test for coronary artery disease. The patients were grouped into Group I (negative angiogram); Group IIA (non-obstructed coronary vessels, positive angiogram) and: Group IIB (obstructed coronary vessels, positive angiogram). The anthropometric measurement, blood pressure, and fasting serum lipid profile and glucose were determined. The serum levels of LRP6 were determined by using the Enzyme-Linked ImmunoSorbent Assay (ELISA).

**Results:** A non-significant higher serum level of LRP6 observed in patients with a positive angiogram  $0.175 \pm 0.074$  ng/ml (Group IIA) and  $0.166 \pm 0.063$  ng/ml (Group IIB) compared with the negative angiogram (Group I:  $0.160 \pm 0.019$  ng/ml). The area under the curve of LRP6 in patients with positive angiogram was significantly lower than that with a normal angiogram.

**Conclusion:** We conclude that the serum level of LRP6 is a good discriminator of patients with coronary artery disease as the area under the curve of the serum levels of LRP6 is significantly decreased as the number of occluded coronary vessels increased.

**Keywords:** Coronary artery angiogram, coronary artery occlusion, low density lipoprotein receptor related gene-6, myocardial ischemia.

### Background

The Wingless/integrated (Wnt) signaling pathways are made of proteins which included the canonical and non-canonical pathways. These pathways are activated by binding a Wnt protein ligand to a receptor related to the Frizzled family. These pathways play a role in the regulation of the cell development, shape and migration [1,2]. Co-receptors, including lipoprotein receptor related protein (LRP) 5/6 are required to facilitate the interaction between the Wnt binding protein and the Frizzled receptor [3]. Activation of a canonical pathway (Wnt/ $\beta$ -catenin signaling) leads to accumulation and phosphorylation of cytosolic  $\beta$ -catenin and then translocated into the nucleus [4,5].

Therefore, LRP6 plays a role in the stability of  $\beta$ -catenin protein and thereby regulates cell metabolism [3,4,6,7]. Wnt receptors have established a role in the different pathways of cell signaling leading to the multiple pharmacological actions, including the release of the intracellular calcium stores as a result of activation non-canonical Wnt/calcium pathway [8]. Activation of the Wnt/ $\beta$ -catenin signaling pathway improves the insulin sensitivity and glucose homeostasis in peripheral tissues particularly the skeletal muscles as it up-regulates the glucose transporters, reduced hepatic glucose production, and promotes hepatic insulin sensitivity [9]. Pharmacological inhibition of the LRP6 / Wnt-binding domains leads to the suppression of the Wnt pathway and its downstream gene regulatory mechanisms and inducing hyperglycemia [10,11]. Therefore, any defect in the LRP6 has been associated with increased risk of dyslipidemia, atherosclerosis and diabetes [12-17].

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Malfunction of LRP6 caused impairment of the internalization of low-density lipoprotein cholesterol and thereby impaired its clearance, which may lead to atherosclerosis [18,19]. Moreover, mutation of the LRP6 induced combined hyperlipidemia in terms of elevated low-density lipoprotein and triglyceride as a result of activation of different hepatic pathways, including lipogenesis, cholesterol biosynthesis and secretion of apolipoprotein B-100 [20]. It is reported that a mutation of the LRP6 was found in families with coronary artery disease and normal lipid profile, which led to the dysfunction of the vascular Wnt signaling and thereby endothelial dysfunction [21]. Early-onset of coronary artery disease as a result of atherosclerosis is observed in individuals with low plasma levels of LRP-6 antagonists without explanation, the mechanism of atherosclerosis [22]. Recently, the co-receptors LRP-5/6 has been found to play an important role in the progression rather than induction of atherosclerosis [23]. There is evidence that impairment of the Wnt signaling pathway may contribute to inflammation, foam cell formation, endothelial dysfunction, pathological angiogenesis and arterial calcification, which are an important process in the formation and stability of the plaque [24]. In experimental animal models of surgical myocardial infarction, it has been found that deletion of the LRP5/6 enhances the myocardial ischemic injury [25]. Most studies demonstrate a link between coronary artery disease and the LRP6 were experimental or genetic.

