

# Correlation Between the Estimated and the Measured Serum Apolipoprotein –B100 in Kurd Subjects: A Trend to Establish a New Formula

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## ABSTRACT

Apolipoprotein B100 (ApoB100) provides a good assessment of atherogenic lipoproteins (very low, intermediate, and low-density lipoproteins (VLDL, IDL, and LDL)). There is evidence that polymorphism of ApoB100 was observed in many conditions and it links with obesity, diabetes mellitus hypertension, and chronic inflammation, which could be related to the broad field of the atherothrombotic process, and could be one of the leading causes of coronary artery disease (CAD). It can be computed using a formula that makes use of a measurement of non-high-density lipoprotein levels. This study aimed to derive an estimated equation of ApoB100 from the measured ApoB100 levels specific to the healthy subjects of Kurd race/ethnicity in the Kurdistan region of Iraq taking into consideration the gender-based effect and the status of fasting and postprandial effects. A total number of 45 healthy subjects (23 males and 22 females) were enrolled in the study. The following measurements were achieved: anthropometric indices, blood pressure, lipid profile, including ApoB100, and blood sugar. The biochemical measurements were carried out at fasting and postprandial states. Specific equations were derived for calculating the levels of ApoB100. Significant differences in the anthropometric indices, blood pressure, and lipid profile were observed between males and females. The calculated ApoB100 levels were significantly less than the measured ApoB100 levels in both genders and fasting and postprandial states. The estimated equations for ApoB100 for females have differed from that for males at fasting and postprandial states. The levels of ApoB100 can be determined instead of measuring it in the laboratory by using a specific equation for healthy Kurd people. These equations are gender and race/ethnicity based. The established equation of estimated ApoB100 levels in males differed from that in females which is attributed to the cardio-metabolic factors and higher levels of systolic and mean arterial blood pressures among males. Apo B level is a quantitative index of plasma atherogenic lipids in hypertensive patients who presented with dyslipidemia.

**KEYWORDS:** Apolipoprotein B100; gender; kurd ethnicity; estimated equation.

## الخلاصة

يوفر Apolipoprotein B100 (ApoB100) تقييماً جيداً للبروتينات الدهنية المسببة للتصلب العصيدي (البروتينات الدهنية المنخفضة جداً والمتوسطة ومنخفضة الكثافة (VLDL و IDL و LDL) هناك دليل على أن تعدد الأشكال لـ ApoB100 قد لوحظ في العديد من الحالات. فهو يرتبط بالسمنة ومرض السكري وارتفاع ضغط الدم، والالتهاب المزمن الذي يمكن أن يكون مرتبطاً بالمجال الواسع لعملية التخثر العصيدي ويمكن أن يكون أحد الأسباب الرئيسية لمرض الشريان التاجي (CAD). يمكن حسابه باستخدام صيغة تستخدم قياس غير مرتفع - مستويات كثافة البروتين الدهني. هدفت هذه الدراسة إلى استنباط معادلة تقديرية لـ ApoB100 من مستويات ApoB100 المقاسة الخاصة بالمواضيع الصحية من العرق في المناطق الكردية/الإثنية في إقليم كردستان العراق مع الأخذ في الاعتبار التأثير الجنساني وحالة الصيام وآثار ما بعد الأكل: تم تسجيل 45 من الأصحاء (23 ذكور و 22 إناث) وتم تحقيق القياسات التالية: المؤشرات وضغط الدم وملف الدهون بما في ذلك ApoB100 وسكر الدم. تم إجراء القياسات البيوكيميائية في حالات الصيام وما بعد الأكل. تم اشتقاق معادلات محددة لحساب مستويات ApoB100. لوحظت فروق معنوية في مؤشرات القياسات البشرية وضغط الدم ونسبة الدهون بين الذكور والإناث. كانت مستويات ApoB100 المحسوبة أقل بكثير من مستويات ApoB100 المقاسة في كلا الجنسين وحالات الصيام وما بعد الأكل. اختلفت المعادلات المقترحة لـ ApoB100 للإناث عن تلك الخاصة بالذكور في حالات الصيام وما بعد الأكل. يمكن تحديد مستويات

