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Breaking the Myth of Lipid Profile Analysis in Fasting State

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Abstract: Pathogens or the toxins produce communicable diseases all others are non-infectious diseases, including most forms of cancer, cardiovascular and genetic disease. Clinical laboratory play an incomparable role in diagnosis of such diseases. Lipid profile is an important parameter among others which used as gold standard to screen a person's cardiac health status. The aim of the study is to breaking the myth of lipid profile in fasting state since patients are in convinced by having to return on a separate visit for a fasting lipid profile and also, laboratories are burdened by a large volume of patients attending for tests in morning. The study was conducted on volunteers to analyze lipid analysis in fasting and non-fasting status. Result proves that there is no statistical significant difference in both fasting and non-fasting levels of lipids. Hence the study concludes lipid profile can be done at any time to screen the lipid status, unless the person is known familial hyperlipidemia.
Keywords: Cardiovascular diseases, fasting, hyperlipidemia, non-fasting, lipid profile.

INTRODUCTION

In recent years, health concern continues to pose a major challenge for developing countries. They have always had to compete with infectious diseases. Most common life style diseases include Diabetes mellitus, Cardiovascular disease, Chronic Kidney disease etc., to deal with these burdens, drug development and alternative treatments are required, as well as measures to ensure that people in need have access to medicines and good standards of care. Modernization has been an accelerating persuade across the world in the last few centuries.

There is a process of adopting Western culture in areas such as industry, health care, technology, law, politics, economics, lifestyle, diet, clothing, language, alphabet, religion, philosophy, and values [1].

Emerging Evidence of Sedentary Behavior

Community indicators of reductions in human energy expenditure and increases in sedentary behavior during the past several decades are predominantly striking. In 2003, nearly 6 in 10 working adults used a computer on their profession and more than 9 in 10 children used computers in school (kindergarten through grade 12 [2]. Other considerable contributors to daily sitting time—watching television and driving personal vehicles—are at all-time highs, with estimates of nearly 4 hours and 1 hour, respectively [3]. Scientists studying the ill effects of this decrease in physical activity have revealed a complex, multifaceted relationship among physical work, energy expenditure, and health [4]. Clinical and basic research has focused on the benefits of incorporating regular bouts of exercise into modern life to adjust to some extent for the loss of the physically active life led by our ancestors [4]. Diabetes onset due to carbohydrate intolerance during pregnancy is called as Gestational Diabetes Mellitus (GDM), irrespective of the treatment with diet or insulin. The risk of developing diabetes in the future is nearly two generations in consideration of GDM.

Women with a history of GDM are at higher risk of future diabetes, predominately type 2 diabetes, as are their children [5]. Screening is indispensable in all pregnant women in India, as the women in India have 11 fold increased risk of developing glucose intolerance during pregnancy compared to Caucasian women [6]. The evidence base that supports this exercise suggestion is considerable. A sedentary lifestyle is defined as major type of lifestyle where an individual does not receive standard amounts of substantial activity. Current epidemiologic evidence suggests that the metabolic and long-term health consequences of habitual sedentary behavior (too much sitting) are different from those associated with a lack of moderate-to-vigorous activity (too little exercise) [7, 8]. This transfer in perception is being clarified through

innovations in technology used to characterize association patterns in populations.

Males - greater risk of heart diseases

Men are at greater risk of heart disease than pre-menopausal women [9]. Once post menopause, it has been argued that a woman's risk is similar to a man even though recent data from the WHO and UN disputes this [9]. If a female has diabetes, she is more probable to develop heart disease than a male in diabetes. Coronary heart diseases are 2 to 5 times more common among middle-aged men than women [10]. In a study done by the World Health Organization, sex contributes to approximately 40% of the deviation in sex ratios of coronary heart disease mortality [11]. Similar study results that gender differences explain nearly half the risk associated with cardiovascular diseases [10]. One of the proposed explanations for gender differences in cardiovascular diseases is hormonal difference. Among women, estrogen is the predominant sex hormone. Protective effects of estrogen on glucose metabolism and hemostatic system, and they may have direct effect in enhancing endothelial cell function. The production of estrogen decreases after menopause and this may change the female lipid metabolism toward a more atherogenic form by decreasing the HDL cholesterol level while increasing LDL and total cholesterol levels.

