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Postprandial dyslipidemia: pathophysiology and cardiovascular disease risk assessment

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ABSTRACT

Although the fed state predominates over the course of a day, the fasting lipid profile has traditionally been used to assess cardiovascular disease (CVD) risk. The nonfasting lipid profile may be more reflective of the daily circulating plasma lipids and simplifies lipid monitoring for patients, laboratories, and clinicians. Nonfasting triglyceride levels are also independently associated with cardiovascular events, leading to several clinical guidelines (e.g. in Denmark, the UK, Europe, and Canada) now recommending nonfasting lipid testing in the primary prevention setting.

Obese and insulin resistant states are associated with intestinal chylomicron overproduction and subsequent remnant lipoprotein accumulation, leading to development of postprandial dyslipidemia in the fed state. Postprandial dyslipidemia is thought to be a major contributor of atherogenesis and shown to be an important CVD risk factor. As intestinal peptides (e.g. glucagon-like-peptide 1; GLP-1) have been shown to regulate chylomicron output, alterations in these signaling pathways in insulin resistant states may play a role in the development and/or progression of postprandial dyslipidemia.

Although several advances have been made in understanding postprandial dyslipidemia in insulin resistance and its association with CVD, several limitations remain. Although nonfasting lipid measurements (i.e. random blood sampling) are now recommended in some countries, a more functional assessment of postprandial lipemia involves ingestion of a high-fat meal with subsequent blood collection over a specified time period (i.e. oral fat tolerance test). However, oral fat tolerance test methodology remains largely unstandardized and reference values to interpret postprandial values remain to be accurately established. Development of standardized methodologies and biomarker profiles for assessment of postprandial dyslipidemia in clinical practice will enable early and accurate identification of those at risk for CVD.



Abbreviations

apoB-48: apolipoprotein B-48

apoB-100: apolipoprotein B-100

CM: chylomicron CVD: cardiovascular disease

DGAT: diacylglycerol acyltransferase

DPP-4: dipeptidyl peptidase-4

FA: fatty acid

HDL-C: high-density lipoprotein cholesterol

GLP-1: glucagon-like peptide-1

GLP-2: glucagon-like peptide-2

LDL-C: low-density lipoprotein cholesterol

LPL: lipoprotein lipase

MCP-1: monocyte chemoattractant protein-1

MTP: microsomal triglyceride transfer protein

OFTT: oral fat tolerance test

PAI-1: plasminogen activator inhibitor-1 (PAI-1)

PCOS: polycystic ovary syndrome

RLP: remnant lipoprotein

T2D: type 2 diabetes

TG: triglyceride

TRL: triglyceride-rich lipoprotein

VLDL: very low-density lipoprotein



POSTPRANDIAL LIPID METABOLISM

With the current eating patterns in Western societies, the fed state predominates over the course of a day, with the typical individual only in the fasted state for a few hours in the early morning (1). Nevertheless, the fasting lipid profile has been a standard assessment of cardiovascular disease (CVD) risk. There are two primary reasons for traditionally measuring fasting triglycerides (TG): to reduce the variability in TG concentration following meal ingestion and to accurately calculate low-density lipoprotein cholesterol (LDL-C) using the Friedewald equation. However, nonfasting (i.e. random blood sample measurement irrespective of time since last meal) TG levels have been reported to fluctuate only modestly within the same individual (2). Additionally, calculated LDL-C has been shown to change minimally after food intake (3) and measured and calculated LDL-C are highly correlated between fasting and nonfasting states (4,5). As nonfasting TG levels are independently associated with cardiovascular events (6), a paradigm shift towards assessing lipid parameters in the nonfasting or postprandial (i.e. blood sample measurement at specified time points following a standardized meal) state is occurring (7). In fact, postprandial TG levels obtained after consuming a standardized high-fat meal, better predict coronary artery disease compared to fasting TG levels (8). Several clinical guidelines have included nonfasting lipid testing in the primary prevention setting, including Denmark in 2009 (3), UK in 2014 (9), as well as the European Atherosclerosis Society (EAS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) (7) and the Canadian Cardiovascular Guidelines in