The importance of this study is to use the serum level of LRP6 as a discriminator of the severity of coronary artery disease. The rationale of this study that patients with myocardial ischemia with a positive coronary angiography have progressive coronary atherosclerosis and the possibility of altered signaling Wnt signaling will arise.

Therefore, this study aimed to determine the serum levels of the LRP6 as co-receptor of Wnt signaling in patients referred to the coronary angiogram taking into considerations the findings of coronary angiography, evidence of dyslipidemia, obesity and co-existed diabetes mellitus.

## Methods

### Study design and the Sampling technique

Ninety-six consecutive patients undergoing coronary angiography for evaluation of coronary artery disease were included in this cross-sectional study. The study was conducted from September 2016 to January 2017 at the Baquba Teaching Hospital, Diyala, Iraq.

### Inclusion and exclusion criteria

The criteria of inclusion are known patients with ischemic heart disease, and they treated with a variety of medications that included anti-ischemic, antiplatelet, lipid-lowering agents and antihypertensive medicines. Patients with cardio-metabolic risk factors, including obesity, type 2 diabetes, were also included. Patients with renal failure, chronic liver disease, terminal illness, pregnancy and lactated mothers were excluded.

### Clinical and anthropometric measures

Consultant cardiologists examined each patient prior to the coronary angiogram, and the following information and measurements were collected; the characteristics data;

anthropometric measurements; blood pressure levels, glycated haemoglobin, fasting serum glucose and lipid profile. Then, the patients referred to non-invasive cardiac investigations, including electrocardiography and echocardiography. Coronary angiogram (using Siemens Artis zeego 03792228, USA) was performed, through femoral artery access, under local anaesthesia using 1% lidocaine, and heparin is recommended in certain situations such as patients with bypass grafting or stenotic valve disease. Angiographic coronary artery disease was defined as >50% of diameter stenosis in any of the major epicardial coronary arteries, while non-obstructed diffuse coronary artery disease was defined as involvement of >20 mm segment in a particular epicardial vessel.

### Biochemical analysis

At the time of catheterization of the femoral artery, samples of the blood were drawn, and the sera were separated by centrifugation for determination of LRP6 levels by using the technique of Enzyme-Linked Immunosorbent Assay (ELISA). With respect to the findings of the coronary angiogram, the patients were grouped into Group I (normal coronary angiogram); Group II (A: non-obstructed atherosclerotic lesion and B: obstructed coronary artery).

### Statistical analysis

The results were expressed as a number, percentage and whenever possible as mean  $\pm$  SD. The serum level of LRP6 as a discriminating and dependent variable was statistically assessed using receiving operating characteristics for measuring the area under the curve and 95% confidence interval, and multivariable linear regression with ANOVA test for assessing the independent variables as predictors. An independent two samples t-test for continuous data and Chi-square test for categorical data were used to compare the data of two groups. For all tests, a two-tailed  $p \leq 0.05$  was considered statistically significant. All calculations and diagrams were made using SPSS version 20 programs for Windows.

## Results

### Characteristics of the patients

Ninety-six patients (71 men and 25 women) included in this study. There is no significant difference in the mean age between study groups (Table 1). At the time of the entry into the study, 31.3% of patients had high blood pressure despite using antihypertensive agents, and 38.5% were diabetic (Table 1). Past medical history revealed that 30 out of 96 (31.3%) of patients had a previous history of percutaneous cardiac catheterization intervention. All the patients were on the treatment with statins (of different generations) and antiplatelets in terms of acetylsalicylic acid and clopidogrel.

### Cardiometabolic risk factors

There are significant high serum levels of cholesterol and low serum levels of high-density lipoprotein-cholesterol in Group II (A) compared with Group I (Table 2). Group II (B) patients had a significant low diastolic blood pressure compared with Group I, and they had significantly higher values of fasting triglyceride, blood sugar and glycated haemoglobin, and low systolic blood pressure compared with Group II A (Table 2).