Among men and women, there are notable differences in body weight, height, body fat distribution, heart rate, stroke volume, and arterial compliance. In the very elderly, age-related large artery pulsatility and stiffness is more pronounced among women than men. This may be caused by the women's smaller body size and arterial dimensions which are independent of menopause [12].

Significance of lipid profile

Lipid profile or lipid study a group of blood tests that serves as an initial broad clinical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases. Lipid panels are commonly ordered as part of a physical exam, master health checkups along with other panels such as the complete blood count (CBC) and basic metabolic panel (BMP).The lipid profile typically includes: Total cholesterol, Triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL), Cholesterol: HDL ratio. Most of the physicians and the laboratories are preferred to be in fasting for the above test. It's always having certain difficulties to be in fasting state for the particular analysis. It's always possible for a healthy male or female who no need to be assist to reach for the analysis. Mostly the difficulty occurs for the elderly individuals who totally depend of their children. If the elder one is diabetic or with some chronic complications, it adds even more difficulty for the procedures. As an added benefit to such patients the study is aimed to analyze the lipid profile in fasting and non-fasting state and compare whether the difference in value changes the medical decision which is strictly followed by the CLSI 88 guideline.

Aim

The aim of the study is to analyze lipid profile in fasting and non-fasting state and compare whether the difference in value changes the medical decision and to provide information of acceptable state of testing.

MATERIALS AND METHODS

Sample Design

The study was conducted on volunteer's age of 40 – 60 yrs who randomly came for the laboratory for analysis of lipid profile at Billroth Hospitals, Shenoy Nagar, Chennai-600030. Eligible patients included both men and women. The study was approved by the institutional ethics committee IEC No: BHL/lab projects/2018/006

Sample Collection

Based on the requisition given by the consultant for lipid profile, the blood samples were collected from the individuals by the Phlebotomist in the OPD of Billroth Hospital, at phlebotomy area. Initially Fasting Blood samples were collected in plain Becton Dickinson (BD) Vacutainer® was allowed to clot and centrifuged at 5000 rpm for 20 min. Later the non-fasting (random or a post prandial) plain blood sample was also requested and taken from the same volunteers for comparison of fasting and non fasting. Parameters were analyzed using fully automated biochemistry analyzer Beckman Coulter AU 480.

METHODS

Total Cholesterol [13]: The Cholesterol reagent utilizes an enzymatic method to measure cholesterol in human serum. Cholesterol esters in a sample are hydrolyzed by cholesterol esterase (CHE). The produced free cholesterol is oxidized by cholesterol oxidase (CHO) to cholestene-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield a chromophore. The red quinoneimine dye formed can be measured spectrophotometrically at 540/600 nm as an increase in absorbance.

Triglycerides [14-16]: This Triglyceride procedure is based on a series of coupled enzymatic reactions. The triglycerides in the sample are hydrolysed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-

phosphate is oxidised by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The formed H_2O_2 reacts with 4-aminophenazone and N, N-bis (4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800nm. The increase in absorbance at 660/800nm is proportional to the triglyceride present in the sample.

HDL-Cholesterol [13]: Anti-human-β-lipoprotein antibody in reagent one binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons). The antigen-antibody complexes formed block enzyme reactions when second reagent is added. HDL-cholesterol is quantified by the presence of an enzyme chromogen system.

LDL-Cholesterol [17-19], VLDL and non-HDL: Parameters are calculated using Friedewald formula.

Statistical Analysis

Data were statistically analyzed based on the CLIA 88 guidelines acceptable limit calculation which is medically used whether the difference in results have impact on medical decision, Difference in fasting and non-fasting were analyzed based on Standard deviation.

