COMPARISON of 12-hour Fasting and 2-hour Post Prandial Sample Collection and Processing of Lipid Profile among Selected Dyslipidemic Patients from Cagayan de Oro, PHILIPPINES

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ABSTRACT

Lipid profile testing continues to be the method of choice for monitoring and predicting cardiovascular risk. Patient adherence to complete 12-hour fasting poses threat to test results validity as it contributes to reduced patient compliance to fasting, and inconvenience from patients with dyslipidemias. The aim of this study is to compare the lipid profile from a 12-hour fasting and 2-hour postprandial over a two-week interval for four weeks. The study followed a descriptive comparative design. Eighty four dyslipidemic subjects were recruited and serum samples were processed. Statistical difference and statistical interaction effects between the analyte measurements across time and patient preparation type was determined. The findings showed that there were no statistical differences between the pairwise comparison of lipid profile measurements between 12-hours fasting and 2-hours postprandial preparation. However, further analysis found a significant interaction between the triglycerides measurements in various time intervals and patient preparation type which indicates that 2-hours postprandial preparation may result in inconsistent triglyceride measurement compared to 12-hour fasting preparation.

KEYWORDS: *Lipid profile, cardiovascular risk, serum samples, dyslipidemias, patient compliance, Philippines*

INTRODUCTION

The practice of fasting in lipid profile has always been associated with several drawbacks. This can be a potential cause of error in the laboratory results and needs to be addressed (Patra et al., 2013). Patient adherence to a 12-hour

fasting regimen poses a threat to test validity, as it contributes to reduced compliance and patient inconvenience among patients with dyslipidemia and related diseases.

A twelve-hour fast is needed because of the theoretical changes that occur in test results for other lipid components during testing after meals (Langsted & Nordestgaard, 2019). There are certain alterations in the postprandial TG and calculated LDL-C (Dipankar & Pawar, 2019). TG after meals stay high for a few hours, and most serum lipid reference values are derived from blood samples collected during fasting (Nigam, 2010). Many laboratories estimate low-density lipoprotein cholesterol by the Friedewald equation, and since TG varies according to fasting status during the fat-tolerance test, the calculated LDL-C is also affected. Current recommendations for treating dyslipidemias in patients recommend the use of lipid profile components such as TC, LDL-C, and TG as primary targets for lipid-lowering therapy. Measurement of these components is usually done after a 12-hour fast (De Vries et al., 2014).

Errors are strictly prohibited in the laboratory and always will be. A study by Plebani (2006) stated that pre-analytical causes account for 46-68.2 percent of all errors. Supported by a study by Rana (2012), which revealed that 70 percent of the errors in the testing procedure occur during the pre-analytical phase of the process. One of the common causes of errors in the pre-analytical phase is the patient not being appropriately prepared for the test (Patra et al., 2013). In another study, it was stated that a patient who fails fasting contributes to this pre-analytical error (Abdollahi, 2014). The drawbacks of fasting can be a potential cause of pre-analytical errors in laboratory results and need to be addressed. According to Lee and Sidiqui (2023), the accuracy of the fasting lipid profile always depends on the patient's compliance.

Patient compliance is influenced by various factors, one of which is diabetes, which is at risk of hypoglycemia, and it cannot be avoided (Langsted & Nordestgaard, 2018). Another factor is that some people are not even truthful regarding their food intake or fasting state (Ghildiyal et al., 2020).

In the Philippines, laboratories still follow a strict rule of 12-14 hours fasting prior to blood collection for lipid profile (Department of Health). This is why most of the disadvantages of fasting in lipid profile are experienced every day, every morning, which also leads to commotion between the laboratory personnel and the patients. However, some countries already recommend

non-fasting preparation for lipid profile; these include Denmark in 2009, the United Kingdom in 2014, as well as Europe and Canada in 2016 (Dipankar & Pawar, 2019). There have been attempts to make the tests simpler by substituting the non-fasting lipid profile for the fasting lipid profile, as it was proposed that lipids and lipoproteins were scarcely altered in the fasting and non-fasting states. When assessing a lipid profile for cardiovascular risk prediction, there is currently no scientific evidence to suggest the advantage of fasting specimens over non-fasting specimens. Despite the emergence of studies challenging the fasting requirement for lipid profile testing, few countries, especially the Philippines, have adopted the rule yet, particularly in hospitals, including in Cagayan De Oro City.

