Glycemia and Blood Lipids

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Abstract

Diabetes mellitus (DM) is a heterogenous disease with a common hyperglycemic manifestation. 90% of DM is due to type 2 diabetes and it has become a common disease worldwide.

The aim of this study was to investigate the relationship between blood glucose level and the concentration of the various blood lipid fractions in non-diabetic and diabetic patients. This study also observed and evaluated the correlation between FBG and HbA1c as a diagnostic tool in diabetes.

This is a retrospective study of data collected in a private hospital from 788 non-diabetic and diabetic patients (451 males and 337 females) aged 18 to 90. Fasting blood glucose, HbA1c assays and lipid profile (total cholesterol, HDL-C, LDL-C, and triglyceride (TG) were analyzed simultaneously in all subjects. Data analysis was performed by SPSS (Version 17). A P values $\leq$ 0.05 was considered as statistically significant between tested groups.

Female patients in borderline and diabetes groups had significantly higher TG, lower HDL-C levels and higher TG/HDL-C ratio ($P<0.05$) when compared with the normal group. Male diabetes group had significantly higher TG, lower HDL-C levels and higher TG/HDL-C ratio ($P<0.05$) when compared with corresponding normal and borderline groups. No significant difference was observed in the rest of tested parameters. A significant correlation between fasting blood glucose and HbA1c ($r^2=0.828$, $P=0.000$) was noted. Patients diagnosed as diabetes in age group $\geq$75 years was 3 times than that of diabetic patients under 45 years of age.

In conclusion, data analyses have indicated that blood glucose levels were significantly correlated with a higher TG, lower HDL-C and higher TG/HDL-C ratio in the borderline and diabetes groups when compared with the normal group despite of sexes. Results showed that HbA1c is more sensitive than FBG to monitor the progress of DM. The incidence of DM progressed with increasing age.

Key words: *Diabetes mellitus, dyslipidemia, lipid profile, HbA1c*

Introduction

Epidemiology and features of diabetes mellitus

Diabetes mellitus (DM) is a heterogenous disease with a common hyperglycemic manifestation. 90% of DM is due to type 2 diabetes. The remaining 10% of DM belongs to type-1 diabetes, which is a detrimental pancreatic islet β cell autoimmune disease, found mainly in the youth with acute onset of the disorder. Hereditary defects, pancreatic tumor, corticosteroid treatment and gestational diabetes are other forms of diabetes.[1] Type 2 diabetes has become a common disease worldwide, with a chronic insidious onset, affecting mainly the middle and old age population of both genders.[2] This type of diabetes is characterized by insulin resistance and hyperinsulinemia or relative insulin deficiency. Insulin is an anabolic hormone that promotes the uptake of glucose into adipose tissue and skeletal muscle. It stimulates the conversion of glucose to glycogen or fat for storage, inhibits liver to produce glucose, and therefore decreases blood glucose level. Patients with DM are at high risk of developing complications such as renal failure, atherosclerotic, neuropathy (nerve damage) and retinopathy which may lead to blindness.[3] In Hong Kong, the mortality from diabetes was about 7.9 and 6.9 for female and male respectively per 100,000 populations in 2010.[4] Globally, the number of people with diabetes was approximately 30 million in 1985, increasing to 135 million and 217 million by 1995 and 2005 respectively. It is anticipated that the morbidity will progress rapidly in the near future.[5] The incidence of the disease has elevated in the developing country currently, due to economic growth and alternations in habit of food, a predilection of eating high calories and high fat western foods. At the same time, the disease has also evolved in the youth group due to genetic factors, over nutrition and obesity which is becoming an important universal social health problem today.[6]

Diabetes mellitus and dyslipidemia

It is generally accepted that age, obesity, physical inactivity, dietary and hereditary causes such as the Pro12 Ala Polymorphism in PPAR-γ and variants in CALPAIN 10 and KCNJ 11 genes are important patho-physiological defects leading to insulin resistance and insulin secretion insufficiency which are the two principle factors in the initiation of type 2 diabetes.[6-7] Insulin resistance declines the synthesis, secretion and activity of lipoprotein lipase (LPL), an important enzyme for triglyceride (TG) hydrolysis which inevitably leads to elevation of blood TG and very low density lipoprotein (VLDL). The synthesis of high density lipoprotein (HDL) is attenuated as a consequence of decreased TG hydrolysis. A decrease in HDL will impair the retrograde transport of endogenesis cholesterol and the metabolism of cholesterol by the liver. Thus, causes dyslipidemia and progress to

microvascular and macrovascular complications of diabetes.[2,8]

Dyslipidemia is a common feature in type 1 and type 2 diabetes, being more remarkable in the latter and also an intractable therapeutic intervention.[9] The incidence of dyslipidemia in type 2 diabetes range from 34.8% in Korea[10], 60% in Beijing , China[11], to 90.7% in South-East Nigeria.[12] The discrepancies in ethnic, genetics, economic status, food habits, disparities in age, body mass index (BMI), obesity, life style and bias in sampling are all possible causes relating to dyslipidemia.[11] Ethnic and dietary customs have been validated for their effects on lipid levels. In Malaysia, the Malays had higher total cholesterol (TC) and LDL-C, while the Chinese had a higher HDL.[9] Similarly, African Americans had the lowest TG, while the Hispanics had a moderate TG and the White had the highest TG.[13] Clinical observations showed that optimized diabetic treatment alleviated blood glucose as well as dyslipidemia and diabetic complications.[14-15] An integrative anti-diabetic, anti-hypertensive and anti-lipid treatment protocol claimed success in lowering TC in 80%; LDL-C, TG, HDL-C in 66.3%, 57.9% and 31.8% respectively of type 2 diabetic patients within the first year but no significant improvement was observed in the second and later years.[16] The dilemma in dyslipidemia management is unknown which deserve further investigations, this reflexes the complexity in the mechanism of dyslipidemia in type 2 diabetes. Diabetic patients have a 2–4 fold higher risk of coronary heart disease (CHD) mortality than non-diabetic patients.[17] CHD is caused by precipitations of LDL-C on blood vessels forming atheromatous plaque that impedes arterial blood flow to organ supplied.

LDL-C is usually considered as “bad cholesterol”. It has a tendency to cause atherosclerosis leading to cardiovascular disease. Atherosclerosis is initiated by inflammation in response to endothelial injury in the artery wall.[18] The injured endothelium becomes highly permeable to LDL particles. As LDL molecules pass through the endothelial cells, they are susceptible to oxidation.[19] The oxidized LDL activates the endothelial cell to express adhesion molecules and release of growth factors, resulting in the recruitment of monocytes/macrophages in the affected area. Macrophages have scavenger receptors, enabling them to ingest oxidized LDL. However, macrophages cannot process the oxidized LDL, and so they become foam cells in the form of lipid droplets within the cytoplasm. If the LDL concentration remains high in blood, more and more LDL particles will deposit in arterial wall. As a result, artherosclerotic plaque will form and becomes a hard cover, which impedes arterial blood flow.[18] HDL-C is usually considered as “good cholesterol”. It is able to transport cholesterol from tissue (e.g. artery atheroma) to the liver where it is catabolized and removed as bile acids, a process known as reverse cholesterol transport.[20] Consequently, low HDL-C level increases the risk of atherosclerosis and cardiovascular disease, while a higher HDL-C level can reduce the onset of

cardiovascular disease.

A seminal hypothesis suggested that a fraction of small and dense LDL-C might also exist in type 2 diabetic dyslipidemia that cannot be detected by ordinarily laboratory methods but can be determined by ultracentrifugation or electrophoresis. These small and dense LDL-C are more artherogenic because of their sensitivity to oxidation. These oxidized LDL-C now known as ox-LDL-C plays a primary and vital role in the formation of artherogenesis. High TG and low HDL enhance the synthesis of these small and dense LDL-C through impaired metabolism in body tissues and the liver which promotes artherogenesis.[21] Moreover, TG is a pro-thrombotic and pro-inflammatory factor which can influence and contribute to the increase risk of CHD.[22] However, further investigations are needed for confirmation of this hypothesis.

