Multiple linear regression model analysis in predicting fasting blood glucose level in healthy subjects

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Abstract. Diabetes mellitus referred to inability to produce or respond to hormone insulin resulted in elevated blood glucose level in human body. The purpose of this study was to investigate the relationship between fasting blood glucose, cholesterol and blood pressure levels in healthy subjects. 211 subjects having age between 23-66 years old were randomly selected among UMP's residents from April 2017 to May 2018. Mann-Whitney Ranksum test determine the significant differences between overall and diabetics subjects. Pearson Correlation compute the associations between fasting blood glucose, lipid profile substances and blood pressure. Linear regression analysis verified the relationship between fasting blood glucose and other parameters, with 95%CI. Fasting blood glucose are significantly difference (p<0.05) with blood pressure and others lipid profile substances except for total cholesterol. All lipid profile substances are significantly difference (p<0.05) with blood pressure level. There is 59%(R²-value) chances in getting correct prediction of diabetes using high density lipo-protein cholesterol, low density lipo-protein cholesterol, triglyceride, systolic blood pressure and triglyceride based on fasting blood glucose value. However, a larger and well-spread cohort with different backgrounds and demographics however is required to validate the finding of this study.

1. Introduction
Diabetes mellitus (DM) is a global issues having 3.8 billion of people diagnosed in 2013 and this number are expected to increase up to 5.9 billion people in 2035[1]. Type 2 DM (T2DM) is a metabolic disorder that occurs due to insulin resistance and/or inability to produce enough insulin from islet β cells in pancreas. Commonly, T2DM is related with the increased blood glucose (BG) level which caused impaired fat metabolism[2].

One of the methods in diagnosing DM is by measuring fasting blood glucose (FBG). Fasting is defined as no caloric intake for at least past 8 hours. FBG level are easily measured, cost effective and save time compared to glycosylated haemoglobin (HbA1C) and two-hours plasma glucose (PG). As HbA1c are too expensive and more related to protein than glucose. Meanwhile, PG are time-consuming even it has better sensitivity towards BG level[3, 4]. FBG level has been used as the most effective early detection and prevention towards DM and are more compatible towards latest demands. The diagnostic
value are as follows: impaired fasting glucose (IFG) also known as pre-diabetes, FBG level is between 5.6-6.9 mmol/L; DM, FBG is more/equal to 7.0 mmol/L. Individuals with pre-diabetes are at high risk of developing DM and its complications[5].

DM is a major health problem worldwide and a well-known risk factor for cardiovascular disease (CVD). CVD also known as heart and blood vessels disease that is related to atherosclerosis, a condition where arteries wall narrower by builds up of bad cholesterol, making it harder for blood flow, thus leads to blood clots that can cause heart attack and stroke. Total cholesterol (TC) is the summation of all cholesterol present in human body consists of good cholesterol which is high density lipoprotein cholesterol (HDL) and bad cholesterol which is low density lipoprotein cholesterol (LDLC). HDLC having function to eliminate bad cholesterol that accumulate and narrowing artery wall[6].

According to NCEP ATP-III, for a normal lipid profile, a person should have TC<5.2 mmol/L; HDLC>1.0 mmol/L; non HDLC<3.4 mmol/L; LDLC<2.6 mmol/L; triglycerides,TG<1.7 mmol/L and/or cholesterol/HDL ratio<4.6 mmol/L. Increased TC, LDLC and TG levels along with decreased HDLC level are associated with major risk factor for CVD. This condition of lipid profile described dyslipidemia. Dyslipidemia was found increases the risk in diabetic patients known as diabetic dyslipidemia[6-9].

In addition, hypertension (HTN) is a well-known risk factor of CVD, DM, stroke and renal disease. According to Framingham study, subjects with systolic blood pressure, SBP≥130mmHg and/or diastolic blood pressure, DBP≥85mmHg classified as HTN[10]. HTN also are closely related to lipid profile as increase blood pressure (BP) resulting an increase in lipid profile and vice versa [11]. Closely related, DM is also a risk factor of CVD as DM has tendency to have low HDLC, high TG and high LDLC level[12-15]. Thus, DM associated with high BP as abnormal lipid profile leads to the increased BP level.