A non-significant higher serum level of LRP6 observed in patients with positive angiogram compared with the negative angiogram. The mean ± SD of serum LRP6 was 0.160 ± 0.019 ng/ml in Group I compared with 0.175 ± 0.074 ng/ml in Group II A and 0.166 ± 0.063 ng/ml in Group IIB (Figure 1). Further statistical analysis using the receiving operating characteristics revealed that the area under the curve of patients having two and three-vessel lesions were below the reference line of 0.5 (Table 3 and Figure 2).

**Multilinear regression test of cardiometabolic risk factors**

Multilinear regression test of cardiometabolic risk factors (as independent factors) and LRP6 (as dependent factor) revealed a non-significant correlation (R=0.246, R<sup>2</sup>=0.061 F=0.86, p=0.523) (Figure 3). The standardized coefficients (β) are -0.137 for body mass index; +0.157 for mean arterial blood pressure; 0.094 for blood glucose; +0.146 for triglyceride; 0.029 for high density lipoprotein-cholesterol; -0.076 for non-high-density lipoprotein -cholesterol.

**Table 1:** Characteristics of the patients

Characteristics	Group I (n=6)	Group II Positive angiogram		Probability
		Group II (A) (n=12)	Group II (B) (n=78)	
Gender (M: F)	1:5	11:1	59:19	*p=0.001, †p=0.002, ‡0.214
Age (year)	58.5±6.5	58.9±9.5	54.0±7.5	*p=0.914, †p=0.152, ‡p=0.107
Smoking				
Current	2	3	18	*p=0.710, †p=0.570, ‡p=0.833
Ex-smoke	0	2	8	*p=0.289, †p=0.410, ‡p=0.511
Family history of CAD	0	0	1	NC
Concomitant illnesses				
Hypertension	1	2	27	*p=1.0, †p=0.369, ‡p=0.215
Diabetes mellitus	2	2	33	*p=423, †p=0.667, ‡p=0.090

The results expressed as number and mean ± SD. Group I: normal angiogram, Group II (A): non-obstructed lesion angiogram and Group II (B): obstructed lesion angiogram. P value was calculated by independent two samples t-test for continuous data and by Chi-square test for categorized data. \* denotes comparison between Group I and Group II (A); † denotes comparison between Group I and Group II (B); ‡ denotes comparison between Group II(A) and Group II (B). M: male, F: female, CAD: coronary artery disease. NC: not calculated because of the zero number.