ApoB100 بدلاً من قياسها في المختبر باستخدام معادلة محددة للأشخاص الأكراد الأصحاء. هذه المعادلات تعتمد على الجنس والعرق / الإثنية. اختلفت المعادلة الموضوعية لمستويات ApoB100 المقدر في الذكور عنها في الإناث والتي تُعزى إلى عوامل التمثيل الغذائي للقلب والمستويات الأعلى من ضغط الدم الانقباضي والمتوسط لدى الذكور. مستوى Apo B هو مؤشر كمي للدهون المسببة لتصلب الشرايين في البلازما لدى مرضى ارتفاع ضغط الدم الذين يعانون من خلل شحميات الدم.

## INTRODUCTION

Apolipoprotein B100 (apoB100) molecule is present in atherogenic particles (very low, intermediate, and low-density lipoproteins (VLDL, IDL, and LDL). Small dense LDL-c is dependent on metabolic risk factors related to obesity, hepatic overproduction of a prob containing lipoproteins, and postprandial hyper-lipidaemia with an accumulation of atherogenic remnants [1]. Therefore, the measurement of ApoB100 provides a good assessment of atherogenic lipoproteins [2,3]. Assessment of non-fasting plasma lipid is routinely recommended because the differences between fasting and postprandial levels are not clinically significant [4]. However, apolipoprotein B levels or LDL particle numbers are significantly better than non-high-density lipoprotein (non-HDL) [5]. Therefore, the levels of ApoB100 can be calculated by using a specific formula. Hermans et al. established an equation to determine the levels of ApoB100 in the plasma through the determination of non-HDL levels [6]. This equation was derived by studying the fasting lipid profile of North-Caucasian subjects with diabetes mellitus. Therefore, this equation shows limitations with respect to race, gender, fasting or postprandial status, diabetes and the procedure of measurement of ApoB100. There is evidence that polymorphism of ApoB100 was observed in many conditions and it links with obesity, diabetes mellitus hypertension [7-9] and chronic inflammation, 10 which could be related to the broad field of the atherothrombotic process, could be one of the leading causes of coronary artery disease (CAD) [11]. similar et al reported that the levels of apolipoproteins showed significant variation according to the race/ethnicity factor and they were independently related to the cardio-metabolic risk factors [12]. The rationale of this study is that the Kurd race/ethnicity may have a variation in the levels of apolipoprotein compared with the reference value and the determination of the ApoB100 is not necessary for the clinical practice. Therefore, this study aimed to derive an estimated equation of apoB100 from the measured apoB100 levels specific to the healthy subjects of Kurd race/ethnicity in the Kurdistan region of Iraq

taking into consideration the gender-based effect and the status of fasting and postprandial effects.

## MATERIALS AND METHODS

This study is an analytical observational, which conducted in the Department of Clinical Analysis, College of Pharmacy at Hawler Medical University, and The Department of Pharmacy at Suleimani Technical Institute, Kurdistan Region-Iraq, from September 2020 to January 2021. The subjects were randomly recruited from the employee working in different institutes (including colleges, technical institutes and the proxy of patients who attended the hospital). The criteria of inclusion were healthy subjects, and they did not on any medical therapy. The criteria of exclusion were any disease related to the endocrine, cardiovascular, renal, and hepatic systems as well as pregnancy and lactated nursing. A total number of 45 subjects (23 males and 22 females) completed the study. All participants were examined and the following clinical and laboratory measurements were carried on.