2016 (10). The nonfasting lipid panel has become the clinical standard in Denmark, offering physicians the option to measure fasting lipids when TG > 4 mmol/L (3), while in Canada it is recommended to obtain a fasting measurement when TG > 4.5 mmol/L (10). Furthermore, the EAS/EFLM guidelines state that nonfasting and fasting measurements should be complementary and not mutually exclusive (7). The option of nonfasting lipid testing has also been included in The 2011 National Heart, Lung, and Blood Institute (NHLBI) Guidelines specific for the pediatric population (11). Assessing the postprandial lipid profile can provide a better indication of an individual's capacity to metabolize lipids following a meal, reflecting their metabolic efficiency.

Following ingestion of a fat-containing meal, cholesterol, monoacylglycerol, and fatty acids (FAs) are absorbed by the small intestine and re-esterified in the enterocyte. Triglyceride-rich lipoproteins, termed chylomicrons (CMs) are formed within the enterocyte, comprised of cholesterol, TG, phospholipids, and apolipoproteins (12). These TG-rich lipoproteins (TRLs), containing apolipoprotein B-48 (apoB-48), are subsequently secreted into lymphatic vessels, entering the blood via the thoracic duct. As CMs travel through the circulation, TGs are hydrolyzed by lipoprotein lipase (LPL), releasing FAs for subsequent uptake by peripheral tissues. As CMs become TG-depleted, they form smaller, cholesteryl ester- and apoE-enriched particles, termed CM remnants. CM remnants then compete with hepatic-derived TRL (i.e. apoB-100-containing very-low density lipoprotein (VLDL)) remnants for clearance by the liver through apoE binding to the LDL receptor or LDL-receptor-related protein. Accumulation of remnant lipoproteins (RLPs), often present in obese and insulin resistant states, is a major CVD risk factor. In fact, T2D patients have a three- to fourfold increased risk of death due to CVD (13),

accounting for the majority of deaths in these patients (14). Patients with type III hyperlipoproteinemia, a genetic disorder characterized by RLP accumulation as a result of impaired RLP removal, are also predisposed to premature atherosclerosis and CVD risk (15).

This state-of-the-art review discusses the current scientific knowledge of postprandial dyslipidemia. The dysregulation of postprandial lipid metabolism in insulin resistant states and subsequent CVD risk, regulation of postprandial lipids by the brain-gut neuroendocrine axis, clinical assessment of postprandial dyslipidemia, as well as current limitations and future research directions will be discussed.

POSTPRANDIAL LIPIDS AS MARKERS OF CARDIOVASCULAR DISEASE RISK

The traditional fasting lipid profile used to assess CVD risk includes TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and LDL-C. However, evidence is lacking that fasting measurements of lipid parameters are superior to nonfasting measurements. Rather, several notable advantages are recognized for nonfasting measurements, including a more reflective measure of the daily average of plasma lipids, simplification of blood sampling for patients, laboratories, and clinicians, as well as improvement of patient compliance with lipid testing (7). Although fasting dyslipidemia is strongly associated with CVD risk, only 47.5% of patients with acute coronary syndrome present with fasting dyslipidemia (16). Additionally, it has been suggested that nonfasting and postprandial lipid parameters may better predict CVD risk compared to fasting measurements (6,17).