Introduction of FBG as a diagnostic tool for diabetes

Normally, the concentration of blood glucose is maintained in a narrow range by hormones that regulate the glucose shift into and out of the circulation. These hormones include insulin, which decreases blood glucose levels, while hormones such as glucagon, cortisol, epinephrine and growth hormone, can cause an increase in blood glucose.[3] Hyperglycemia is the hallmark of diabetes, generally acknowledged before the onset of symptoms and complications of diabetes[2] and usually the prime diagnostic evidence of the disease for most patients. Fasting blood glucose (FBG), post-prandial glucose (PPG) and oral glucose tolerance test (OGTT) are the traditional methods for use in the past.[23] FBG is widely accepted as a diagnostic testing for diabetes. Advantages include ease of performance, non-expensive and available in most laboratories[24] with an unequivocal diagnostic criteria (FPG <6.1mmol/L indicate normal, 6.1-6.9 mmol/L suggest impaired fasting glucose, ≥7.0 mmol/L suggest diabetes).[2] However FBG is subject to certain disadvantages and limitations. FBG represents the moment of blood glucose level and the patients have to suffer an overnight fasting. The result is affected by incompliance in fasting, over activity, stress and drugs. Long standing of blood samples can cause glucose degradation due to erythrocyte glycolysis or the effect of other blood components, the rate of glycolysis is higher in samples with leukocytosis or bacterial contamination. Hence, it is necessary to use a tube containing glycolytic inhibitor, usually fluoride, to collect blood sample.[24]

Introduction of HbA1c as a diagnostic tool for diabetes

In 1958, Huisman and Meyering first used a chromatographic column to separate HbA1c from other types of haemoglobin.[25] HbA1c, also known as A1c, is currently proposed as a laboratory method for diagnosis and long-term control in diabetes.[1] HbA1c is a form of glycated hemoglobin when blood glucose irreversibly conjugates to the N-terminal valine of the β chain of hemoglobin within the erythrocytes by non-enzymatic process. During the

glycosylation process, an unstable aldiminic product (Schiff base, pre-HbA1c) is formed primarily and then it undergoes molecular rearrangement to form a stable ketoamine, HbA1c.[3] The level of HbA1c is proportional to the concentration of blood glucose and can maintain stability for 8-12 weeks. Once glycated haemoglobins are formed, they remain that type. So HbA1c reflects the average level of blood glucose during 120-day lifespan of the erythrocytes.[26] Hence, a more reliable parameter for monitoring the chronic course of the illness, response to treatment and emerge of complications.[27] Some observational studies[28] and clinical trials[29] showed good correlations between HbA1c and microvascular complications in diabetes, as well as retinopathy. More importantly, the HbA1c level is a predictor of the risk of microvascular and macrovascular complications and a decrease in HbA1c level reduce the morbidity of microvascular and macrovascular complications in diabetes.[29-30] Hence, HBA1c was proposed as a gold standard for diagnosis of diabetes by the American Diabetic Association (ADA) in 2008.[31] Furthermore, most factors that alter FBG did not significantly affect HbA1c results. Acute illness and short lifestyle changes (exercise, ingestion of food, stress) did not affect HbA1c value significantly. There are no requirements of fasting, glucose ingestion or 2-hour waiting in HbA1c examination.[32] Moreover, the whole blood samples are stable for 1 week at 4℃ and for at least 1 year at -70℃.[33] However, HbA1c has several disadvantages. First, it is affected by intra-individual discrepancies including ethnic, genetic, age and pregnancy.[26] Second, it is significantly correlated with the structure and life span of the erythrocytes, as seen in diseases such as hemolytic anemia, the hemoglobinopathies, as well as in disorders such as uremia, iron deficiency anemia and nutritional anemia.[32] Third, it lacks sensitivity in an acute hyperglycemic state such as type 1 diabetes and gestational diabetes. Fourth, non-standardized performance and method may influence the examination result.[26]

There are many methods for the detection of HbA1c. These methods separate HbA1c from other haemoglobin fractions based on electrical charge differences (e.g. electrophoresis, ion-exchange chromatography and high-performance liquid chromatography (HPLC)), chemical difference (e.g. spectrophotometry), or structural differences (e.g. affinity chromatography and immunoassay).[3] In the past twenty years, laboratory techniques and new methods improved the HbA1c measurement, giving it more reliable. However, the results achieved by the variability of methodology and instrument cannot be reproducible from different laboratories. Thus, values achieved by different methods are non-comparable. It was emphasized the need to standardize HbA1c measurement globally in order to allow comparative results between different laboratories. The advisable method for HbA1c is ion exchange HPLC or immunological assay, where the normal HbA1c range is approximately 4.0-6.1%.[24] The HPLC ion-exchange method has been

recommended as a reference method. It is fully automatic, but its equipment costs are high and it cannot be applied to all laboratories. HbA1c is the domain with extensive interest and investigation currently. The ADA proposed a cut-off point of ≥6.5% as confirmed diabetes.[31] However, much controversy still exists regarding the optimum cut-off point in different conditions of diabetes.

There is no clear evidence to suggest whether FBG or HbA1c was superior to the other at present.[24] FBG, and HbA1c represent different underlying processes for detecting the same morbid hyperglycemia, it would not be surprise that disparities may exist between these two parameters at different occasions and pathological changes in the course of the disease. Hence, further thorough investigations are reasonable and obligatory in future aspects.

Materials and Methods

Place and timeframe of the study

A retrospective study was conducted in the clinical laboratory department of Tsuen Wan Adventist Hospital, Hong Kong SAR, China between January 2010 and May 2012.

Sample size

Seven hundred and eighty-eight cases from non-diabetic and diabetic patients, aged 18 to 90, 451 males, and female 337 were registered. The sex and age of individuals accompanying with the results of fasting blood glucose, HbA1c, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglyceride were retrieved for data analysis. In this cohort, these 788 cases were further divided into four age groups namely 0-44 years; 45—64 years; 65-74 years and ≥75 years according to CDC[34] for further analysis.

Inclusion and Exclusion criteria

Inclusion criteria: According to World Health Organization[35], HbA1c levels and fasting blood glucose (FBG) and were categorized into three groups.

Group1 Normal control group: HbA1c < 6.0% and FBG < 6.1 mmol/L.
Group2 Borderline group or suspected diabetic group: Either HbA1c = 6.0 - 6.4% or FBG = 6.1 - 6.9 mmol/L.
Group3 Diabetic group: Either HbA1c ≥ 6.5% or FBG ≥ 7.0 mmol/L.

Exclusion criteria:

a. Patients were non-fasting or fasting less than 8 hours before blood taking.
b. Patients who had been admitted suffered from diabetes and had related drugs management.

Study approval

The results generated from present study were solely to fulfill the M.Sc. requirements and did not affect the subsequent clinical management of the patients, no conflict of interest, and of no commercial value. Therefore, ethical committee approval was waived.

Patient sample and data security

All tested samples were stored at -20℃. All testing results were kept in the LIS and only authorized person can access to the data.

Samples analysis method

Sample collection: All patients had to fast overnight for at least 8 hours before blood taken. The venous blood was collected in EDTA tube for HbA1c test, serum separator gel tube was used for analysis of lipid profile. Fluoride tube was drawn for fasting blood glucose.

Glucose:

The glucose level was detected by an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method and measured by SIEMENS Dimension RxL clinical chemistry system.

Principle: In the reaction, glucose was phosphorylated by adenosine-5'-triphosphate (ATP) in the presence of hexokinase and magnesium to form glucose-6-phosphate and ADP. Then, the glucose-6-phosphate was oxidized by enzyme glucose-6-phosphate dehydrogenase (G-6-PDH) to 6-phosphogluconate in the presence of nicotinamide-adinine (NAD). The amount of NADH formed was proportional to glucose concentration in the specimen and measured by absorbance at 340nm and 383nm.[36]

Reagent: Glucose reagent was supplied by Siemens Hong Kong

Glycated Hemoglobin:

The determination of HbA1c in whole EDTA blood was analyzed by ion-exchange high-performance liquid chromatography (HPLC) of the Bio-Rad D-10 Dual Program.

Principle: The whole blood samples were diluted automatically by Bio-Rad D-10 and injected into the column, an analytical cartridge. The D-10 transferred elution buffer gradient of increasing ionic strength to the column. The different hemoglobin fractions were separated based on their ionic interaction with the material in the column. Then the separated hemoglobin passed through the photometer, and was measured by absorbance at 415 nm.[37]

Reagent: HbA1c reagent was supplied by BioRad Hong Kong

Lipid profile

Total cholesterol:

Total cholesterol was measured by SIEMENS RxL Dimension clinical chemistry system.

Principle: Total cholesterol in serum comprises of cholesterol esters and free cholesterol. Cholesterol esters were primarily hydrolyzed by cholesterol esterase to produce free cholesterol. Subsequently, free cholesterol was oxidized to produce cholest-4-ene-3-one and H_2O_2 by cholesterol oxidase. Through the action of horseradish peroxidase, the H_2O_2 oxidized N,N diethylaniline -HCL/4-aminoantipyrine (DEA-HCL/AAP) to form oxidized DEA-HCL/AAP that absorbs at 540nm. The absorbance was proportional to the total cholesterol concentration in the sample.[38]

Reagent: Cholesterol reagent was supplied

by Siemens Hong Kong

High density lipoprotein cholesterol:
High density lipoprotein cholesterol was measured by SIEMENS Dimension RxL clinical chemistry system. The method did not require pretreatment or ultracentrifugation manipulation.