The study aimed to compare lipid profiles (TC, TG, HDL-C, and LDL-C) in subjects with dyslipidemias who had undergone both a 12-hour fast and a 2-hour postprandial lipid profile. The results of the subject's lipid profile will be compared (12-hour fasting compared to 2-hour postprandial results) to determine if there is no significance in the differences between the results. This will provide evidence regarding the feasibility of substituting a brief period of fasting or non-fasting for the 12-hour fasting testing for lipid profile. The results of this research will provide a vital understanding of specimen collection for lipid profile tests. With this, it can eliminate the inconvenience and possible interferences encountered during pre-sample collection to ensure proper testing.

METHODS

The study was performed on 84 test subjects with dyslipidemia from Cagayan De Oro City, Philippines. The test subjects were recruited using a screening form to properly select the right subjects for the study. The height and weight of the subjects were assessed for the Body Mass Index (BMI). Two blood samples were collected from the subjects. One after an overnight fasting of 12 hours (complete fasting except for water), and after 2-3 days, another blood collection 2 hours after breakfast. Collections were repeated after two weeks and after four weeks.

Once the specimen was collected and prepared, a lipid profile was run using a standard routine lipid profile procedure using Roche Diagnostics Cobas C111 Chemistry Analyzer. LDL-C was calculated using the Friedewald equation (LDL-C = [TC] - [HDL-C] - [TG/5]). Reference values were based on levels mandated by the National Cholesterol Education Program (NCEP): < 200 mg/dL for total cholesterol, < 150 mg/dL for triglycerides, > 60 mg/dL for high-density

lipoprotein, and < 100 mg/dL for low-density lipoprotein. Results for the lipid profile (TC, TG, HDL-C, and LDL-C) from both 12-hour fasting and 2-hour postprandial samples were recorded in Microsoft Excel.

The data for the 12-hour fasting and 2-hour postprandial were analyzed using an independent samples T-test to see if there was a significant difference in results from the lipid profile coming from those who had 12-hour fasting and those who had 2-hour postprandial. The results were also analyzed using the repeated measures Analysis of Variance (ANOVA) to test the difference within subjects' baseline, two weeks, four weeks, and the interaction between time interval and the specimen's fasting type.

RESULTS

Lipid Profile values of the subjects

Table 1

Mean Lipid values of 12-hour fasting specimens from subjects with known dyslipidemias (N = 84)

Parameter	$\bar{x}$ (mg/dL)	SD
Triglycerides		
Baseline	148.7	84.0
2nd week	153.6	77.9
4th week	162.0	69.6
Average	154.8	74.0
Low-density Lipoprotein-cholesterol		
Baseline	130.2	68.7
2nd week	131.9	80.8
4th week	134.7	77.3
Average	132.3	69.8
High-density Lipoprotein-cholesterol		
Baseline	66.1	29.4
2nd week	67.6	23.8

Parameter	$\bar{x}$ (mg/dL)	SD
4th week	69.0	17.6
Average	67.6	21.5
Total Cholesterol		
Baseline	226.1	90.4
2nd week	230.2	99.8
4th week	236.0	89.1
Average	230.8	87.9

The mean serum lipid measurements for 12-hour fasting specimens are shown in Table 1. For the baseline, the mean TC was 226.1 mg/dL +90.4, after two weeks, the mean TC was 230.2 mg/dL +99.8, and finally after four weeks, the mean TC was 236.0 mg/dL +89.1. The overall mean of TC for the 12-hour fasting is 230.8 mg/dL +87.9. The mean TG measurements were 148.7 mg/dL +84, 153.6 mg/dL +77.9, and 162 mg/dL +69.6 for the baseline, after two weeks, and after four weeks, respectively. For HDL-C, its mean value for baseline was 66.1 mg/dL +29.4, 67.6 mg/dL +23.8 after two weeks, and 69 mg/dL +17.6 after four weeks, with an overall mean of 67.6 mg/dl +21.5. The mean value for LDL-C at baseline was 130.2 mg/dL +68.7, 131.9 mg/dL +80.8, after two weeks, and 134.7 mg/dL +77.3, with an overall mean of 132.3 mg/dL +69.8.