Table-1: Comparative Analysis of Total Cholesterol Levels in Fasting and Non-Fasting

COMPARATIVE ANALYSIS OF TOTAL CHOLESTEROL LEVELS IN FASTING AND NON FASTING						
S.No	**Volunteer Name**	**Age**	**Sex**	**Cholesterol F**	**Cholesterol NF**	**Acceptable limit**
1	Sabanayagam	24	M	161	139	15.8
2	Sivakamasundari	28	F	139	139	0.0
3	Gunasekaran	41	M	217	222	-2.3
4	Dhanalakshmi	57	F	223	211	5.7
5	Pradeep Kumar	29	M	146	141	3.5
6	Subramanian	74	M	154	145	6.2
7	Sundaram	53	M	278	240	15.8
8	Selvam	64	M	203	180	12.8
9	Peer Mohammed	80	M	223	226	-1.3
10	Suresh	42	M	237	228	3.9
11	Chandrasekhar	69	M	213	210	1.4
12	Dhema	47	F	193	197	-2.0
13	Shahul	48	M	183	201	-9.0
14	Sampath	30	M	132	121	9.1
15	Malar	47	F	196	194	1.0
16	Akanksha Rai	23	F	166	189	-12.2
17	Sarathi	37	M	185	177	4.5
18	Mohammed Shamimulla	63	M	211	214	-1.4
19	Jayaprabha	40	F	185	186	-0.5
20	Prathyusha	22	F	144	148	-2.7
21	Priyanka	23	F	142	134	6.0
			Mean	**187.1904762**	**182.952381**	**2.594736374**
			STD	**38.08624299**	**36.33108337**	**7.115950855**
			CV%	**0.203462504**	**0.198582184**	**2.742456199**

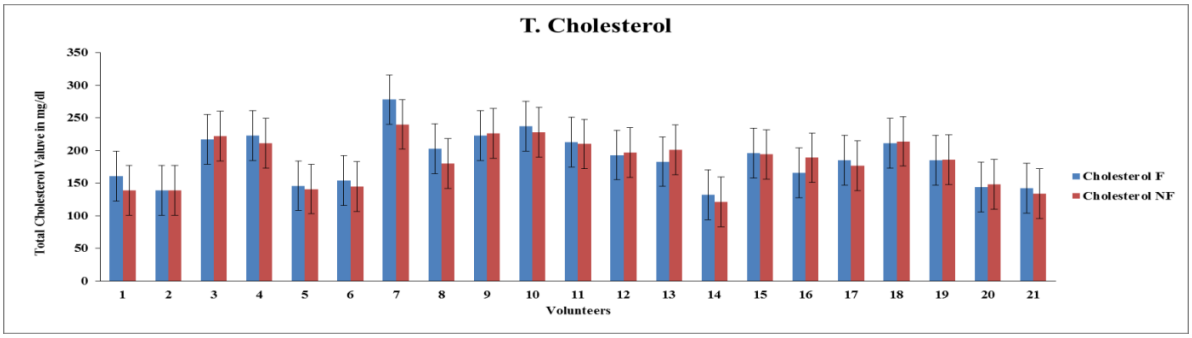

Fig-1: Comparative Analysis of Total Cholesterol Levels in Fasting and Non-Fasting

Legend: Each bar represents the value of Cholesterol among volunteers in fasting and the parallel bar indicates the Non fasting levels, The significance difference is based on the standard deviation of the same.