Principle: In the presence of magnesium sulfate, chylomicrons, VLDL, LDL reacted with dextran sulfate to form water soluble complexes. These complexes did not react with the polyethylene glycol (PEG)-modified cholesterol esterase and PEG-cholesterol oxidase in the reaction. Meanwhile, the HDL cholesterol esters were hydrolyzed by PEG-Cholesterol esterase to produce free cholesterols. The free HDL cholesterols were oxidized to Δ4-cholestenone and H_2O_2 by PEG-cholesterol oxidase in the presence of oxygen. By the action of peroxidase, H_2O_2 reacted with 4-aminoantipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxya niline to form a colored product that could be measured by absorbance at 600nm and 700nm.[39]

Reagent: High density lipoprotein cholesterol reagent was supplied by Siemens Hong Kong

Low density lipoprotein cholesterol:
Low density lipoprotein cholesterol was measured directly by SIEMENS Dimension clinical chemistry system, without the need of pretreatment or ultracentrifugation manipulation.

Principle: Detergent 1 solubilized only non-LDL lipoprotein particles. The cholesterols released reacting with cholesterol esterase and cholesterol was oxidased to form a non-colored product. Then, the remaining LDL particles were solubilized by detergent 2. The soluble LDL cholesterol reacted with cholesterol esterase and cholesterol was oxidased to form cholestenone and H_2O_2. In the presence of N,N-bis(4-sulfobutyl)-m-toluidine, disodium salt (DSBmT) and 4-aminoantipyrine (4-AA), H_2O_2 reacts with peroxidase to form a colored product which was measured by absorbance at 540nm and 700nm. The absorbance was proportional to the concentration of LDL-C in sample.[40]

Reagent: Low density lipoprotein cholesterol reagent was supplied by Siemens Hong Kong

Triglycerides:
Triglycerides were measured by SIEMENS Dimension RxL clinical chemistry system.

Principle: In the reaction triglycerides were converted into free glycerol and fatty acids by lipoprotein lipase (LPL) enzyme. In the presence of glycerol kinase, glycerols were phosphorylated by ATP to glycerol-3-phosphate which was then oxidized to dihydroxyacetone phosphate and H_2O_2 by glycerol-3-phosphate oxidase. The H_2O_2, aminoantipyrine and 4-chlorophenol were catalyzed by peroxidase to produce quinoneimine which was measured by absorbance at 510nm and 700nm. The absorbance was proportional to the triglyceride concentration in the sample.[41]

Reagent: Triglycerides reagent was supplied by Siemens Hong Kong

Statistical analysis

The statistical analysis of data in this project was performed by SPSS version 17 (Chicago, IL USA). Various correlations were analyzed by Pearson's correlation test. The different parameters between females and males were analyzed by independent samples t-test. One-way ANOVA and post hoc Dunnett's multiple comparison tests were used to analyze the significance of value for cholesterol, HDL-C, LDL-C and triglyceride in different groups. The mean difference is considered as statistically significant when P values ≤ 0.05.

Results

Comparing testing results between genders

In our study, 451 males and 337 females were recruited. Independent samples t-test was utilized to analyze the different parameters including FBG, HbA1c and lipid profile between females and males. A significant increase in FBG (6.5 ± 2.47), LDL-C (3.21 ± 0.851), triglycerides (1.58 ± 1.195), and TG/HDL ratio (1.48 ± 1.505) in males ($P<0.05$) were found by comparing with females. The level of HDL-C (1.49 ± 0.425) in females was significantly higher than males (1.26 ± 0.376). There was no significant difference ($P>0.05$) in HbA1c (6.4 ± 1.38 vs 6.6 ± 1.58) and total cholesterol (5.0 ± 0.87 vs 5.0 ± 0.92) between females and males. Table 1 shows the comparisons of HbA1c, FBG and lipid profile between males and females.

Remarks: Values insides the bracket represents mean ±standard deviation (SD)

Comparing results of lipid profiles between different groups

Seven hundred and eighty-eight subjects were categorized by gender and then further subdivided into 3 groups based on the following criteria: Subjects with HbA1c <6.0% and FBG< 6.1 mmol/L were classified as group 1 (normal control group). Subjects with either HbA1c= 6.0 - 6.4% or FBG = 6.1 - 6.9 mmol/L, were classified as group 2 (borderline group or suspected diabetic group). Subjects with either HbA1c $\geq$ 6.5% or FBG $\geq$ 7.0 mmol/L were classified as group 3 (diabetic group).

Total cholesterol:

It was observed that the mean values of total cholesterol in the female group 1 to 3 were 5.0 mmol/L, 5.1 mmol/L and 4.9 mmol/L respectively (Table 2), and the mean values of total cholesterol (5.0 mmol/L) were the same in all male subgroups (Table 3). By one-way ANOVA analysis in tables 2 and 3, no significant difference in total cholesterol levels was observed between the three groups in both females and males ($P>0.05$).

Triglycerides:

The mean values of triglycerides in group 1 to 3 of females were 0.95 mmol/L, 1.39 mmol/L and 1.68 mmol/L respectively. By using one-way ANOVA analysis in the females, group 2 and group 3 patients had significantly higher triglycerides levels ($P<0.05$) than that of group 1 patients as

Table 1 shows the comparisons of HbA1c, FBG and lipid profile between males and females.

	Female (N=337)		Male (N=451)			
	Mean	SD	Mean	SD	T	P value[a]
HbA1c (%)	6.4	1.38	6.6	1.58	-1.77	0.08
FBG (mmo/L)	6.1	1.73	6.5	2.47	-2.54	<0.05*
Cholesterol (mmol/L)	5.0	0.87	5.0	0.92	-0.46	0.65
Triglycerides (mmol/L)	1.29	1.089	1.58	1.195	-3.55	<0.05*
HDL-C (mmol/L)	1.49	0.425	1.26	0.376	8.10	<0.05*
LDL-C (mmol/L)	3.03	0.758	3.21	0.851	-3.05	<0.05*
TG/HDL Ratio	1.06	1.281	1.48	1.505	-4.15	<0.05*

a. Assessing gender differences by t-test

Table 2 shows comparisons of lipid profile in females among 3 groups by one-way ANOVA

	Female				
	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG/HDL-C
Group 1 (N=156)	5.0 ± 0.73	0.95 ± 0.601	1.66 ± 0.418	2.97 ± 0.665	0.69 ± 0.682
Group 2 (N=67)	5.1 ± 0.76	1.39 ± 1.039	1.43 ± 0.463	3.20 ± 0.688	1.23 ± 1.482
Group 3 (N=114)	4.9 ± 1.08	1.68 ± 1.443	1.29 ± 0.304	3.01 ± 0.897	1.46 ± 1.608

	P value				
	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG/HDL-C
Group 1[a] vs Group 2[b]	1.00	<0.05*	<0.05*	0.11	<0.05*
Group 1[a] vs Group 3[c]	0.72	<0.05*	<0.05*	1.00	<0.05*
Group 2[b] vs Group 3[c]	0.22	0.22	0.06	0.32	0.70

* The difference is significant when P values ≤ 0.05.

a. Group 1: Normal control group

b. Group 2: Borderline diabetic group or suspected diabetic group

c. Group 3: Diabetes group

shown in table 2. For male patients, the mean values of triglycerides were 1.33 mmol/L, 1.46 mmol/L and 1.97 mmol/L in group 1 to 3 respectively. It was indicated that the group 3 of male patients had significantly higher ($P<0.05$) levels of triglycerides than group 1 and group 2, but there was no significant difference ($P=1.00$) in triglycerides levels between group 1 and group 2 (Table 3). A consecutive rising in the mean values of triglycerides from group 1 to 3 patients were observed in both females and males (Figures 1b and 2b).

High density lipoprotein cholesterol:

The mean values of HDL-C in group 1 to 3 of female patients were 1.66 mmol/L, 1.43 mmol/L and 1.29 mmol/L respectively. One-way ANOVA analysis demonstrated that there were significantly lower ($P<0.05$) HDL-C levels in group 2 and group 3 female patients than that in group 1(Table 2). For group 1 to 3 male patients, the mean values of HDL-C were 1.36 mmol/L, 1.26 mmol/L and 1.14 mmol/L respectively. There were significantly lower ($P<0.05$) levels of HDL-C in group 3 than group 1 and group 2 patients, although no significant difference ($P=0.06$) in HDL-C levels between group 1 and group 2 patients was found by using one-way ANOVA analysis (Table 3). A consecutive decrease in HDL-C means values from group 1 to 3 patients were observed in both females and males (Figures 1c and 2c).

Low density lipoprotein cholesterol:

The mean values of LDL-C in group 1 to 3 of female patients were 2.97 mmol/L, 3.20 mmol/L and 3.01 mmol/L respectively (Table 2). The mean values of LDL-C in group 1 to 3 male patients were 3.22 mmol/L, 3.22 mmol/L and 3.18mmol/L respectively (Table 3). By one-way ANOVA analysis, no significant difference ($P>0.05$) in LDL-C values was observed between the three groups for both females and males.