**Table 2:** Cardiometabolic risk factors

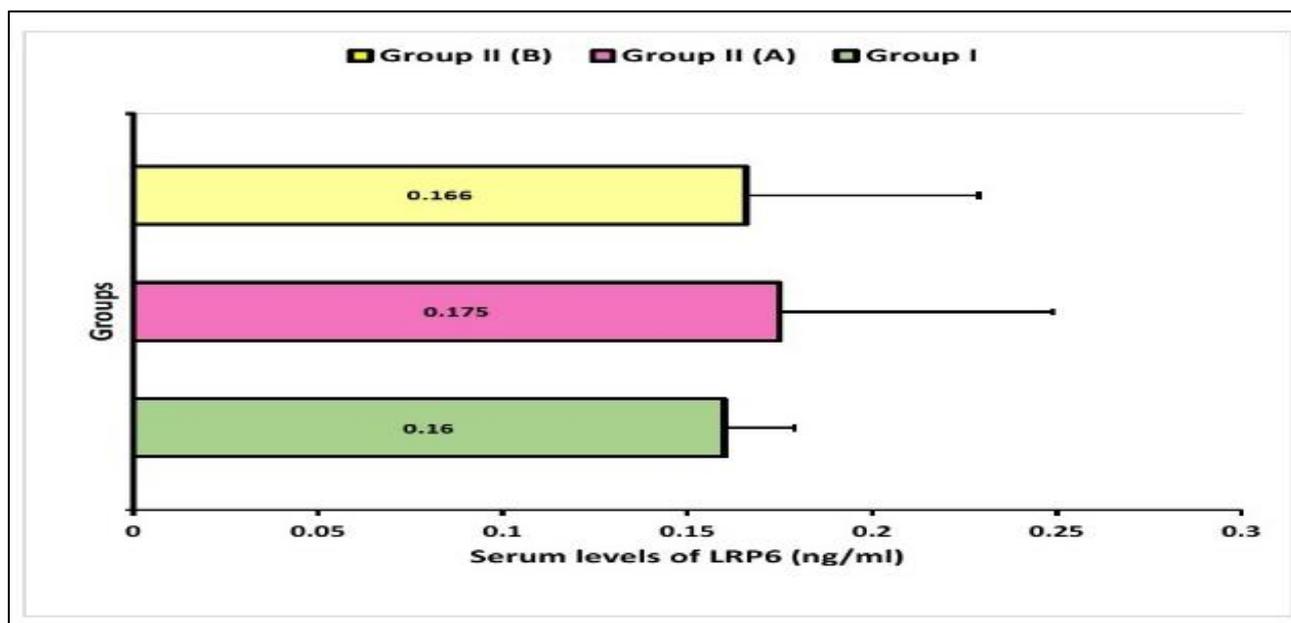
Risk factor	Normal angiogram	Positive angiogram		Probability
	Group I (n=6)	Group II (A) (n=12)	Group II (B) (n=78)	
Body mass index (kg/m <sup>2</sup> )	32.5±5.5	28.2±3.5	29.5±4.1	*p=0.126, †p=0.241, ‡p=0.284
Systolic blood pressure (mmHg)	151.7±26.4	150.5±25.9	139.1±19.5	*p=0.930, †p=0.302, ‡p=0.014
Diastolic blood pressure (mmHg)	91.7±4.1	85.3±9.2	85.4±9.1	*p=0.058, †p=0.010, ‡p=0.956
Mean arterial blood pressure (mmHg)	111.6±10.9	107.0±12.3	103.3±10.4	*p=0.429, †p=0.122, ‡p=0.342
Fasting serum lipid profile (mmol)				
Total cholesterol	4.01±1.39	2.93±0.85	3.49±1.02	*p=0.021, †p=0.510, ‡p=0.065
Triglyceride	1.65±1.56	1.45±1.56	1.77±0.94	*p=0.814, †p=0.889, ‡p=0.039
High density lipoprotein-cholesterol	1.04±0.12	0.81±0.27	0.97±0.29	*p=0.041, †p=0.346, ‡p=0.089
Low density lipoprotein-cholesterol	2.22±0.98	1.46±0.98	1.72±0.80	*p=0.225, †p=0.389, ‡p=0.252
Non-high density lipoprotein-cholesterol	2.97±1.34	2.12±0.65	2.52±0.9	*p=0.298, †p=0.551, ‡p=0.094
Fasting serum glucose (mmol)	6.11±2.44	5.43±1.24	6.57±3.21	*p=0.542, †p=0.067, ‡p=0.031
Glycated hemoglobin (mmol)	36.1±16.8	31.4±8.6	39.5±22.3	*p=0.543, †p=0.656, ‡p=0.027

The results expressed as mean ± SD. P value was calculated by independent two samples t-test for continuous data. Group I: normal angiogram, Group II (A): non-obstructed lesion angiogram and Group II (B): obstructed lesion angiogram. \* denotes comparison between Group I and Group II (A); † denotes comparison between Group I and Group II (B); ‡ denotes comparison between Group II(A) and Group II (B).

**Table 3:** Serum level of low density lipoprotein receptor related protein-6 as a discriminating variable positive angiogram including Group IIA (non-obstructed coronary lesion) and IIB (obstructed coronary lesion).