Table-2: Comparative Analysis of Serum Triglycerides Levels in Fasting and Non-Fasting

COMPARATIVE ANALYSIS OF SERUM TRIGLYCERIDES LEVELS IN FASTING AND NON FASTING						
S.No	**Volunteer Name**	**Age**	**Sex**	**TGL F**	**TGL NF**	**Acceptable limit**
1	Sabanayagam	24	M	146	188	-22.3
2	Sivakamasundari	28	F	116	152	-23.7
3	Gunasekaran	41	M	208	179	16.2
4	Dhanalakshmi	57	F	141	159	-11.3
5	Pradeep Kumar	29	M	138	132	4.5
6	Subramanian	74	M	160	242	-33.9
7	Sundaram	53	M	131	203	-35.5
8	Selvam	64	M	292	338	-13.6
9	Peer Mohammed	80	M	141	170	-17.1
10	Suresh	42	M	137	155	-11.6
11	Chandrasekhar	69	M	109	115	-5.2
12	Dhema	47	F	94	96	-2.1
13	Shahul	48	M	156	173	-9.8
14	Sampath	30	M	129	143	-9.8
15	Malar	47	F	197	224	-12.1
16	Akanksha Rai	23	F	62	71	-12.7
17	Sarathi	37	M	126	166	-24.1
18	Mohammed Shamimulla	63	M	109	123	-11.4
19	Jayaprabha	40	F	124	159	-22.0
20	Prathyusha	22	F	52	77	-32.5
21	Priyanka	23	F	50	44	13.6
			Mean	**134.1904762**	**157.5714286**	**-13.15246141**
			STD	**53.93108477**	**64.26474261**	**13.82132514**
			CV%	**0.401899496**	**0.407845148**	**-1.050854643**

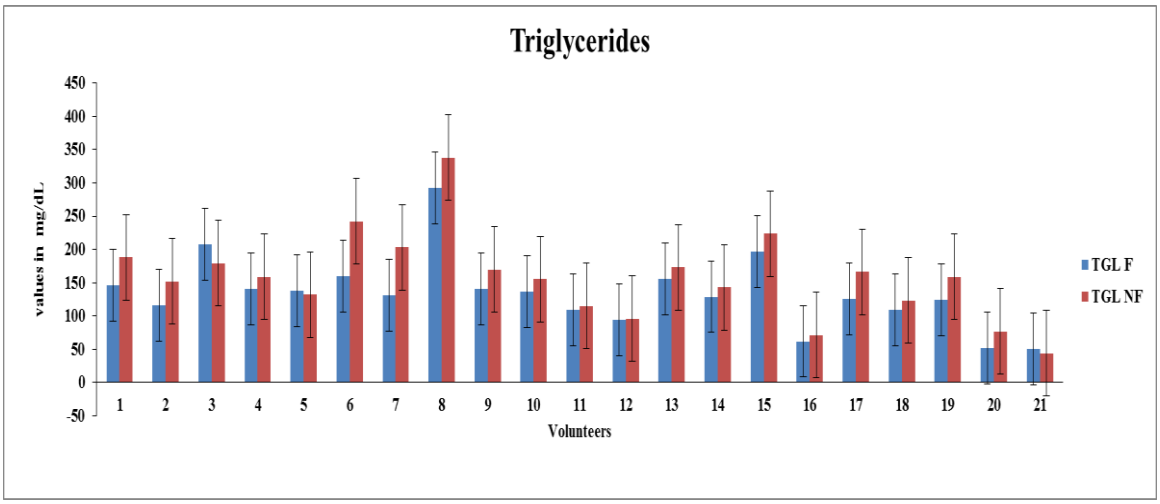

Fig-2: Comparative Analysis of Serum Triglycerides Levels Infasting and Non-Fasting

Legend: Each bar represents the value of triglycerides among volunteers in fasting and the parallel bars indicate the Non-fasting levels, the significance difference is based on the Standard deviation of the same.