Triglyceride / High density lipoprotein cholesterol ratio:

The mean values of TG/HDL ratio in group 1 to 3 were 0.69, 1.23 and 1.46 respectively in the female group. One-way ANOVA analysis demonstrated that the group 2 and group 3 patients had significantly higher ($P<0.05$) TG/HDL ratio than group 1 (Table 2). The mean values of TG/HDL ratio were 1.17, 1.35 and 1.94 respectively in the male group 1 to 3. One-way ANOVA analysis showed group 3 had significantly higher ($P<0.05$) TG/HDL ratio than group 1 and group 2, but no significant difference ($P=0.83$) was found between group 1 and group 2 (Table 3). A consecutive rising in mean values of TG/HDL ratio from group 1 to 3 was detected in both females and males (Figures 1e and 2e).

Table 3 shows comparisons of lipid profile in males among 3 groups by one-way ANOVA

	Male				
	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG/HDL-C
Group 1 (N=172)	5.0 ± 0.83	1.33 ± 0.843	1.36 ± 0.454	3.22 ± 0.824	1.17 ± 0.999
Group 2 (N=127)	5.0 ± 0.85	1.46 ± 0.939	1.26 ± 0.320	3.22 ± 0.813	1.35 ± 1.346
Group 3 (N=152)	5.0 ± 1.07	1.97 ± 1.573	1.14 ± 0.275	3.18 ± 0.915	1.94 ± 1.941
	P value				
	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG/HDL-C
Group 1[a] vs Group 2[b]	1.00	1.00	0.06	1.00	0.83
Group 1[a] vs Group 3[c]	1.00	<0.05*	<0.05*	1.00	<0.05*
Group 2[b] vs Group 3[c]	1.00	<0.05*	<0.05*	1.00	<0.05*

* The difference is significant when P values ≤ 0.05.

a. Group 1: Normal control group

b. Group 2: Borderline diabetic group or suspected diabetic group

c. Group 3: Diabetes group

Correlation between HbA1c and other assays

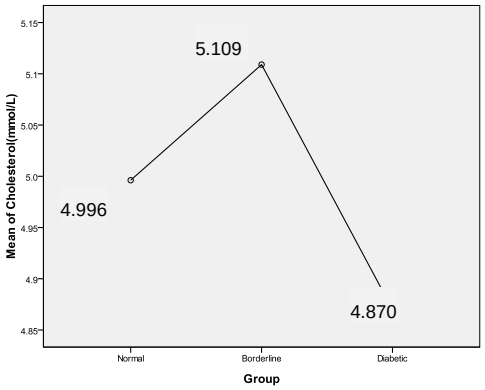
Pearson's correlation test in figure 3 demonstrated a significant correlation between FBG and HbA1c (r^2=0.828, P<0.05). Moreover, HbA1c showed significant correlations with triglycerides (r^2=0.236, P<0.05), TG/HDL ratio (r^2=0.225, P<0.05) and an inverse correlation with HDL-C (r^2= -0.245, P<0.05). However, the value of HbA1c in figure 4 did not influence the results of cholesterol (r^2= -0.002, P=0.95) and LDL-C (r^2=0.015, P=0.68).

Correlation between fasting blood glucose and lipid profile

The results presented in figure 5 indicated that FBG also demonstrated a significant correlation with triglycerides (r^2=0.285, P<0.05), TG/HDL ratio (r^2=0.267, P<0.05) and inverse correlation with HDL-C (r^2= -0.247, p<0.05). Nevertheless, there was no significant influence of glucose value on the results of cholesterol (r^2=0.014, P=0.70) and LDL-C (r^2=0.014, P=0.69).

Figure 1 shows the mean values of lipid profile in females in normal (Group 1), borderline (Group 2) and diabetes (Group 3).

(a)



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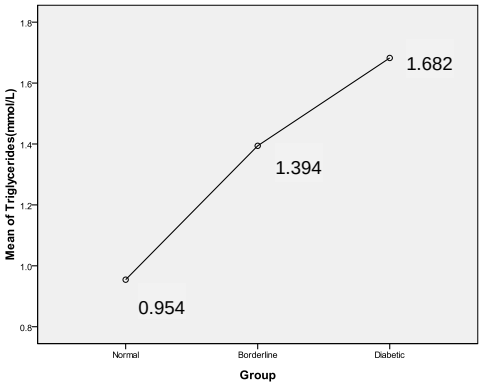
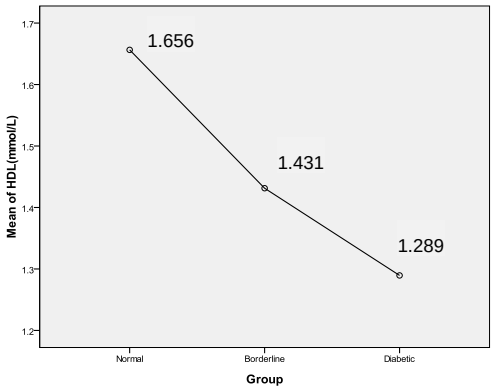
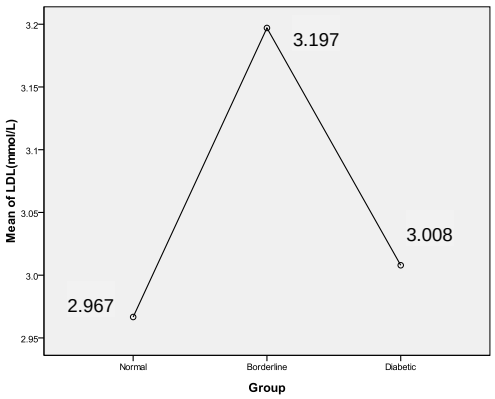


(c)



(d)



(e)

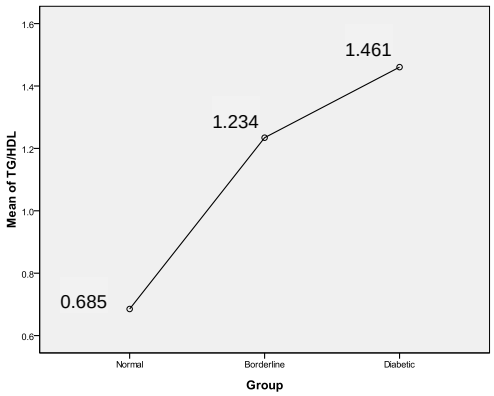
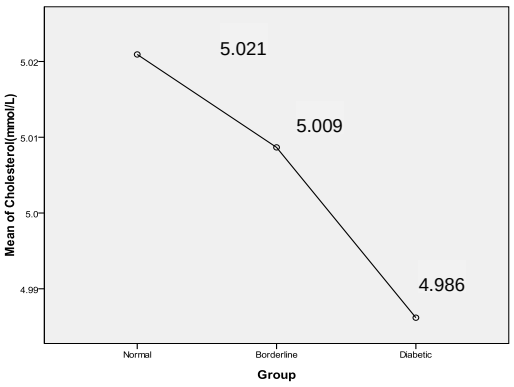
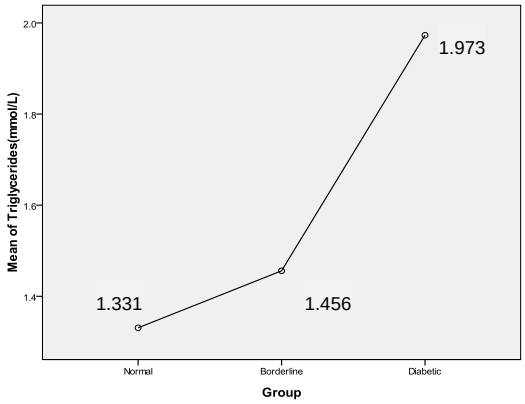


Figure 2 shows the mean values of lipid profile in males in normal (Group 1), borderline (Group 2) and diabetes (Group 3).

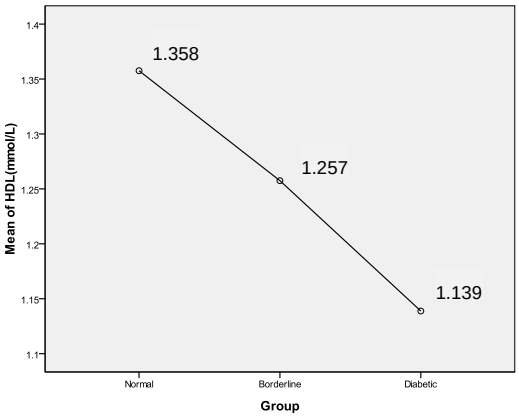
(a)



(b)



(c)



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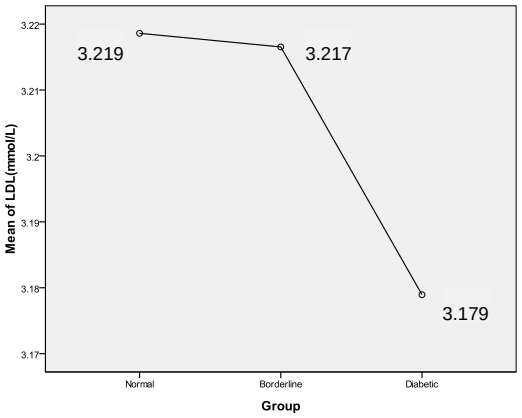