Coronary blood vessel	Area under the curve	95% confidence interval
Non-obstructed	0.507	0.324-0.690
One vessel obstruction	0.542	0.408-0.676
Two vessels obstruction	0.492	0.354-0.630
Three vessels obstruction	0.462	0.325-0.599

Null hypothesis: true are=0.5. Any value below 0.5 indicates a significant low levels of Serum level of low density lipoprotein receptor related protein-6



**Figure 1** Serum levels of low density lipoprotein receptor related protein-6 (LRP6) according to the result of the coronary artery angiography. Group I: normal angiogram, Group IIA: non-obstructed lesion angiogram and Group IIB: obstructed lesion angiogram. The probability of statistical difference between groups is  $> 0.5$

#### Multilinear regression test of cardiometabolic risk factors

Multilinear regression test of cardiometabolic risk factors (as independent factors) and LRP6 (as dependent factor) revealed a non-significant correlation ( $R=0.246$ ,  $R^2=0.061$ ,  $F=0.86$ ,  $p=0.523$ ) (Figure 3). The standardized coefficients ( $\beta$ ) are -0.137 for body mass index; +0.157 for mean arterial blood pressure; 0.094 for blood glucose; +0.146 for triglyceride; 0.029 for high density lipoprotein-cholesterol; -0.076 for non-high-density lipoprotein -cholesterol.