Values of lipid profile parameters TC, TG, and LDL-C were abnormal as expected with dyslipidemic subjects; HDL-C, although normal, was almost below 60 mg/dL (mean of 67.6). The values were compared with the NCEP guidelines. The results aligned with the study of AL-Mahdawi et al. (2021), where they compared the 2 hours after a meal and fasting lipid profiles in dyslipidemic patients. The values were also elevated in their study compared with the NCEP guidelines.

The study implies that dyslipidemic subjects have an imbalance of lipid metabolism, such as TC, TG, HDL-C, and LDL-C. Increased results of TC, TG, LDL-C, and decreased results of HDL-C may indicate a greater risk for cardiovascular diseases. It supports the study of Mahalle et al. (2014), which found that among the dyslipidemic patients they tested, 54.6 percent had low HDL-C, 63 percent had elevated TG, and 23 percent had increased TC.

Table 2

Mean Lipid values of 2 hours postprandial specimens from subjects with known dyslipidemias (N =84)

Parameter	$\underline{x}$ (mg/dL)	SD
Triglycerides		
Baseline	175.9	90.9
2nd week	172.2	83.6
4th week	177.6	75.7
Average	175.2	80.0
Low-density Lipoprotein-cholesterol		
Baseline	130.7	70.2
2nd week	128.0	78.0
4th week	130.5	74.6
Average	129.7	68.4
High-density Lipoprotein-cholesterol		
Baseline	69.6	32.1
2nd week	70.1	22.2
4th week	73.0	20.0
Average	70.9	22.5
Total Cholesterol		
Baseline	235.6	94.1
2nd week	232.5	95.2
4th week	239.0	85.7
Average	235.7	87.3

The data in Table 2 shows the mean results of serum lipids of 2-hours post-prandial specimens. The mean TC was 235.6 mg/dL +94.1 at baseline, 232.5 mg/dL +95.2 after two weeks, and 239 mg/dL +85.7 after four weeks, with an overall mean of 235.7 mg/dL +87.3. The mean TG was 175.9 mg/dL +90.9, 172.2 mg/dL +83.6, and 177.6 mg/dL +75.7 for baseline, after two weeks, and after four weeks, respectively. The overall mean of TG was 175.2 mg/dL +80. The mean HDL-C at baseline was 69.6 mg/dL +32.1, 70.1 mg/dL +22.2 after two weeks, and 73 mg/dL +20 after four weeks, with an overall mean of 70.9 mg/dL +22.5. The mean LDL-C at baseline was 130.7 mg/dL +70.2, 128 mg/dL +78 after two weeks, and 130.5 mg/dL +74.6 after four weeks, with an overall mean of 129.7 mg/dL +68.4.

In the 2-hour postprandial specimens, lipid profile values showed a noticeable increase. Just as with the 12-hour fast, the values were also abnormal

compared to the NCEP reference range. Abnormal values were expected due to the subject being a dyslipidemic. TG showed the highest elevation of two hours after a meal. The findings imply that although affected by diet in the 2-hour postprandial specimen, lipid profile values remain abnormal in dyslipidemic patients, as with the 12-hour postprandial preparation.

The data support the study by AL-Mahdawi et al. (2021), which found elevated levels in dyslipidemia, both in fasting and non-fasting preparations. It also supports the findings of Ghildiyal et al. (2020), where both fasting and non-fasting values of lipid profile were elevated. The result implies that the lipid profile parameters were still abnormal in either pre-sample collection preparation, 12-hour fasting preparation, or 2-hour postprandial.