(e)

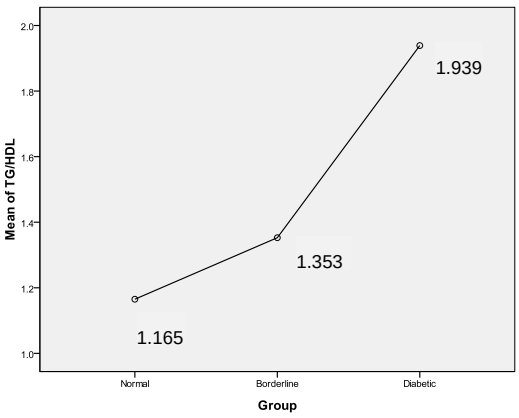


Figure 3 shows correlation between FBG and HbA1c

r^2=0.828, P<0.05

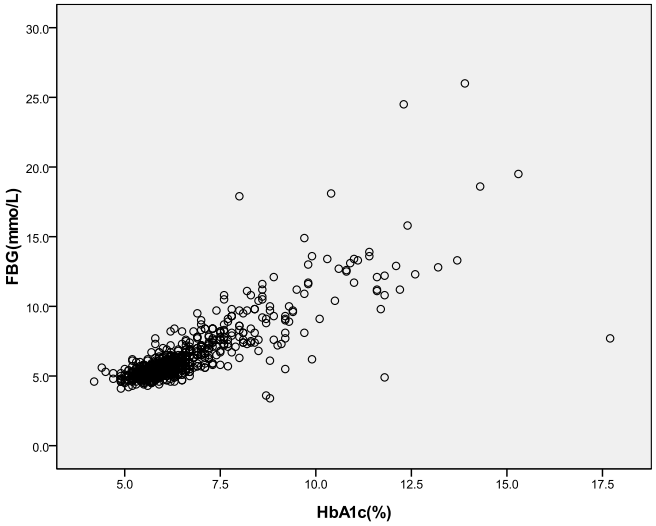
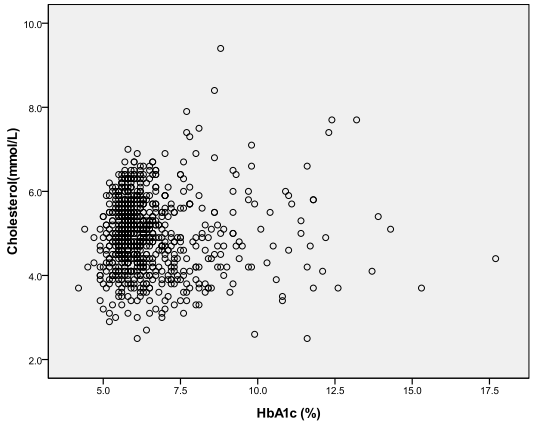
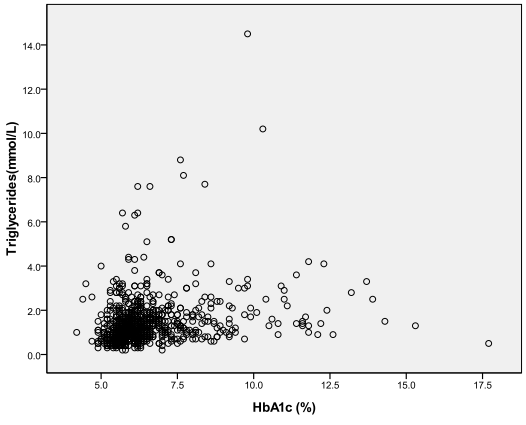


Figure 4 shows Correlations between HbA1c and lipid profile

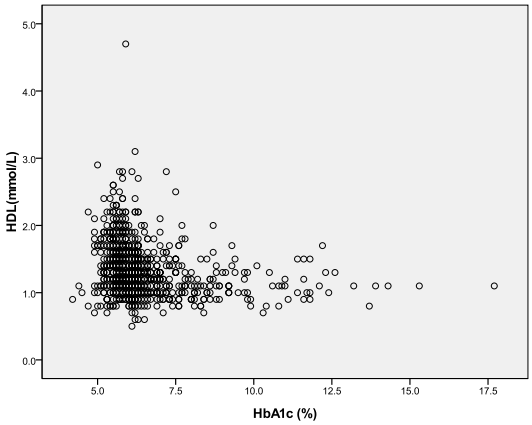
r^2= -0.002, P=0.95



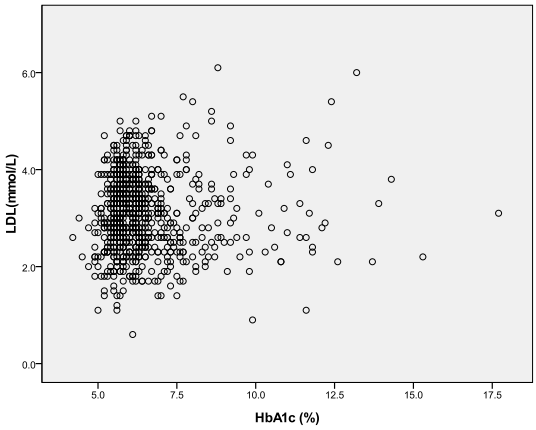
r^2=0.236, P<0.05



r^2=0.236, P<0.05



r^2=0.015, P=0.68



r^2= 0.225, P<0.05

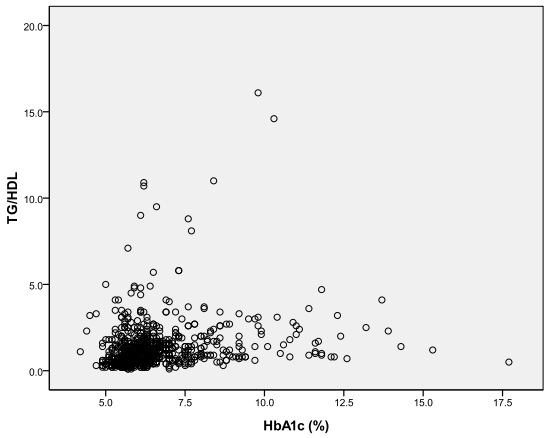
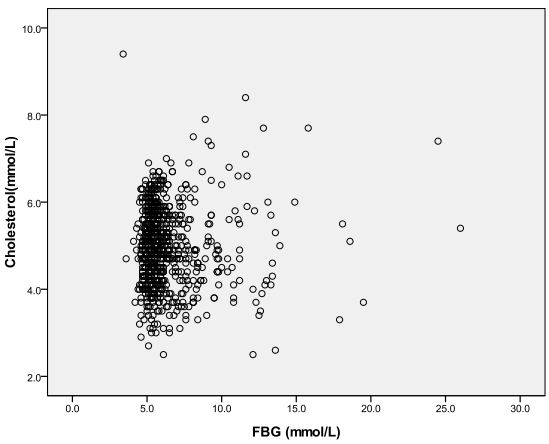
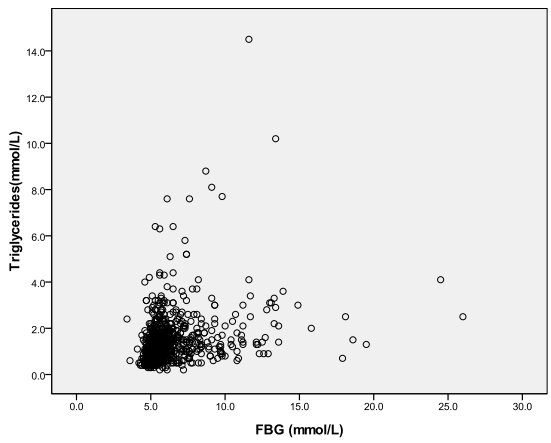