Previous research has been reported that FBG are correlates with heart rate variability (HRV), waist circumferences (WC), DM, HTN and lipid profile. Even so, only DM, HTN and lipid profile will be highlighted in this study[16-20]. With this background, dyslipidemia along with raised in BG and BP does correlates and links as a risk factors for CVD. High BP is also a risk factor for DM. Bur et al. has suggested that FBG together with HbA1C is an alternative to detect DM in HTN[20]. Contradict, Kuwabara et al. found that high FBG is an independent risk to develop HTN in Japanese population[16]. Thus, this study was conducted to measure the blood cholesterol and BP levels among normal and diabetic; and to determine the correlation between blood cholesterol and BP levels with FBG level for normal and diabetic. Also to investigate the blood cholesterol and BP levels that can predict the FBG level and how can these predictor parameters be used to aid the prevention of DM disease?

2. Methods
This research study was conducted at University Health Centre, University Malaysia Pahang (UMP), Malaysia from April 1, 2017 to May 30, 2018. Informed written consent was obtained after a brief explanation on the blood test procedure and the objective of the study to the volunteers. Ethical clearance for this study was obtained.

2.1. Subjects
A total of 211 healthy volunteers which have not been diagnosed with DM or any disease related to glucose metabolism; 120 males and 91 females; aged between 23-66 years old have been recruited randomly for this study. Volunteers recruited were the UMP’s staffs and students from both Gambang and Pekan campus. From 211 volunteers, 69 were suspected to have IFG and DM (diabetic) after the blood test analyses whereas 142 with normal FBG (non-diabetic), classified by American Diabetic Association (ADA) criteria[21]. Those who on medication that affects glucose metabolism, lipid lowering and antihypertensive within duration of 3 months were excluded[7, 22]. Those with gestation, lactation and serious cardiovascular disease (CVD) were also excluded[23].
2.2. Data collection

Figure 1 shows the flow process of data collection. All 211 volunteers were instructed to fast overnight before their taking blood test on the next morning. The fasting conditions require the volunteers to avoid consumption of caffeine and sugary water, no use of tobacco, no medication and no anti-diabetic medications[24-27]. After obtaining volunteers' consent, body mass index (BMI) and blood pressure (BP) was measured. Prior study found that 75% of spontaneous fall in BP occurred within 10 minutes. Thus, BP was measured after volunteers has rested for 10 minutes to ensure resting/normal BP[7] and increase the accuracy of the measurement. According to the International Diabetes Federation (IDF), HTN was defined as SBP≥130 mmHg and DBP≥85 mmHg[10]. Mean value from two measurements was used for analysis. Then, 7ml of vein's blood was drawn for FBG and lipid profile measurements. All vein blood samples were collected by University Health Centre University Malaysia Pahang clinical staffs, then the samples were analysed by Pantai Premier Pathology laboratory.

2.3. Data statistical analysis

Based on Figure 2, the process flow is shown to discover the relationship between blood cholesterol and BP levels with FBG level. Statistical analysis was performed by using MATLAB R2016a™ software. The first step in data statistical analysis is computing Mann-Whitney Ranksum Test. This test is to determine the significant differences (p-value) between group (diabetic and non-diabetic) and parameters (blood cholesterol and BP levels with FBG level). Then, the associations between parameters are computed by using Pearson correlation method, to obtain $R^2$-value. Next, linear regression analysis is used to verify the relationship between FBG and other parameters, with 95% confidence interval (CI). A p-value<0.05 was considered as statistically significance.
3. Results
TC is the summation of all cholesterol present in our body as shown in equation (1) and (2). LDLC known as bad cholesterol is a cholesterol that accumulates and narrowing human blood arteries. Meanwhile, HDLC is a good cholesterol that eliminates the bad cholesterol[28].

\[
TC = HDLC + \text{NonHDLC} \tag{1}
\]
\[
TC = HDLC + LDLC + \text{other substance (remain unknown)} \tag{2}
\]

Table 1 shows the clinical and demographic characteristics of overall, diabetic and non-diabetic subjects.