### Procedure

Anthropometric measurements were determined, including weight, height, waist circumference and hip circumference. Weight was measured with a personal scale balance (CAMRY®- China: Capacity: 150 Kg/Graduations: 1.000 g) after the participants were asked to remove their shoes and jacket. Two kilograms of weight were subtracted for wearing clothes, and height was measured to the nearest 0.1 cm. Waist circumference was measured with a constant metallic tape to the nearest 0.1 cm midway between the lower rib margin and the upper iliac spine, which in most instances was at the level of the umbilicus. Hip circumference was measured at the tip of the trochanter femoris. The body mass index (BMI) was calculated by using the following equation:  $BMI = \text{weight (kg)} / \text{height (m)}^3$  Waist-to-hip or to-height ratios were calculated simply by dividing the waist circumference (cm) by the hip (cm) or to the height (cm). The blood pressure was measured from the left arm while subjects remained in a seated position after 15 min of rest with the Digital Blood Pressure Monitor (Cuff Size: 13.5 - 19.5 cm, Measuring Range: 0-

300 mmHg (CITIZEN SYSTEMS JAPAN CO., LTD/ Model No.: CH-618)) at the same level as the heart. The mean arterial blood pressure was calculated by using the following equation:

Mean arterial blood pressure (mmHg)=Diastolic blood pressure +  $\frac{1}{3}$  (systolic blood pressure minus diastolic blood pressure).

Then blood samples for biochemical tests were collected in the fasting state and 2 hours after a meal (which represented the postprandial state). Venous blood was sampled from the antecubital vein and collected into plain tubes, and the sera were separated by centrifugation at 3000 rpm for 20 minutes. The serum samples were frozen at  $-20^{\circ}$  C for further biochemical analysis (within two weeks of collection). The following biochemical tests were measured; serum glucose, total cholesterol, triglycerides, high-density and low-density lipoproteins, apolipoprotein-B100 and uric acid. All the biochemical measurements were done by using specific kits (purchased from Roche Diagnostics GmbH D-68298, Mannheim, Germany) and utilizing the Cobas e 411 analyzers (the manufacturer: Roche Diagnostics GmbH D-68298, Mannheim, Germany).

The calculated apolipoprotein B-100 (mg/dl) was determined by using the following equation:  $(0.65 \times \text{non-HDL-C}[\text{mg/dl}] + 6.3 \text{ mg/dl})$  based on fasting or non-fasting lipids [5].

### Statistical analysis

The results are expressed as means $\pm$ SDs. The data were analyzed using two-tailed, paired and two independent samples Student's t-test and simple correlation (Pearson's) test taking the probability (p-value) of  $\leq 0.05$  as the lowest limit of significance. An equation of regression ( $y=a+bx$ ) is used to interpret the correlation between measured and calculated values of apolipoprotein-B100. An integrated equation of summation of regression equations of fasting and postprandial apolipoprotein-B100 was calculated to express the value of apolipoprotein-B100 of the fasting or postprandial lipid values.

## RESULTS AND DISCUSSION

The anthropometric measurements of the subjects enrolled in the study are shown in table 1. Significant differences were observed concerning the gender factor. Males had significantly higher values of age, weight, height, waist circumference

and waist-to-hip ratio than the corresponding values of females. Significant high systolic and mean arterial blood pressures were observed among males compared with corresponding levels in females (Table 1). Table 2 showed the results of the determinants of glucose, lipid profile and uric acid with respect to the fasting state and gender. Significant differences between fasting and postprandial levels of serum glucose and lipid profile were observed in both genders. The serum levels of apolipoprotein B-100 and uric acid did not show significant changes between fasting and postprandial state in both genders (Table 2). Table 3 showed a non-significant difference between the levels of the measured and calculated serum apolipoprotein-B100 at the fasting and postprandial states in both genders. The calculated apolipoprotein-B100 levels attended low levels compared with the measured apolipoprotein-B100 levels. Figure 1 showed strong positive significant correlations between the measured and the calculated serum apolipoprotein-B100 levels in the fasting state ( $r=0.947$ ,  $p<0.001$ ) and postprandial state ( $r=0.876$ ,  $p<0.001$ ) in females.