The potential atherogenicity of TGs and TRLs in the postprandial state was first proposed by Zilversmit in 1979 (18), stating that LPL-mediated lipolysis of CMs results in

cholesteryl ester-enriched CM remnants, which are subsequently retained by arterial smooth muscle cells (18). This widely accepted hypothesis regarding the contribution of nonfasting lipids to CVD risk has been further supported by numerous prospective studies further evaluating this link. An 11.4 year follow-up study in women found that postprandial TG levels, but not fasting TG levels, are independently associated with incident cardiovascular events (6). Additional prospective studies have reported an association between nonfasting TG levels and increased risk of coronary heart disease (19), ischemic stroke (2), as well as myocardial infarction, ischemic heart disease, and death (20). Furthermore, Langsted et al reported a significant association between nonfasting levels of TG and cholesterol with myocardial infarction risk, as well as a significant association between nonfasting TG and total mortality (21). Additional postprandial lipid parameters, including markers of intestinally-derived lipoproteins (i.e. apoB-48) and RLPs, have also been reported to be significantly associated with CVD risk. Karpe et al reported postprandial levels of CM remnants (apoB-48 in the Svedberg flotation (Sf) 20-60 subfraction) following ingestion of a mixed-meal correlate with 5-year progression of coronary atherosclerosis in postinfarction patients (22). Postprandial RLP cholesterol (RLP-C) (i.e. total remnant cholesterol, including intestinal and hepatic derived) was more strongly associated with common carotid artery intima-media thickness (i.e. CIMT, a surrogate marker for atherosclerosis) compared to fasting RLP-C in healthy middle-aged men (17). Similarly, Higashi and colleagues found significantly higher RLP-C in patients with coronary artery disease compared to those without (23).

Accumulation of RLPs is thought to be a main contributor to the link between postprandial lipid measures and CVD risk. RLPs less than 70 nm in diameter are able to contribute to atherosclerosis due to their ability to deliver cholesterol

to the arterial wall (24). It has been widely reported that CM remnants are able to penetrate the arterial wall and are subsequently retained in the subendothelial space (25–28). Fully hydrolyzed CM remnants contain approximately forty times more cholesterol than LDL particles (29,30) and may be preferentially retained relative to other lipoproteins (26,31). Thus, it is not surprisingly that CM remnants substantially contribute to the cholesterol deposition within the intima. Lipolysis products of TRLs (i.e. RLPs) have been shown to increase endothelial permeability through rearrangement of tight and adherens junctions of endothelial cells (32). Increased permeability may in turn contribute to increased diffusion of RLPs and LDL into the subendothelial space via paracellular transport (32). Upon entry to the subendothelial space of arterial walls, CM remnants induce the development of atherosclerotic lesions (31,33). RLPs may augment atherosclerosis via several mechanisms including stimulation of the inflammatory state through increased expression of leukocyte activation markers and proinflammatory genes, as well as complement system activation (34). Inflammation is a key characteristic of atherosclerotic progression and requires monocytes to adhere to endothelial cells and enter the vascular wall (35). CM remnants in rats have been shown to induce mRNA expression and protein secretion of monocyte chemoattractant protein-1 (MCP-1), which stimulates monocyte migration, and thus plays a critical role in atherosclerosis development (36). Endothelial cell apoptosis (37), as well as increased production of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, an important regulator of thrombus formation (38), have also been reported in response to CM remnants. Therefore, understanding the regulation of CM metabolism and how CM remnant accumulation associates with CVD risk in insulin resistant states is critical to slow atherosclerotic CVD progression.

THE ROLE OF THE COMPLEX BRAIN-GUT AXIS IN REGULATING POSTPRANDIAL LIPID METABOLISM

The intestine was once thought to be a relatively passive organ in regards to lipid handling, however intestinal lipid absorption and lipoprotein secretion are now recognized as complex, regulated processes. The significant changes in gut hormone secretion and reversion of T2D following gastric bypass surgery (39,40) further highlights the critical role of the intestine in metabolic regulation. Two hormones secreted in equimolar amounts from enteroendocrine L-cells following nutrient ingestion, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), paradoxically pose opposite effects on intestinal lipoprotein output (41). While GLP-1R agonists have been shown to blunt CM output in animal (42) and human studies (43,44), administering GLP-2 has been shown to stimulate secretion of stored TG and preformed CMs, in a process requiring nitric oxide signaling (45,46).