Figure 5 shows correlations between FBG and lipid profile

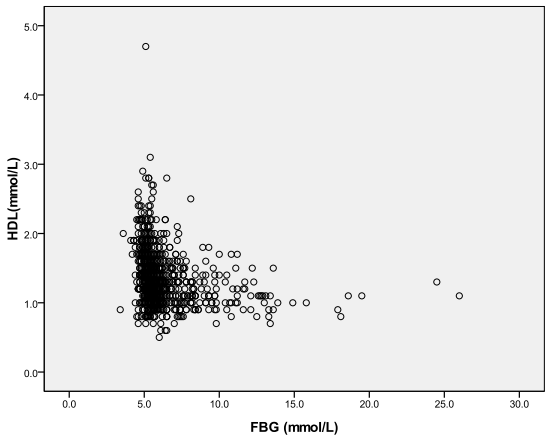
r^2=0.014, P=0.70



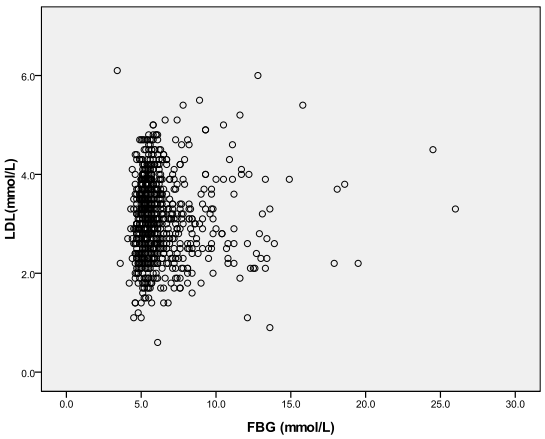
r^2=0.285, P<0.05



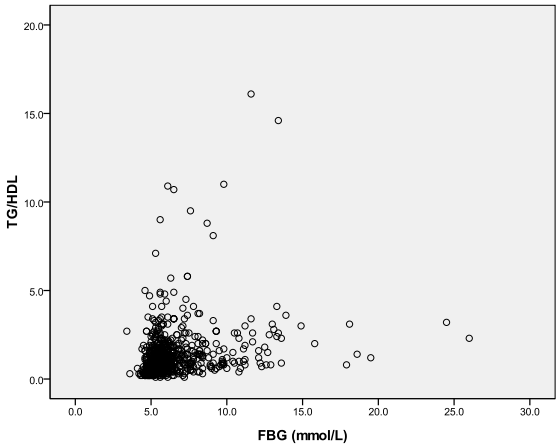
r^2= -0.247, P<0.05



r^2=0.014, P=0.69



r^2=0.267, P<0.05

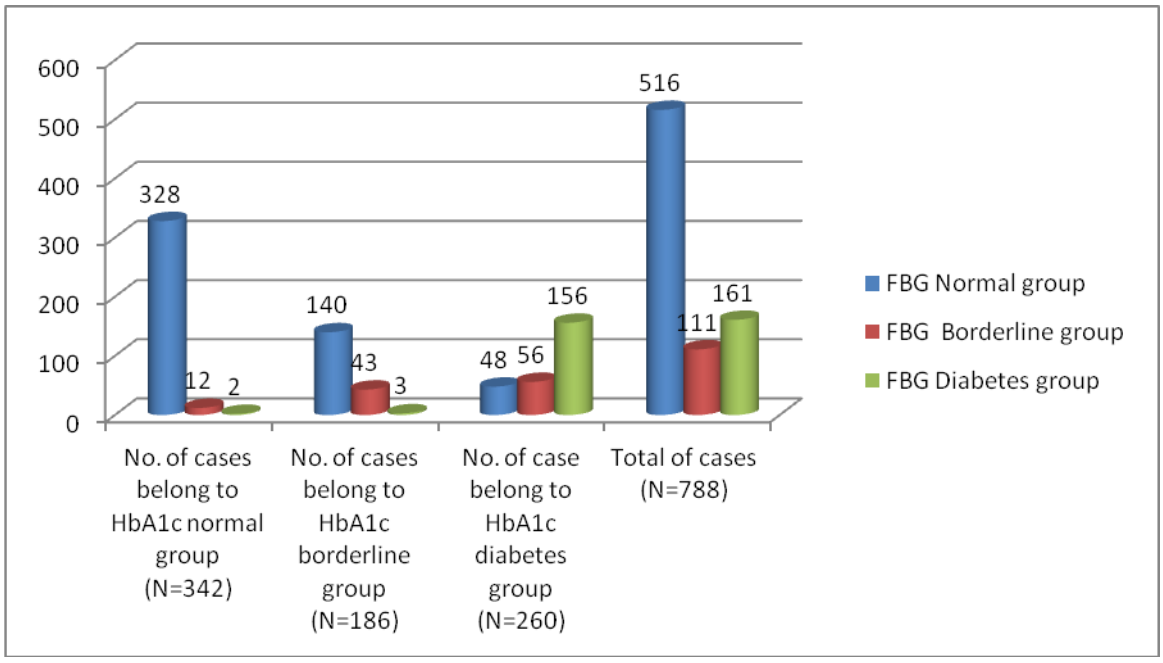


Re-categorization based on HbA1c and FBG levels

When these 788 subjects were categorized by using HbA1c and FBG separately as the criteria for classification, i.e. FBG which fulfilled the criteria FBG < 6.1 mmol/L, FBG 6.1 – 6.9 mmol/L and FBG ≥7.0 mmol/L were classified as FBG based group 1 (normal control group), group 2 (borderline diabetic group or suspected diabetic group) and group 3 (diabetic group) respectively. Similarly, HbA1c in these 788 subjects fulfilled the criteria of HbA1c < 6.0%, HbA1c 6.0 - 6.4% and HbA1c ≥ 6.5% were classified as HbA1c based group 1 (normal control group), group 2 (borderline diabetic group or suspected diabetic group) and group 3 (diabetic group) respectively. A discrepancy between the FBG based group and the HbA1c based group was obviously observed in our data. As recorded, the incidence of FBG based group 1 to 3 were 516, 111, 161 cases respectively (Figure 6). On the contrary, when HbA1c was adopted as the classification method, the prevalence in HbA1c based group 1 to 3 was 342, 186,

260 cases respectively (Figure 7). HbA1c based group had a higher prevalence in group 2 and 3 as compared with FBG based group and it was opposite in group 1 (FBG based group 516 cases versus HbA1c based group 342 cases). We also observed that a portion of the individuals in the FBG based criteria subgroups were allocated to a higher level group when they were settled into the HbA1c based criteria. Therefore, the sensitivity of HbA1c was higher than FBG when it was used as a biomarker for diabetes.

Figure 6 shows the correlation of FBG and HbA1c in the FBG based criteria in normal, borderline and diabetes groups



Age distribution

The age range of patients included was 18 to 90 years old. Among a total of 788 patients, 235 (29.8%) were 0-44 years; 436 (55.3%) were 45-64 years; 69 (8.8%) were 65-74 years and 48 (6.1%) were ≥75 years. Those age groups were further divided into 3 groups: normal control group, borderline group and diabetes group according to 2.3.1 (Figure 8). The percentage of patients diagnosed as diabetes in age group ≥75 years (66.7%) was more than three times that of patient under aged 45 years (17%).

Figure 7 shows the correlation of HbA1c and FBG in the HbA1c based criteria in normal, borderline and diabetes groups

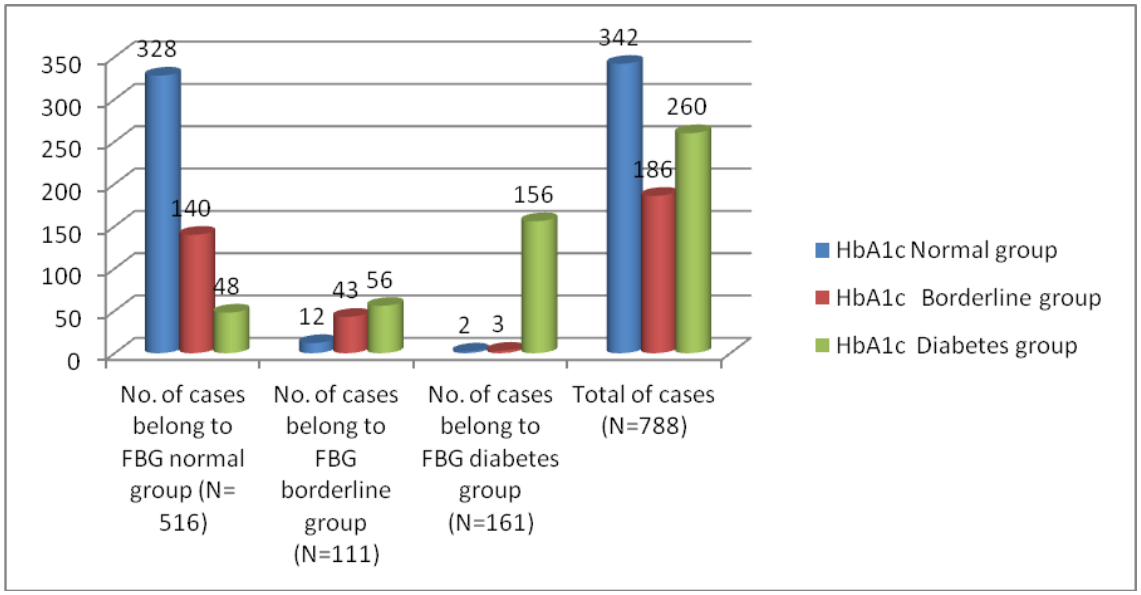
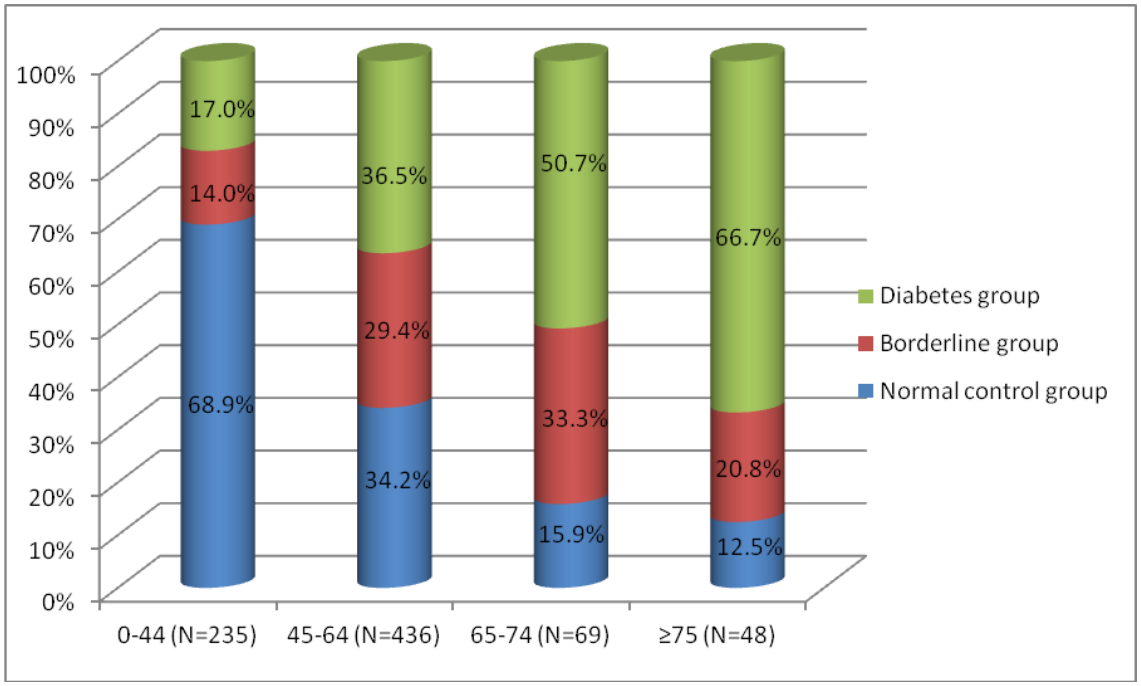