The regression equation of the fasting state is:

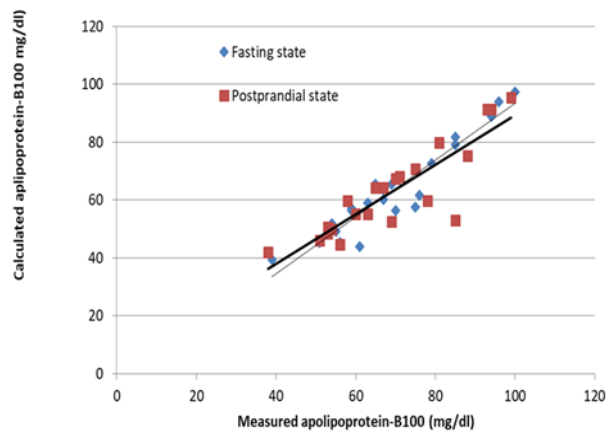
Calculated apolipoprotein-B100 level =  $(0.855 \times \text{measured apolipoprotein-B100 level})$  minus 4.944.

The regression equation of the postprandial state is:

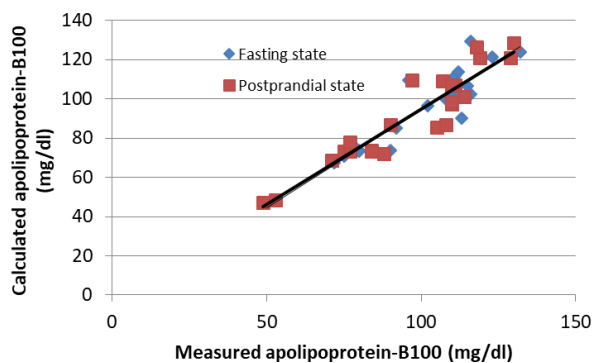
Calculated apolipoprotein-B100 level =  $(0.984 \times \text{measured apolipoprotein-B100 level})$  plus 3.832

By summation of the above equations, the calculated apolipoprotein-B100 at fasting or postprandial is equal to:  $(0.920 \times \text{measured apolipoprotein-B100 level})$  minus 1.112.

Figure 2 showed significant correlations between the measured and the calculated serum apolipoprotein-B100 levels at the fasting state ( $r=0.937$ ,  $p<0.001$ ) and postprandial state ( $r=0.935$ ,  $p<0.001$ ) in males.



**Figure 1.** Correlation between measured and calculated apolipoprotein-B100 in females at fasting and postprandial states.



**Figure 2.** Correlation between measured and calculated apolipoprotein-B100 in males at fasting and postprandial states.

The regression equation ( $Y$  (dependent variable) =  $a + bX$  ( $X$  = independent variable)) of fasting state Calculated apolipoprotein-B100 level =  $(1.001 \times \text{measured apolipoprotein-B100 level}) - 5.592$ . The regression equation of the postprandial state is: Calculated apolipoprotein-B100 level =  $(0.973 \times \text{measured apolipoprotein-B100 level}) - 2.727$ . By summation the above equations, the calculated apolipoprotein-B100 at fasting or postprandial is

equal to:  $(0.987 \times \text{measured apolipoprotein-B100 level}) - 4.160$ .

The results of this study have shown that the established estimated equations of ApoB100 for healthy Kurds have differed from those derived on the other population and the lipid profile values have shown significant differences between fasting and postprandial states, which resembles the results of previous studies [13]. The established equation of estimated ApoB100 levels in males differed from that in females and this may be attributed to the cardio-metabolic factors. In Table 1, males have significantly higher values of weight, waist circumference and waist-to-hip ratio and all these values were within normal reference values. In one study conducted in Italy, the levels of ApoB100 among Roma women (but not among non-Roma women) were found to be an independent predictor of metabolic syndrome [14]. Moreover, central obesity and higher serum poB100 levels were considered predictors of dyslipidemia (particularly hypercholesterolemia that will occur in the future [15] Significantly higher levels of systolic and mean arterial blood pressures among males may also be the reason for the difference in the estimated equations of ApoB100 level between males and females, this was also reported by another study [16]. In young individuals the serum levels of ApoB100 did not relate to the levels of arterial blood pressure but it's associated with familial cardiovascular events in white girls [17]. Another contributing factor associated with the alteration of ApoB100 is dyslipidemia. The levels of atherogenic lipids are higher among males and at the postprandial state (Table 2).