| Parameter          | Overall (n=211) | Diabetic (n=69) | Non-diabetic (n=142) | P-value    |
|--------------------|-----------------|-----------------|----------------------|------------|
| FBG\(^a\) (mmol/L) | 5.37 ± 1.03     | 6.47 ± 1.09     | 4.84 ± 0.39          | <0.05**    |
| TC\(^b\) (mmol/L)  | 5.19 ± 0.95     | 5.04 ± 1.00     | 5.26 ± 0.92          | >0.05      |
| HDLC\(^c\) (mmol/L)| 1.27 ± 0.27     | 1.00 ± 0.14     | 3.85 ± 0.95          | <0.05**    |
| Non HDLC (mmol/L)  | 3.92 ± 0.96     | 4.04 ± 0.96     | 1.40 ± 0.22          | >0.05      |
| LDLC\(^d\) (mmol/L)| 3.32 ± 0.91     | 3.22 ± 0.96     | 3.38 ± 0.89          | >0.05      |
| TG\(^e\) (mmol/L)  | 1.34 ± 0.88     | 1.91 ± 1.24     | 1.07 ± 0.41          | <0.05**    |
| SBP\(^f\) (mmHg)   | 128.69 ± 14.57  | 133.30 ± 15.65  | 126.44 ± 13.51       | <0.05**    |
| DBP\(^g\) (mmHg)   | 81.26 ± 12.08   | 84.41 ± 13.57   | 79.73 ± 11.01        | <0.05**    |
| Age                | 41.91 ± 7.39    | 43.97 ± 6.69    | 40.92 ± 7.53         | <0.05**    |
| BMI\(^h\) (kg/m\(^2\)) | 26.43 ± 5.02 | 28.26 ± 4.34 | 25.73 ± 4.62 | <0.05** |
| Sex (Male/Female)  | 120 / 91        | 45/24           | 75/67                |            |

**Statistical significance attributed to results with p < 0.05.

\(^a\)Fasting Blood Glucose
\(^b\)Total Cholesterol
\(^c\)High Density Lipoprotein
\(^d\)Low Density Lipoprotein
\(^e\)Triglyceride
\(^f\)Systolic Blood Pressure
\(^g\)Diastolic Blood Pressure

Table 1 shows the mean and standard deviation of the overall volunteers were compared and the association for each cholesterol and BP levels between diabetic and normal group were identified. In general, most of subjects having normal levels of FBG, TC, HDLC, TG, SBP, DBP however non HDLC, LDLC and BMI levels are above recommended value. HDLC level for diabetic is lower than non-diabetic (p<0.05). Age (p<0.05), TG (p<0.05), SBP (p<0.05), DBP (p<0.05) and BMI (p<0.05) levels were found higher in diabetic compared to non-diabetic subjects. The average BMI for diabetic subjects is 28 which classified as pre-obese. TC, non HDLC and LDLC were not significantly different between diabetic and non-diabetic subjects.
Correlation analysis for clinical parameters were summarized and tabulated in Table 2.

| Parameter | TC      | HDLC    | Non HDLC | LDLC    | TG      | SBP     | DBP     |
|-----------|---------|---------|----------|---------|---------|---------|---------|
| FBG       | -0.1722h| -0.7193h| 0.0338h  | -0.1131h| 0.4006h | 0.3563h | 0.2928h |
|           | <0.05*  | <0.05***| <0.05*** | <0.05***| <0.05***| <0.05***| <0.05***|
| TC        | 0.1278h | 0.9568h | 0.9293h  | 0.2367h | 0.0644h | 0.0972h |
|           | <0.05***| <0.05***| <0.05*** | <0.05***| <0.05***| <0.05***| <0.05***|
| HDLC      | -0.1570h| -0.0223h| -0.3867h | -0.2159h| -0.1971h|         |
|           | <0.05***| <0.05***| <0.05*** | <0.05***| <0.05***| <0.05***| <0.05***|
| Non HDLC  | 0.9293h | 0.3494h | 0.1235h  | 0.1516h |
|           | <0.05***| <0.05***| <0.05*** | <0.05***| <0.05***| <0.05***| <0.05***|
| LDLC      | 0.0424h | 0.0495h | 0.0777h  |
|           | <0.05***| <0.05***| <0.05*** |
| TG        | 0.1908h | 0.1825h |
|           | <0.05***| <0.05***| <0.05*** |
| SBP       | 0.8238h |
|           | <0.05***|

Pearson coefficient correlation. **Statistical significance attributed to results with p < 0.05.

Table 1 shows the mean and standard deviation of the overall volunteers were compared and the association for each cholesterol and BP levels between diabetic and normal group were identified. In general, most of subjects having normal levels of FBG, TC, HDLC, TG, SBP, DBP however non HDLC, LDLC and BMI levels are above recommended value. HDLC level for diabetic is lower than non-diabetic (p<0.05). Age (p<0.05), TG (p<0.05), SBP (p<0.05), DBP (p<0.05) and BMI (p<0.05) levels were found higher in diabetic compared to non-diabetic subjects. The average BMI for diabetic subjects is 28 which classified as pre-obese. TC, non HDLC and LDLC were not significantly different between diabetic and non-diabetic subjects.