Comparison of the Lipid Profile of the Subjects of 12-hour fasting and 2-hour postprandial

The pre-sampling collection method used in the study was compared. Comparisons were carried out between the 12-hour fasting and 2-hour postprandial in the baseline, after two weeks, and after four weeks.

Table 3

Pairwise Comparison of Serum Lipid Measurements Between 12-hour Fasting and 2-hours Post-Prandial specimens from subjects with known dyslipidemias (N=84)

Parameter	12-hour fasting (mg/dL)		2-hours post-prandial (mg/dL)		Mean Difference	t	p	Remarks
	$\bar{x}$	SD	$\bar{x}$	SD				
Triglycerides								
Baseline	148.7	84.0	175.9	90.9	-27.2	-2.011	0.05	Not Significant (NS)
2nd week	153.6	77.9	172.2	83.6	-18.7	-1.496	0.14	NS
4th week	162.0	69.6	177.6	75.7	-15.6	-1.387	0.17	NS

Average	154.8	74.0	175.2	80.0	-20.5	-1.721	0.09	NS
Low Density Lipoprotein								
Baseline	130.2	68.7	130.7	70.2	-0.6	-0.053	0.96	NS
2nd week	131.9	80.8	128.0	78.0	4.0	0.322	0.75	NS
4th week	134.7	77.3	130.5	74.6	4.2	0.357	0.72	NS
Average	132.3	69.8	129.7	68.4	2.5	0.237	0.81	NS
High Density Lipoprotein								
Baseline	66.1	29.4	69.6	32.1	-3.5	-0.734	0.46	NS
2nd week	67.6	23.8	70.1	22.2	-2.5	-0.714	0.48	NS
4th week	69.0	17.6	73.0	20.0	-4.1	-1.400	0.16	NS
Average	67.6	21.5	70.9	22.5	-3.4	-0.993	0.32	NS
Total Cholesterol								
Baseline	226.1	90.4	235.6	94.1	-9.5	-0.667	0.51	NS
2nd week	230.2	99.8	232.5	95.2	-2.3	-0.154	0.88	NS
4th week	236.0	89.1	239.0	85.7	-3.0	-0.222	0.82	NS
Average	230.8	87.98	235.7	87.3	-4.9	-0.365	0.72	NS

Statistically significant at 0.05 alpha

In the study findings shown in Table 3, differences in results in TC, HDL-C, and LDL-C between 12-hour fasting and 2-hour postprandial were not significant. These changes were minimal, which can be observed by a low mean difference between the three parameters. This means that the effects of food on TC, HDL-C, and LDL-C were minimal. This implies that the TC, HDL-C, and LDL-C can be tested regardless of the pre-sample collection method followed, whether it be 12-hour fasting or 2-hour postprandial. Since food intake after 2 hours is minimal, this could improve patient compliance, especially during

testing for TC, HDL-C, and LDL-C, as these parameters are used to evaluate or monitor cardiovascular disease risk.

According to Langsted and Nordestgaard (2018), there is substantial evidence linking LDL-C and TC to atherosclerotic cardiovascular disease and reducing both has been shown to significantly reduce the risk of both cardiovascular disease and death. For the TG values, it is shown that there was a noticeable mean difference between 12-hour fasting and 2-hour postprandial. This large mean difference (-20.5) suggests that TG measurement was affected by diet. This increase in mean difference was not necessarily caused by a clinical disorder as this was true to both healthy and in dyslipidemic patients from the studies of Dipankar and Pawar (2019), which reported a fasting and non-fasting TG of 121.16 mg/dl and 126.18 mg/dl respectively in healthy young adults, and to the study of Ghildiyal et al. (2020) where their TG levels were 230.53 and 281.92 for the fasting and non-fasting respectively in abnormal patients. The findings imply that TG measurement in dyslipidemics using 2-hour postprandial is not feasible.

Most of the findings support the large-scale study of Anne Langsted and Borge Nordestgaard (2019) in Copenhagen general population in Denmark which shows that lipids, lipoproteins, and apolipoproteins change minimally in response to normal food intake.