GLP-1, a potent incretin, mediates several effects involved in regulating glycemia, including glucose-dependent insulin secretion (47). Pharmaceutical agents have thus been developed to exploit these beneficial effects by either preventing endogenous GLP-1 degradation through inhibiting dipeptidyl peptidase-4 (DPP-4), or stimulating GLP-1 receptors (GLP-1R). In the scientific community, these agents have been used as tools to elucidate functions and subsequent mechanisms of GLP-1. Animal and human studies using these agents have reported profound effects, not only on glycemic regulation, but on lipid metabolism. Hsieh et al reported that injecting a GLP-1R agonist intraperitoneally reduced postprandial TRL-TG and apoB-48 concentration compared to vehicle-treated controls in both healthy mice and hamsters (42). Furthermore,

3 weeks of DPP-4 inhibitor treatment in an insulin-resistant hamster model reduced TG excursions following a fat-load (42), supporting the inhibitory effects of GLP-1 on postprandial lipemia in both healthy and insulin resistant states. GLP-1-mediated lipid regulation has also been confirmed in healthy and T2D humans. A study of normolipidemic, normoglycemic men found that a GLP-1R agonist significantly reduced TRL-apoB-48 accumulation in the postprandial state (44). A single oral dose of the DPP-4 inhibitor sitagliptin decreased chylomicron production in healthy men, independent of changes in insulin, glucagon, glucose, and free FAs (48). Furthermore, a double-blind cross-over study of T2D patients treated with a DPP-4 inhibitor had reduced postprandial TG and apoB-48 after an oral lipid tolerance test compared to placebo-treated controls (49).

The mechanisms by which GLP-1 regulates lipid metabolism are incompletely understood. Improvements in postprandial lipemia with GLP-1R agonists and DPP-4 inhibitors could result from a number of mechanisms, including reduced lipid absorption, decreased CM secretion, and/or enhanced clearance from the circulation. Although GLP-1 is a potent stimulator of insulin secretion and insulin decreases CM secretion (50,51), the effects of the GLP-1R agonist exenatide on postprandial CM production are maintained under pancreatic clamp conditions (44). Regulation of intestinal lipoprotein production by exenatide or a GLP-1 infusion can also occur independent of changes in gastric emptying as demonstrated by bypassing the stomach (44,52). Additionally, fractional catabolic rates of intestinal lipoproteins in response to exenatide and DPP-4 inhibitors do not appear to be significantly affected, supporting the view that reduced CM accumulation is not due to impaired clearance (44,48,53). GLP-1R stimulation may regulate lipid metabolism by decreasing the rate of intestinal lipid absorption

and subsequently limiting lipid availability within the enterocyte for CM production. Reduced absorption of radiolabeled dietary TG has been observed in hamsters (41) and rats (52) that received a GLP-1 infusion. This may result from a reduction in pancreatic exocrine secretions including lipases (54).

More recently, a brain-gut axis has been proposed to explain the effects of GLP-1R agonists on postprandial lipoprotein production. GLP-1-producing neurons have been identified in the nucleus of the solitary tract of the brain stem, which project to hypothalamic nuclei that express the GLP-1R, including the arcuate (ARC), paraventricular (PVN), and dorsomedial (DMH) nuclei (55). A recent study demonstrated that injecting the GLP-1R agonist exendin-4 into the third ventricle of the brain reduced plasma and TRL-TG and TRL-apoB48 levels in fat-loaded hamsters (56). These effects could be prevented by adrenergic receptor blockers as well as central delivery of an antagonist to melanocortin-4 receptors (MC4R), which are known to activate the intermediolateral nuclei (IML) of the spinal cord to mediate sympathetic outflow to the periphery (57). These findings support the involvement of MC4R signaling and sympathetic pathways in the brain-gut axis that GLP-1R agonists may use to regulate postprandial lipid output (56). This study further demonstrated that GLP-1R agonists may work by reducing jejunal FA absorption as well as decreasing microsomal triglyceride transfer protein (MTP) activity, which is required for apoB-48 lipidation (56). Despite the potential for a brain-gut axis for intestinal lipid regulation, peripheral exendin-4 was found to sufficiently reduce CM production, independent of central GLP-1R signaling (56).