Table-3: Comparative Analysis of Serum HDL Cholesterol Levels in Fasting and Non-Fasting

COMPARATIVE ANALYSIS OF SERUM HDL CHOLESTEROL LEVELS IN FASTING AND NON FASTING						
S.No	**Volunteer Name**	**Age**	**Sex**	**HDL F**	**HDL NF**	**Acceptable limit**
1	Sabanayagam	24	M	35.4	33	7.3
2	Sivakamasundari	28	F	40.9	39	4.9
3	Gunasekaran	41	M	39.2	37	5.9
4	Dhanalakshmi	57	F	50.6	45	12.4
5	Pradeep Kumar	29	M	36.5	32	14.1
6	Subramanian	74	M	42.7	37	15.4
7	Sundaram	53	M	58	48	20.8
8	Selvam	64	M	51.8	44	17.7
9	Peer Mohammed	80	M	48.1	45	6.9
10	Suresh	42	M	47	45	4.4
11	Chandrasekhar	69	M	38	36	5.6
12	Dhema	47	F	50.4	50	0.8
13	Shahul	48	M	38	41	-7.3
14	Sampath	30	M	32.6	30.1	8.3
15	Malar	47	F	39	37	5.4
16	Akanksha Rai	23	F	53.8	57	-5.6
17	Sarathi	37	M	34	32	6.3
18	Mohammed Shamimulla	63	M	43.3	43	0.7
19	Jayaprabha	40	F	40.1	38	5.5
20	Prathyusha	22	F	49	49	0.0
21	Priyanka	23	F	59	55	7.3
			Mean	**44.16190476**	**41.57619048**	**6.513284541**
			STD	**7.809832021**	**7.519435136**	**6.943646829**
			CV%	**0.176845452**	**0.180859166**	**1.066074541**

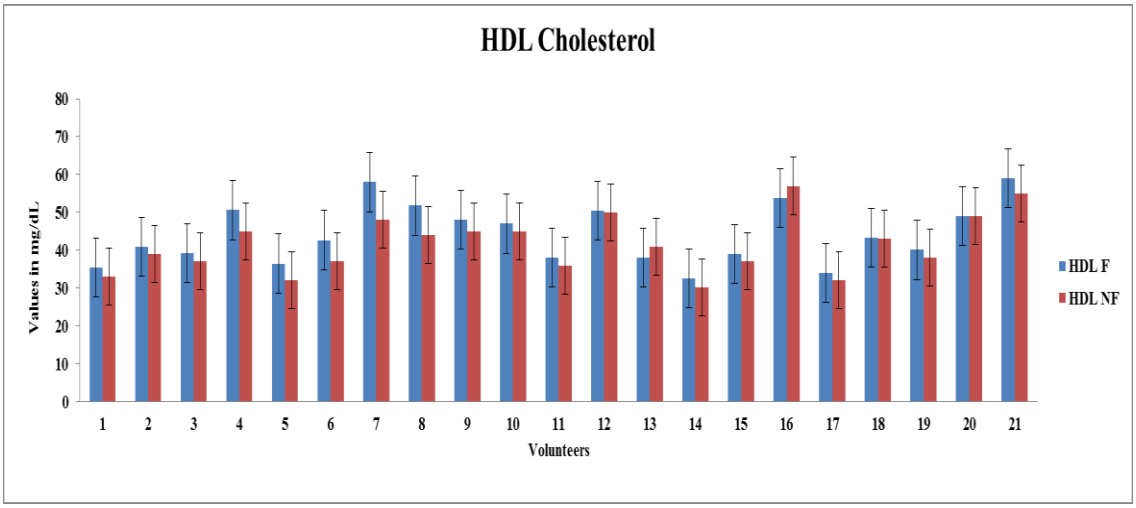

Fig-3: Comparative Analysis of Serum HDL Cholesterol Levels In Fasting And Non-Fasting

Legend::Each bar represent the value of HDL Cholesterol among volunteers in fasting and the parallel bar indicate the Non fasting levels, the significance difference is based on the Standard deviation of the same.