Figure 8 shows the percentage of diabetes incidence rate in 788 patients among 4 difference age groups.



Discussions

Derivation of classification and criteria in use

Diabetes mellitus is a common clinical symptom. More than 90% are type 2 diabetes. It is a disease with a chronic course and insidious onset due to impairment in metabolism related to hereditary and environmental causes.[1] The course of the disease may be classified as a pre-diabetic or borderline diabetic stage, and confirmed diabetic stage which has vital clinical significance in relevance to treatment and prognosis.[1] Accordingly, the data in our cohort is analogously classified as non-diabetes (normal control group) , borderline diabetic group (suspected diabetic group) and confirmed diabetic group in this study.

FBG and HbA1c are the two parameters adopted for measurement of blood glucose level in this study. FBG is the classical assay used for more than a century. It has an acknowledged diagnostic criteria universally adopted; FBG < 6.1mmol/L as non-diabetic, FBG 6.1 - 6.9 mmol/L as borderline diabetes, and FBG ≥ 7.0mmol/L as confirmed diabetes.[1,35] This is concordance with the criteria adopted in this study. In the mid 1980's HbA1c was suggested for use as criteria for the diagnosis of diabetes.[26] HbA1c ≥ 6.5% was recommended as the criteria for confirmed diabetes and also a cut-off point for microvascular complication by the World Health Organization (WHO) in 2011. HbA1c values between 6.0 and 6.5% were proposed by the International Expert Committee as high risk Diabetes Mellitus which requires diabetic prevention intervention.[35] The correlation of FBG with HbA1c and the optimal cut-off point of HbA1c for staging and screening purpose is the realm of much dispute in recent years.[42-43] Several cross sectional epidemiological surveys were reported currently. They claimed a cut-off point of HbA1c ≥6.1 – 6.2%[24] or HbA1c ≥ 6.3%[44] as the criteria for early diagnosis of diabetes. A report declared that HbA1c 5.5 – 6.5% can capture a large population of people at high risk of diabetes.[23] The similar cut-off point of HbA1c 5.7 – 6.4% as criteria for pre-diabetes was proposed in a report.[45] The criteria of HbA1c adopted in this study i.e. HbA1c < 6.0% as non-diabetes (normal control group), HbA1c 6.0 – 6.4% as borderline diabetes, and HbA1c ≥ 6.5% as confirmed diabetes are originated from WHO proposals.

Lipid profile disparities related to genders

Discrepancy in lipid profile between both genders was observed in our study. By the independent sample t-test analysis TG, LDL-C, and TG/HDL-C ratio were significantly higher in the males than the females and the level of HDL-C in females was significantly higher than males but no significant difference in total cholesterol level between males and females. Wu reported male subjects had higher TG, LDL-C and lower HDL-C than female subjects, which were consistent with the findings of this study.[46] Meanwhile, higher fat distribution on the abdomen was also observed in the males. Cai L. also reported

the higher prevalence of dyslipidemia in man than in woman which increased with age.[11] Khan suggested the levels of total cholesterol and HDL-C were significantly higher and TG significantly lower in females as compared to males in type 2 diabetes.[17] These observations all indicate disparities in lipid profile do exist between males and females, while the cause is hitherto ambiguous.

Female hormone was proposed as a vital factor in this aspect which claimed to have the effect of cardioprotection to the females against hypertension and hyperlipidemia.[47] This protecting effect will subside after menopause. As a consequence, a higher prevalence of hypertension, hyperlipidemia and cardiovascular disease may occur in females at this age. Central obesity is documented to be intimately related to metabolic syndrome, hyperlipidemia and diabetes. It was observed that such body stature is more commonly seen in males than females.[46] Females in Hong Kong have the habit to take less food and low fat diet to reduce weight and keep slim in order to maintain beauty and good body stature. These are some of the reasons which may affect the condition of the lipid profile in the females. While more research is needed to clarify the mechanism.

Dyslipidemia in diabetes

Type 2 diabetes is typically represented in diabetes mellitus especially so in the mid and aged population. Accordingly, the majority of the diabetic cases in this study were type 2 diabetes. Type 2 diabetes is a complex metabolic disease, persistent hyperglycemia will evolve to insulin resistance and insulin secretion impairment and progress to dyslipidemia in the course of diabetes.[8] As reported the incidence of dyslipidemia in type 2 diabetes range from 34.8 – 90.7% which differs in different countries.[10,12] Dyslipidemia is a common clinical manifestation due to multiple causes which can be subdivided into a hereditary cause such as familial hypercholesterolemia and familial hypertriglyceridemia and a secondary cause commonly represented in hyperthyroidism, nephrotic syndrome , autoimmune disease (type 1 diabetes), and metabolic disease (type 2 diabetes).[48] Dyslipidemia may be expressed as a single rise in total cholesterol, TG, or LDL-C, or a combine rise in total cholesterol，TG and LDL-C which differs according to the etiology and mechanism of the underlying disease.[49] It was reported that the most common pattern of dyslipidemia in type 2 diabetic patients was elevated TG level and decreased in HDL-C level[17,50], a moderate elevation in total cholesterol and a mild rise in LDL-C was also observed in some reports[11] but less common and significant. The data of this study also demonstrated an elevation in TG level and a decrease in HDL-C level with no significant increase in total cholesterol and LDL-C levels which is the common manifestation of dyslipidemia in type 2 diabetes. It was also observed that the condition of dyslipidemia was correlated to blood glucose level as detected by FBG and HbA1c methods. A consecutive rise in TG mean values and a consecutive decrease in HDL-C mean values from normal,

borderline to diabetic group were also observed in this study. These findings are consistent with most observations reported from different countries.[17,51]

Another study reported that TG/HDL-C ratio in type 2 diabetes may be more sensitive than TG and HDL-C used solely in the prediction of diabetic dyslipidemia.[52] Our observation indicated that TG/HDL-C ratio was also significantly correlated with diabetic stage and showed a higher magnitude value than TG but had no statistical significance. Since an increase in TG level and a decrease in HDL-C level is the common manifestation of dyslipidemia in diabetes, TG/HDL-C ratio may be a useful tool for identification of people at diabetic risk.