**Table 1. Anthropometric measurements distribution with respect to gender.**

Characteristics	Female (n=22)	Male (n=23)	P value
Age (year)	37.1±6.0	42.1±9.9	0.028
Weight (kg)	58.7±7.3	70.0±6.8	<0.001
Height (m)	1.63±6.3	1.76±6.7	<0.001
<b>Body mass index (kg/m<sup>2</sup>)</b>	22.1±2.2	22.5±1.7	0.464
Waist circumference (cm)	76.7±8.1	86.3±8.4	<0.001
Hip circumference (cm)	99.3±5.8	100.2±4.3	0.547
Waist to hip ratio	0.77±0.06	0.86±0.08	<0.001
Waist to height ratio	0.471±0.048	0.490±0.048	0.182
Systolic blood pressure	109.7±12.2	126.2±19.7	0.002
Diastolic blood pressure	74.9±6.8	76.7±8.8	0.447
Mean blood pressure	86.5±7.6	93.1±11.2	0.025

The results are expressed as mean ± SD.

**Table 2. Serum levels of bio-metabolic determinants in respect to both genders' fasting and postprandial state.**

Variables	Female (n=22)			Male (n=23)		
	Fasting	Postprandial	P value	Fasting	Postprandial	P value
Glucose (mg/dl)	90.3±5.0	90.0±13.7	0.009	94.9±7.7	98.0±12.3	0.476
TC (mg/dl)	138.1±26.1	134.7±23.8	0.005	171.2±30.8	169.5±30.6	0.230
TG (mg/dl)	65.5±26.7	96.8±40.5	<0.001	126.1±95.1	177.3±34.4	0.002
HDL-c (mg/dl)	50.5±16.5	47.6±16.6	<0.001	40.7±12.5	39.1±12.9	<0.001
LDL-c(mg/dl)	72.9±20.3	69.0±18.8	<0.001	104.1±26.4	99.7±24.8	<0.001
Apolipoprotein B100 (mg/dl)	69.3±15.7	69.1±16.1	0.797	96.7±21.8	96.3±22.3	0.754
Uric acid (mg/dl)	3.64±0.93	3.67±1.0	0.678	5.76±1.04	5.7±1.1	0.601

The results are expressed as mean ± SD.

**Table 3. Comparison between the measured and the calculated apolipoprotein- B100 at fasting and postprandial states in both genders.**

Variables	Female (n=22)		Male (n=23)	
	Fasting	Postprandial	Fasting	Postprandial
Apolipoprotein B100(measured)	69.3±15.7	69.1±16.1	96.7±21.8	96.3±22.3
Apolipoprotein B100 (calculated)	63.2±16.3	62.9±15.7	91.1±23.2	91.0±23.0
	(p<0.001)	(p=0.003)	(p=0.001)	(p=0.005)

The results are expressed as mean ± SD.

Serban *et al.* found that the levels of Apo B reflected the atherogenic lipids and suggested that Apo B level is a quantitative index of plasma atherogenic lipids in hypertensive patients who presented with dyslipidemia [18]. The postprandial levels of ApoB100 are significantly altered than the corresponding values of fasting levels in both genders. This finding is in agreement with an Otokozawa et al study who found that postprandial levels of ApoB-48 were increased by more than 100% of the fasting state levels in healthy subjects [19]. The best fit lines of the correlations between estimated and the measured levels of ApoB100 in both genders make the results of this study reliable and the derived equations can be used to calculate the ApoB100 instead of measuring it in the healthy Kurd population. In such a study, a small sample size is considered a limitation.

## CONCLUSIONS

It concludes that the levels of ApoB100 can be determined instead of measuring it in the laboratory by using a specific equation for healthy Kurd people. These equations are gender and race/ethnicity-based. The established equation of estimated ApoB100 levels in males differed from that in females which is attributed to the cardio-metabolic factors and higher levels of systolic and mean arterial blood pressures among males. Apo B level is a quantitative index of plasma atherogenic lipids in hypertensive patients who presented with

dyslipidemia.

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