Result from Mann-Whitney Ranksum test was summarized and tabulated as in Table 2 which provide the information on association between blood cholesterol and BP levels with FBG level. By referring to p-value, FBG are significantly difference with BP and others lipid profile except for TC. All lipid profile is significantly difference with BP level.

Table 2 also shows the relationship between FBG, cholesterol and BP level. The correlation results were obtained based on Pearson correlation, R indicates that HDLC, LDLC, TG, SBP and DBP does correlates with FBG level. Non HDLC shows weak relation with FBG level as the R value is close to zero. HDLC (R=-0.7193) level shows strongest correlation with FBG level followed by TG (R=0.4006), SBP (R=0.3563), DBP (R=0.2928) and LDLC (R=-0.1131) which show moderate and acceptable correlations.

Table 3 shows the linear regression model to predict FBG value and the relationship between FBG, lipid profile and blood pressure. $R^2$ statistic is a proportion of the variance parameter in the outcome parameter that has been explained by the regression model. So, $R^2$ acts as a measurements tool that explained how much FBG value influenced by the blood cholesterol and BP levels. As the $R^2$ increases with addition of lipid profile and BP in a regression model, it means that the accuracy of the model to
predict the FBG value increases. $R^2$ value ranges between 0% and 100% (best model fit). In Table 3 below, notice that $R^2$ is increased with every additional predictor parameter added to the model in which model No. 6 has the highest $R^2$ value with 59%.

Table 3. Multiple variable linear regression model for predicting FBG value.

| sNo. | Independent variables in model      | $R^2$ (%) |
|------|------------------------------------|-----------|
| 1    | HDLC                               | 52        |
| 2    | LDLC                               | 1         |
| 3    | TG                                 | 16        |
| 4    | SBP                                | 13        |
| 5    | HDLC + TG + SBP                    | 57        |
| 6    | HDLC + TG + SBP + LDLC             | 59        |

Figure 3 shows the normalize graph for multiple variable linear regression model No. 6 in predicting FBG level. Model No. 6 can be proved as statistically significant as all confidence interval (CI) corresponding to the predictor variable in this model does not include 0 as shown in Table 4. In addition, every single predictor parameter is considered significant as $p<0.05$.

Figure 3. Relationship between FBG and selected parameters (HDLC, LDLC, TG, SBP).

Table 4. Multiple variable linear regression model for predicting FBG value.

| Predictor | Estimated regression coefficient | SE of regression coefficient | t Statistic | p-value | 95% CI for Regression Coefficient |
|-----------|---------------------------------|-----------------------------|-------------|---------|----------------------------------|
| Intercept | 0.0866                           | 0.0069                      | 12.6110     | <0.05   | (0.07 to 0.10)                   |
| HDLC      | -0.5658                          | 0.0439                      | -12.9030    | <0.05   | (-0.65 to -0.48)                |
| LDLC      | -0.1021                          | 0.0320                      | -3.1955     | <0.05   | (-0.17 to -0.04)                |
| TG        | 0.0428                           | 0.0168                      | 2.5481      | <0.05   | (0.01 to 0.08)                   |
| SBP       | 0.3424                           | 0.0773                      | 4.4288      | <0.05   | (0.19 to 0.49)                   |

Number of observations: 211, Error degrees of freedom: 206
Root Mean Squared Error: 0.0084
R-squared: 0.592, Adjusted R-Squared 0.584
F-statistic vs. constant model: 74.7, $p<0.05$
4. Discussion

Regression analysis show that the best model predictor is obtained consists of HDLC, TG, SBP and LDLC levels as a predictor of FBG value with $R^2=0.59\%$. It means that the model can predict possibility of the subject in having diabetes if all those predictor parameter values is known or measure without taking OGTT. Thus, 59% chances are correct in predicting diabetes or pre-diabetes based on FBG levels. By referring Table 4, it shows that HDLC (-0.5658) and LDLC (-0.1021) levels are inversely proportional to FBG value as both levels are having negative estimated regression coefficient. Contradict to TG (0.0428) and SBP(0.3424) levels that having positive estimated regression coefficient leading to increasing levels of TG and SBP resulted in increasing value of FBG. Previous study stated that an inverse relationship between TG and HDLC in diabetic patients as low HDLC level leads to high TG level and vice versa. Diabetic dyslipidemia is a condition where people with DM will have high LDLC and TG level, but low HDLC compared to people without DM[29-31].