Comparison of the Lipid Profile of the Subjects over time

Table 4

Comparison between 12-hour fasting and 2-hours post-prandial specimen serum TG within time interval measurements among subjects with known dyslipidemias (N=84)

Parameter	Baseline (mg/dL)		2nd week (mg/dL)		4th week (mg/dL)		Within subject effect*	Between subject effect	Interaction effect*
TAG	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	p-value	p-value	p-value
12 hr fasting	148.7	84.0	153.6	77.9	162.0	69.6	0.001	0.190	0.041
2 hr post-prandial	175.9	90.9	172.2	83.6	177.6	75.7			

*Statistically significant at 0.05 alpha;*With Greenhouse-Geisser Correction*

The information contained in Table 4 shows the comparison of TG between 12-hour fasting and 2-hour postprandial over time. In all periods,

2-hour postprandial TG values were elevated compared to the 12-hour fasting. Statistical analysis, as indicated within the subject effect ($p<.05$), shows that there was a significant difference over time for TG. Although there were no pairwise differences as shown in the between-subject effect ($p>0.05$), which suggests that there was no significant difference between the 12-hour fasting and 2-hour postprandial, the interaction effect ($p<.05$) suggests that there was an interaction between the 2-hour postprandial preparation in various time intervals. This implies that a 2-hour postprandial lipid profile will vary over time and may result in inconsistent TG measurements. This variation may be caused by the food that the subject eats prior to specimen collection. Components of food will always vary from time to time and are also based on the preference of the subject.

The findings in this area align with the study of Dipankar and Pawar (2019), where they compared the lipid profile of healthy test subjects. They found that the mean TG for 12-hour fasting was 121.16 mg/dL and 126.18 mg/dL ($p<.05$), which was considered significant. This also supports the study conducted by Kaur and Harnam (2008), with the findings where they observed that TG levels increased postprandially compared to the fasting state. In their study group, the mean fasting TG was 173.4 mg/dL while the mean postprandial TG was 227.4 mg/dL.

An increase of triglycerides in the postprandial state was also mentioned by Langsteed and Nordestgaard (2018), stating that large-scale studies found a maximal increase of triglycerides of 3.0 mmol/L or 27 mg/dL, a few hours after normal food intake.

Table 5 shows the comparison of serum LDL-C between the 12-hour fasting and 2-hour postprandial specimens over time. Statistical analysis shows that differences in the mean LDL-C over time were minimal and not significant, as shown in the within-subject effect ($p=0.754$). Also, there were no pairwise differences between the 12-hour fasting and the 2-hour postprandial conditions, as shown by the between-subjects effect ($p=0.913$). Statistical analysis indicates that fasting time for LDL-C does not have significant interaction with the interval of results, as shown in the interaction effect ($p>.05$). This data implies that LDL-C can also be calculated using the Friedewald equation from 2-hour postprandial specimens. It suggests that LDL-C was not affected by diet, which can improve the testing for lipid profile since LDL-C is considered a target for lipid-lowering therapies. It also suggests that LDL-C can be measured in

dyslipidemic subjects using a 2-hour postprandial specimen.

Table 5

Comparison Between 12-hour fasting and 2-hours post-prandial specimen serum LDL-C within time interval measurements among subjects with known dyslipidemias (N=84)

Parameter	Baseline (mg/dl)		2nd week (mg/dl)		4th week (mg/dl)		Within subject effect*	Between subject effect	Interaction effect*
LDL	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	p-value	p-value	p-value
12-hr fasting	130.2	68.7	131.9	80.8	134.7	77.3	0.754	0.913	0.772
2-hr post-prandial	130.7	70.2	128.0	78.0	130.5	74.6			

*Statistically significant at 0.05 alpha;*With Greenhouse-Geisser Correction*

Notably, Langsted and Nordestgaard (2018) emphasized that there are ongoing debates about the use of the Friedewald equation, which should be calculated using a fasting TG measurement. However, their data show that calculated LDL-C changes only minimally in response to normal food intake. They added that increased non-fasting LDL-C predicts increased risk of cardiovascular events. According to Solnica et al. (2020), the LDL-C level is a primary lipid determinant of cardiovascular risk because of the important predictive role that LDL-C plays in atherogenesis.