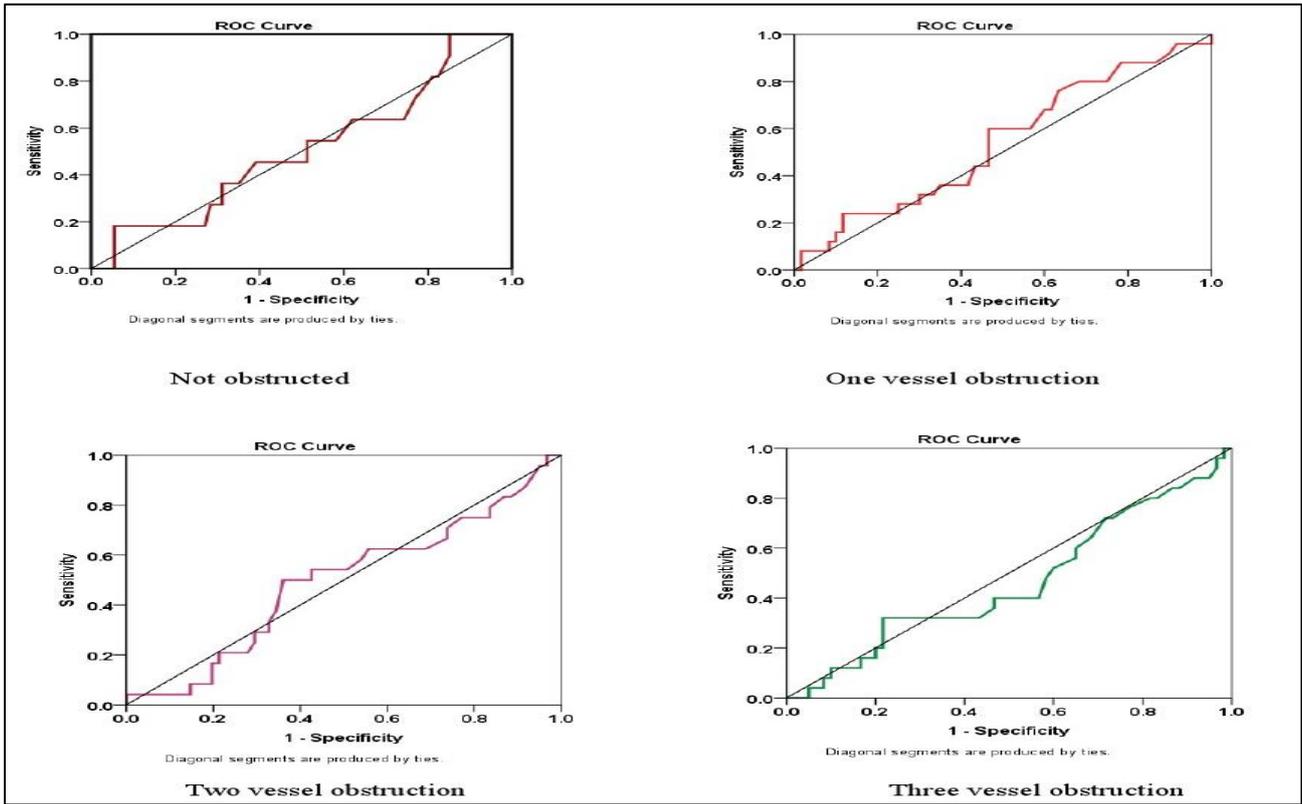
#### Discussion

The results of this study show that serum level of LRP6 is a useful predictor of the severity of coronary artery disease that assessed by a coronary angiogram and this prediction does not relate to the modifiable cardiometabolic risk factors. The changes in the serum LRP6 don't relate to the age factor, as there are no significant differences in the age between the studied groups [26]. The discrepancy in the results of the modifiable risk factors, including the blood pressure, fasting serum lipid profile and glucose due to the medications used by the patients. However, alteration in the LRP6 levels can occur in familial coronary artery disease with normal lipid profile levels and may be due to gene mutation of Wnt signaling pathway [17]. There is no doubt that the serum LRP6 is a predictor of diabetes mellitus, but its level in diabetic patients did not differ from control subjects [16,27]. Therefore, our results that showed the serum level of LRP6 is not correlated and predicted the non-modifiable factors (Figure 3) are in agreement with others as our patients have abnormal values of modifiable factors and they were on the treatment. It is important to emphasize here that identification of the mutation of the LRP6 is not carried on in this study, and it is possible that there is a mutation of LRP6 among the participants, and this speculation reflected on the non-significant correlations that