From observations, HDLC, LDLC, TG and BP levels have high significance difference with FBG and all the predictor parameters (i.e. the independent parameters) show correlation with FBG level. HDLC level shows the strong negative correlation with FBG with $R=-0.7193$. This means linear correlation between HDLC and FBG. Contradict with the research held in Puducherry in 2016, HDLC were not statistically significant and obtained low R value ($R=0.017$)[32]. However, in 2018, Heitham Awadalla et al. reported that about 67% diabetic patient is having low HDLC level and 33% more is having high cholesterol and TG level[29]. Thus, it can be concluded that HDLC alone is strong enough to be the predictor parameter of FBG value.

HDLC level are statistically significant and has lower HDLC in diabetic (1.00 mmol/L) compared to non-diabetic (1.40 mmol/L) subjects. The results perfectly followed recommendations by NCEP ATP-III where HDLC>1.0 mmol/L. Even Lin Xu et. al. (2010) proved that IFG (HDLC = 1.56 mmol/L) and normal fasting glucose (NFG) (HDLC = 1.61 mmol/L) are significantly difference although both group has HDLC value higher than recommended value[33]. The differences might be due to the different region of subjects recruited as differences region or country having different lifestyle, food consumption, demographic characteristic and diseases.

In this study, TG level shows positive correlation, $R=0.4006$ with FBG. This implies that FBG associate directly with TG level as found in previous study. Based on the finding, the correlation between FBG and TG in HTN is $R=0.22$ while without HTN is $R=0.36$[7].

Age, TG, BP and BMI were found higher in diabetic compared to non-diabetic subjects. Either obesity and/or HTN are often present in DM patient[34]. Previous study stated that high BP is increasing with age[35] and high BP increase risk of getting abnormal BG level[15]. Human blood vessels become more fragile with increasing age and resulting in higher tension in transportation of blood, consequently increase BP level, thus increase the BG level. In addition, older people tend to participate less in physical activities which can decrease the blood glucose level[36]. This study shows that average BMI for diabetic subjects are 28 which categorized as pre-obese which similarly found by previous study where about 72% of T2DM were obese with BMI>25[37].

From this study, SBP ($R=0.3563$) and DBP ($R=0.2928$) levels show positive correlation with FBG. Back in 2010, 58% with DM in Hong Kong has high BP and 44% with high BP has abnormal BG level[35]. Australian Healthy Survey in 2011/2012 has conclude that 33.3% adult has high BP and 11% from that are having DM[38]. Similarly in the recent research study found that patient with HTN has higher FBG level than normal people[7]. This can be concluded that high BP level shows in increasing BG level. As a result, there is possibilities of getting HTN when BG level is high.

TC, non HDLC and LDLC levels show insignificant between diabetic and non-diabetic subjects. Prior findings mentioned that TC [39] and LDLC[33] level are not significantly difference between normal and diabetic subjects. TC alone is having advantages because it includes all cholesterol in human body but TC can also overestimates HDLC value which is the good cholesterol[40]. Having uncertainty of high TC value was influenced by bad cholesterol or good cholesterol shows that TC value are non-effective parameters to be counted as predictor to BG level. High TC level with high HDLC level are not risky compared to high TC level with high non HDLC and/or high LDLC level. Recent study in 2018 found that TG, LDLC levels were increased significantly with FBG level[41]. LDLC level gives impact to the BG level as shown in this study. Thus, the outcomes of prior study supported that.
Although random sampling approach was employed and the fact that small number of volunteers were recruited among UMP's residents in this study, few assumptions were made on lifestyle and daily food consumption then adjusted to represent Malaysia cohort in general. Besides, the information via survey might be biased as volunteers mostly has awareness regarding health which may differ in result from person without awareness. In addition, risk factors i.e. physical activities, dietary, alcohol and tobacco consumptions were excluded in this study.

5. Conclusion
In a nutshell, the proposed regression model from this study are still in track as result suggested that FBG level can be predicted by measuring HDLC, TG, LDLC and SBP. In other words, there is 59% chances in getting correct prediction of diabetes (via FBG) i.e. p-value < 0.05. Consequently, it helps in determine relationship between FBG and the predictors (i.e. independent factor such as lipid profile substances, BP, etc.) thus this will help to develop future work in diagnosis and/or prevention type 2 diabetes. It is highly recommended for subjects with high FBG level to have regular medical check-up including checking BP level and getting the blood tests that measures the lipid profile as an initial prevention in developing CVD, DM and HTN. The risk of getting these diseases are higher in the elderly. However, further study in a larger cohort with different demographic profiles that represents the overall Malaysia cohort is necessary to validate the prior finding of this study.

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