Dyslipidemia was reported as a causal factor in atherogenesis, a morbidity related to cardiovascular disease (CVD) and coronary heart disease (CHD) and LDL-C is perceived as the pivotal agent in this process.[53] It was documented that dyslipidemia with a high TG and low HDL-C level in type 2 diabetes is an independent risk factor of CVD and has a 2-4 fold higher CVD mortality rate than non-diabetic individuals.[54] It was debated in the past why and how a high TG and low HDL-C with no significant rise in total cholesterol and LDL-C can evolve to atherogenesis. This mystery was solved only recently through investigations which indicated that LDL-C is not a homogenous substance as what was conceived formerly. Actually, about 15 distinct subpopulation of LDL-C particles may be grouped on a structural and metabolic bases into a minimum of three major subclass i.e. light and large LDL-C, intermediate LDL-C and small and dense LDL-C.[55-56] Further investigations indicate small and dense LDL-C are more atherogenic, they exhibit prolong residence time in the plasma and is easily oxidized to form oxidized small and dense LDL-C (ox-LDL-C) which has higher affinity and detrimental effect to human arterial wall and activated the monocyte-derived macrophage resulting in the formation of cholesterol foam and evolve to artherosclerotic plaque.[56-57] In addition, apoprotein β, a major protein of ox-LDL-C is susceptible to glycation in patients with diabetes. As a result, clearance of ox-LDL-C is depressed, the plasma half-life of these particles are increased which contributes to artherogenesis.[13,21] This small and dense LDL-C cannot be detected through ordinarily clinical laboratory methods hence unacknowledged in the past. Recent success in investigation indicated that small and dense LDL-C is an integral part of the hyperlipidemia in type 2 diabetes that was not detected in the past.[58] Moreover, there was intimate correlation with the hyperglycemia of the disease regardless of plasma lipid level.[57]

High TG level is also significantly related to type 2 diabetes. A high TG level in type 2 diabetes is a major determinant of LDL-C size, which is often accompanied with a high content of small and dense LDL-C[21] but undetectable by ordinary laboratory procedures. The size of LDL-C can now be

detected by using gel electrophoresis and ultracentrifugation techniques.[57] It is also apparent that low HDL-C as manifested in most type 2 diabetic patients is related to TG level. A high TG level impacts lipid metabolism in the adipose tissue and the liver. As the consequence, HDL-C becomes smaller in size and is removed faster from the blood circulation resulting in a low plasma HDL-C leve.[47]

To date it is more reasonable to suggest a high TG, small and dense LDL-C and low HDL-C levels are the real triad signature of dyslipidemia in type 2 diabetes, while small and dense LDL-C which was not detected and ignored in the past, in fact, is the crucial agent leading to atherogenesis which is the major cause of mortality in type 2 diabetes.

Discrepancy between FBG and HbA1c

FBG and HbA1c are the two typical modalities in use for the measurement of blood glucose level in this era. The correlation between FBG and HbA1c is of concern and interest universally. This study demonstrated a highly significant correlation between FBG and HbA1c by Pearson's correlation test. The results are consistent with previous studies.[24,32] Although FBG was significantly correlated with HbA1c by statistical analysis, inconsistency between FBG and HbA1c was observed in our data when the blood glucose level of the 788 subjects were re-categorized by using HbA1c and FBG methods separately as the criteria for classification and divided into a normal control, borderline group and diabetic group. A portion of the cases were not allocated to the same group when the subjects were classified into three groups based on FBG and HbA1c levels separately. Inconsistence between FBG and HbA1c was also observed and recently reported in the literature. A study of 1464 individuals classified as pre-diabetes, 1/3 of the cases were not allocated to the same group. FBG and HbA1c showed poor correlation. The overall agreement for allocation in the non-diabetic, pre-diabetic and suspected diabetic group was fair.[59] The overlap between FBG and HbA1c based category was low, ranging from 10.4 – 28% suggesting poor concordance between the two groups was also reported recently[60], but the underlying cause was seldom discussed in the reports. Another study reported the weakness of correlation between FBG and HbA1c with most likely reason due to the strong within–individual variability of FBG which is less considered in HbA1c.[61]

The data from this study indicated that FBG based criteria subgroup of group 1 has a higher incidence than HbA1c based group 1 (516 versus 342), but 188 of the 516 cases in the FBG based group 1 were allocated to group 2 to 3 (borderline or diabetic group) by the HbA1c based criteria (Figure 6 and 7). In the meanwhile when HbA1c < 6.0% was adopted as the criteria for classification in this HbA1c based criteria subgroup, the consistency between HbA1c and FBG rose to 95.91% (328 of 342 cases), 328 of 342 cases in HbA1c group 1 were allocated to FBG group 1 (Figure 7). It is possible that a part of the cases may have been treated with diabetic drugs before blood examination. It

is acknowledged that FBG represents the moment of FBG while HbA1c represent the blood glucose within 8 – 12 weeks. Theoretically, diabetic drug alleviates blood glucose level faster as reflected by FBG than the HbA1c assay. It is also possible that HbA1c may be capable of detecting the abnormal status in blood glucose level earlier than FBG, and more sensitive than FBG. As when FBG level of the patient is still within normal range, the HbA1c level may already have reached borderline or diabetic stage. Besides, most of the data in this study came from body check-up individuals whom were considered to be healthy, and so the second possibility is estimated to be the major cause of this discrepancy.

HbA1c represents an average glucose level including postprandial blood glucose level and fasting glucose level within 8-12 weeks. The postprandial glucose level is higher than the fasting blood glucose level. Rholfing also reported a strong correlation between postprandial blood glucose level with the HbA1c level.[61] Fasting blood glucose may conceal the impaired glucose tolerance, a pre-diabetes condition which is detected by oral glucose tolerance test (OGTT). OGTT test is a good predictor of early diabetes and more sensitive than FBG in diagnosis of pre-diabetes and diabetes.[3,24] Postprandial blood glucose level is usually increased before an increase in fasting blood glucose. Therefore, the postprandial blood glucose is an early indicator of impaired glucose metabolism and a sensitive predictor of developing diabetes.[32] HbA1c represents non-fasting blood glucose and hence has some similarity in property with postprandial glucose and OGTT. Apparently, HbA1c should also possess a higher sensitivity than FBG in the detection of early diabetic stage. This notion can solve the mystery in our observation why a portion of individuals in the FBG based criteria in group 1 were allocated to a higher (borderline or diabetic) group when HbA1c is adopted as the criteria for classification. We speculate that this special group of individuals may actually be early pre-diabetic patients but not detectable by FBG method. As a consequence, our finding demonstrated HbA1c is superior to FBG when applied as a screening purpose for diabetes because it may be more sensitive than FBG and patients can be detected at an earlier diabetic stage. However, future investigation is demanded to justify this hypothesis.

Because of the limitation in this study, we cannot really calculate the sensitivity of FBG and HbA1c test. The sensitivity is used to measure the proportion of sick people who are correctly diagnosed as sick by the test. The formula for sensitivity calculation: sensitivity = number of true positives ÷ (number of true positives + number of false negatives).[62] In this study, we cannot correctly identify the true pre-diabetes, diabetes and true healthy. Further investigation is necessary. For examples, 1.The individuals in normal and borderline group perform the OGTT test to identify the patients with pre-diabetes and diabetes. 2. The peoples in normal and borderline group

follow up for several years to observe the status of developing diabetes.

FBG and HbA1c represent two separate methods and principles which may also be applied for the measurement of blood glucose[43] both methods have advantages and limitations.[32] It has been debated for several decades whether FBG or HbA1c is more appropriate for use as a diagnostic tool in the different stages of diabetes. As reported, HbA1c measures blood glucose within 8 – 12 weeks is accordingly a more stable and reliable parameter for use in the confirmed diabetic patients during the chronic stage of the disease which may act as a guideline for treatment and it is also documented to be intimately related to the risk of microvascular and macrovascular complications in diabetes than a single or episode measurement by FBG assay.[63] This is the concept generally accepted currently. The American Diabetes Association subsequently adopted the use of the HbA1c for diagnosis of diabetes in 2010.[64] Besides using as a biomarker for pre-diabetes and screening purpose, HbA1c seems to be more sensitive than FBG assay as observed in this study. However, other studies suggested FBG and HbA1c when used together can significantly increase the efficiency of diabetes and pre-diabetic detection rate.[63]

Age distribution

The patient data were further divided into four groups in this study: aged 0-44 years; 45-64 years; 65-74 years and ≥75 years according to CDC 2011 criteria. Those age groups were further divided into 3 groups: normal control group, borderline group and diabetes group according to 2.3.2 Selection criteria. It was found that the percentage of patients diagnosed as diabetes in age group ≥75 years (66.7%) was more than three times that of patients under the age of 45 years (17%). According to the statistics of the Centers for Disease Control and Prevention (CDC) for the percentage of Civilian, Noninstitutionalized Population with Diagnosed Diabetes, by Age in the United States (1980-2010), the percentage of diabetes group in age more than 74 years (20.1%) was more than 11 times that of citizens below age of 45 years (1.8%). The finding of this study was in consistent with CDC's study. Therefore, there was a trend in the development of diabetes mellitus in the increasing age. In addition, it was interested to discover that there was a huge rise in percentage from aged 0-44 years (14%) to aged 45-64 years (29.4%) in the borderline group. People should continue monitoring their HbA1c values and aware of their diet management after the aged of 45 years.

Conclusion

Data analyses have indicated that blood glucose values were significantly correlated with higher TG, lower HDL-C levels, and higher TG/HDL-C ratio in the borderline and diabetes-groups when compared with the normal groups despite of sexes. Strict dietary management is recommended to these groups of patients for the control of DM and development of complication. Results showed that HbA1c is more sensitive than FBG to monitor the progress of DM. The

incidence rate of DM progressed with increasing age.

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