RESULTS & DISCUSSION

Medical diagnosis and the treatment are ultimately based on the report generated for the clinical laboratory and the parameters selected for testing. The current illness and the management of disease completely based on the quality diagnosis. Hence the laboratory plays an inevitable role in providing the error free reports. Based on the previous reports, the errors in the laboratory are common among that the critical phase of occurring error is pre analytical which is proved as 46-68%. The testing in fasting and non-fasting is always a big challenge for the elderly and the patients in diabetes. The information provided by the patient may be false, some time there is hiding of information about fasting and basal state is also a challenging task for a clinical laboratory to release a proper report. All these type of ignorance will lead to the wrong interpretation of clinical results while reporting of particular analyte. Sometimes testing and retesting of the same analyte was done for the proper interpretation of the disease, which adds the patients for unnecessary burden to financial, physical and psychological.

One among the parameter which is always preferred to be in fasting is lipid profile which has its greater advantage in screening and diagnosis of CVD, ASVD and hyperlipidemia. Lipid profile has important implications in clinical practice. Requiring patients to fast causes patients increases stress, potential hypoglycemia in patients with diabetes mellitus, increased transportation costs, and potentially missed days of work. In addition, the inconvenience of fasting may also delay treatment or diagnosis of hyperlipidemia if patients are unable to fast before the clinic visits. Even after reach the laboratory the waiting time from billing to sample collection will affect the fasting state; it also varies on working and non working day. Non-fasting lipid profiles would improve patient satisfaction and potentially avoid delays in screening, diagnosis and treatment.

Difference in fasting and non-fasting were analyzed based on standard deviation. Figure-1: represents the comparative analysis of total cholesterol levels in fasting and non-fasting. Results proven that no statistical difference seen while compare with the fasting and non-fasting total cholesterol levels. Apart froma case of known familial genetic hyperlipidemia or premature atherosclerotic cardio vascular diseases. The difference in values were statistically analyzed using $p<0.05$ as significant. The comparative analysis of serum triglyceride levels in fasting and non-fasting shows there is no major considerable difference found in both the state. Even in serum triglyceride levels were not acceptable in the same case of familial hyperlipidemia. While analyzing serum HDL cholesterol levels the results indicate that there is no significant variation in both the state as in the above said parameters. Even in calculated parameters LDL Cholesterol / VLDL and Non HDL are in similar trend. All these three parameters were obtained usually by Friedewald's formula based on standard calculation. All the variation analysis was verified based on the medically accepted limit which already exists in field of Clinical diagnosis. The data is subjected to the standard formula for acceptable limit in which medical decision will be calculated / analyzed. If the value is deviated above or below the published guideline will definitely have medical impact which leads the clinicians to mislead the patient's treatment.

Requirement of fasting is required in screening and following patients with family history of genetic hyperlipidemia or premature ASVD and in diagnosing hypertriglyceridemia. Non-Fasting is accepted in estimating initial primary prevention in an untreated patient and in clarifying diagnosis of metabolic syndrome. For patients with a family history of premature Atherosclerotic Cardiovascular disease (ASVD) or features suggestive of familial hyperlipidemia, screening and follow-up should perfectly be performed with fasting lipid panels. Among those with a non-fasting triglyceride level ≥200mg/dl, a follow-up fasting lipid panel should be performed. Those who resent with secondary causes of hyperlipidemia (due to diet, drugs, diseases, or disorders of metabolism) should have a fasting lipid panel performed. Indeed, it may be important for those about to initiate therapy with estrogenic hormones, steroids, retinoic acid, or certain antineoplastic agents to understand their propensity for severe hypertriglyceridemia and subsequent risk of pancreatitis [20]. Results of total lipid profile found no difference while compare with the fasting and non-fasting levels. Except in a case of familial / genetic hyperlipidemia or premature atherosclerotic cardio vascular disease.

CONCLUSION

This pilot study proves that a lipid profile can be tested at any time irrespective of time and food intake. The variation in both food and time will never affect test results this was confirmed based on the medical acceptable limit and the statistical analysis. This ultimately prevents males with cardiac disorder and other heart diseases without delay in treatment. Is should be avoided if the person is known case of hereditary hyperlipidemia and the CVD.

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