Table 6

Comparison Between 12-hour fasting and 2-hours post-prandial specimen serum HDL-C within time interval measurements among subjects with known dyslipidemias (N=84)

Parameter	Baseline (mg/dl)		2nd week (mg/dl)		4th week (mg/dl)		Within subject effect*	Between subject effect	Interaction effect*
HDL- cholesterol	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	p-value	p-value	p-value
12-hr fasting	66.1	29.4	67.6	23.8	69.0	17.6	0.123	0.322	0.130
2-hr Post- prandial	69.6	32.1	70.1	22.2	73.0	20.0			

*Statistically significant at 0.05 alpha;*With Greenhouse-Geisser Correction*

Statistical analysis (Table 6) shows that there was a minimal difference between the baseline, two weeks, and four weeks of HDL-C measurement, and these differences are not significant as shown in the within-subject effect ($p>.05$). There were no pairwise differences between the 12-hour fasting and

2-hour postprandial, as shown in the between-subject effect (p>.05), and fasting time had no significant interaction with the interval of results, as shown in the interaction effect (p>.05). This means that the interval of results is not significantly influenced by either the 12-hour fasting or the 2-hour postprandial preparation. These findings imply that HDL-C, which can also serve as a risk estimator, can be measured in dyslipidemic subjects using a 2-hour postprandial specimen. The measurement was not affected by diet and will not vary over time.

This finding agrees with the work of Umakanth (2018), where he stated that TC and HDL-C are used for several risk estimators, both of which vary a little between the fasting and non-fasting state. This is also supported by the study of Dipankar & Pawar (2019). Their HDL-C results from fasting were 45.08 mg/dl and postprandial were 43.84 mg/dl, where the difference was caused by normal food intake, minimal, and is not significant.

Table 7

Comparison Between 12-hour fasting and 2-hours post-prandial specimen serum TC within time interval measurements among subjects with known dyslipidemias (n=84)

Parameter	Baseline (mg/dl)		2nd week (mg/dl)		4th week (mg/dl)		Within subject effect*	Between Subject effect	Interaction effect*
Total Cholesterol	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	p-value	p-value	p-value
12-hr fasting	226.1	90.4	230.2	99.8	236.0	89.1	0.176	0.716	0.499
2-hr post-prandial	235.6	94.1	232.5	95.2	239.0	85.7			

*Statistically significant at 0.05 alpha;*With Greenhouse-Geisser Correction*

The information in Table 7 shows the comparison of TC between the 12-hour fasting and 2-hour postprandial specimen over time. Mean TC from 2-hour postprandial specimen in baseline, after two weeks, and after four weeks were higher than that of the 12-hour fasting. Statistical analysis shows that there was no significant difference between the baseline, two weeks, and four weeks of total cholesterol measurement as shown in within subject effect (p>05). There were no pairwise differences between the 12-hour fasting and 2-hour postprandial as shown in the between subject effect (p = 0.716). In addition, as shown in the interaction effect (p=0.499), it suggests that fasting time of TC has no significant interactions the interval results. Results show that there was no significant difference between the 2-hour postprandial and the 12-hour fasting

time when measuring the total cholesterol levels in dyslipidemic subjects. This implies that TC measurement can be done in dyslipidemic patients using 2-hour postprandial preparation. It is also not greatly affected by food intake. This is a good indicator that TC can be used any time for checking and monitoring dyslipidemic patients.

The findings were also in agreement with that of Dipankar and Pawar (2019) where their measured total cholesterol in fasting and postprandial was 192.1 mg/dl and 194.98 mg/dl respectively. It shows minimal changes and are unimportant. According to Umakanth (2018), together with HDL, total cholesterol is risk estimators for cardiovascular disease. In addition, based on the statistical analysis, interval of results is not influenced by both 12-hour fasting and 2-hour postprandial preparation.

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