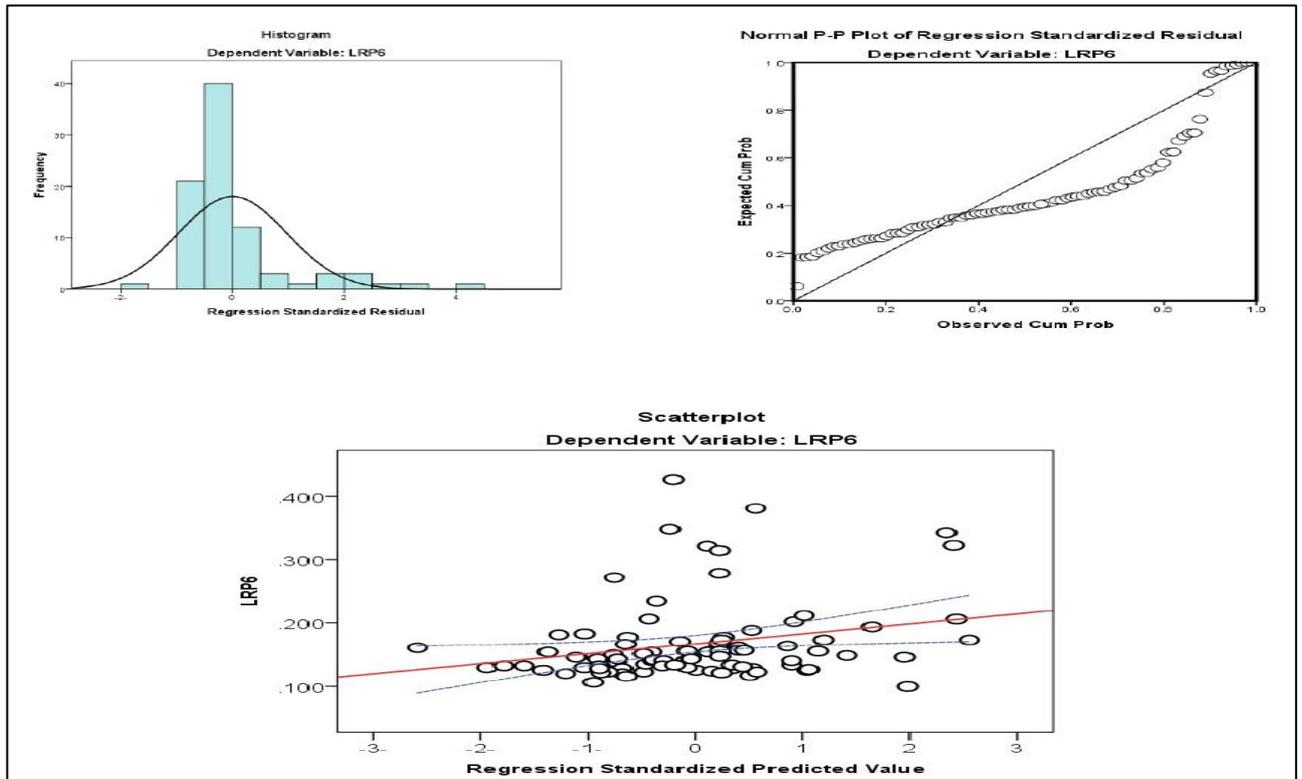
illustrated in figure 3. Moreover, malfunction of LRP6 did not produce specific abnormalities in the lipid profile, and as mentioned before, it induced combined hyperlipidemia [20]. The interesting finding in this study is the association between the serum levels of LRP6 and the number of obstructed coronary vessels (Table 3 and Figure 2). The area under the curve of LRP6 tended to be less than 0.5, which indicated that patients with two-three coronary artery obstruction have low serum levels. Cheng et al. found that LRP6 promotes the osteochondrogenic cell differentiation and it limits the arteriosclerosis calcification in an experimental animal model, that is, a decrease in the LRP6 levels plays a role in the reducing the vascular smooth muscle lineage [28]. Another experimental study showed that when the activity of LRP6 is reduced, the medial hyperplasia of the artery occurs due to the loss of vascular muscle differentiation [29]. The possible explanation of the loss of the LRP6 activity and the coronary artery disease in human is due to the mutation of the LRP6 gene [30,31]. Polymorphism of the LRP6 gene was also associated with a sporadic coronary artery disease [32]. One of the limitations of the study is the small sample size of Group I and Group II A because the design of this study was depended on the findings of a coronary angiogram. The other limitation of the study is the existence of cardio-metabolic risk factors which cannot be avoided because it is hard to recruit ischemic heart disease patients without risk factors and it is unethical to do a coronary angiogram to healthy subjects.

#### Conclusion

We conclude that serum levels of LRP6 are a good discriminator of patients with coronary artery disease as the area under the curve of the serum levels of LRP6 is significantly decreased as the number of occluded coronary vessels increased.



**Figure 2** Area of the under the curve of the serum levels of low density lipoprotein receptor related protein-6 (LRP6) according to the angiograph findings characterized by the number of the obstructed coronary artery.



**Figure 3** Multivariable liner regression plot taking the serum level of LRP6 as dependent factor and the cardiometabolic risk factors; body mass index, mean arterial blood pressure, blood glucose, triglyceride, high density and non-high density lipoproteins as independent factors. The serum cholesterol variable is excluded by the test because of non-linearity.

**Abbreviations**

Wnt: Wingless/integrated LRP6: Human lipoprotein receptor-related protein -6 ELISA: Enzyme-Linked ImmunoSorbent Assay

**Declarations****Acknowledgment**

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**Availability of data and materials**

Data will be available by emailing alnimermarwan@gmail.com

**Authors' contributions**

AHA Concepts, Design, Clinical studies, data acquisition, manuscript review; ZJK Clinical studies, data acquisition, manuscript review; MSMA Definition of intellectual content, literature search, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review, guarantor.

**Ethics approval and consent to participate**

We conducted the research following the Declaration of Helsinki, and the protocol was approved by the Ethic Committee, Faculty of Medicine, University of Diyala, Iraq (Ref: 03/16817/03.December.2017). Confidentiality was assured with signed informed consent.

**Consent for publication**

Not applicable

**Competing interest**

The authors declare that they have no competing interests.

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