Postprandial lipid regulation by GLP-1 has primarily been studied using pharmaceutical agents which either mimic GLP-1 action or pharmacologically increase its concentration by blocking its inhibition. However, it is important

to understand if physiological levels of endogenous GLP-1 are also able to regulate intestinal lipoprotein production. GLP-1R antagonism in chow-fed hamsters augmented TRL-apoB-48 120min after a fat load (42), indicating that endogenous GLP-1R signaling is able to modulate postprandial lipid metabolism. Furthermore, GLP-1R^{-/-} mice had enhanced plasma and TRL-TG compared to GLP-1R^{+/+} littermate controls, despite similar gastric emptying rates (42). However, current evidence for the ability of physiological levels of GLP-1 to signal through the proposed central pathway is limited. Central treatment with the DPP-4 inhibitor MK-0626 reduced TRL-TG and this effect was negated by central GLP-1R antagonism, implicating endogenously produced central GLP-1 (56). However, there is a lack of evidence for the presence of DPP-4 in the brain, and central GLP-1R antagonism alone has no effect of CM secretion compared to vehicle-treated controls (56). There is some evidence that DPP-4 inhibitors may have additional mechanisms of action beyond inhibiting endogenous GLP-1 degradation, such as the stimulatory effects of sitagliptin on L-cell GLP-1 secretion (58). Similar DPP-4-independent mechanisms may account for the effects of central MK-0626 treatment on postprandial lipemia – however, further studies are needed to delineate the role of endogenous central GLP-1 in regulating chylomicron secretion.

DYSREGULATION OF POSTPRANDIAL LIPID METABOLISM IN INSULIN RESISTANT STATES

Dyslipidemia is commonly present in insulin resistant and T2D patients, which as previously discussed, leads to an increased risk of CVD due to the presence of a pro-atherogenic environment (59). It is therefore not surprising that CVD accounts for the majority of deaths in T2D patients (60). In fact, increased CVD risk is already

present in non-diabetic subjects with impaired glucose tolerance or impaired fasting glucose (61). While dyslipidemia associated with T2D has traditionally been described as a combination of elevated TG, reduced HDL-C, and a shift towards small, dense LDL particles, more recent studies describe increased intestinally-derived lipoproteins in T2D patients (62). As nonfasting dyslipidemia independently predicts CVD events (6), it is critical to understand the link between insulin resistance and dyslipidemia in the postprandial state.

Postprandial dyslipidemia in insulin resistant states has been examined in both human and animal studies. Postprandial elevations of hepatic and intestinal lipoproteins are evident in T2D patients, despite normal TG levels in the fasting state (63). Duez et al reported an increased production rate of intestinal apoB-48-containing lipoproteins in hyperinsulinemic men compared to those with normal insulin levels, although no significant difference in CM clearance was evident (64). An increased CM production rate was similarly seen in T2D patients, however, decreased catabolism of CMs was also evident (65). Furthermore, a recent study by Wang et al reported elevated postprandial TG concentration in insulin resistant, abdominally obese adults, compared to abdominally obese adults without insulin resistance and non-abdominally obese controls (66), further highlighting the association between insulin resistance and postprandial lipid abnormalities. In addition to elevated postprandial TG concentrations, a prolonged elevation of TG concentration has also been seen in T2D patients. For example, after an oral fat load, TG levels peaked at 2 hours in healthy controls, but were still elevated at 4 hours in T2D patients (67).

Several animal studies provide further insight into potential mechanisms associated with postprandial dyslipidemia in insulin resistance. Dysregulation of insulin signaling at the level of

the enterocyte may contribute to postprandial dyslipidemia, as CM secretion is reduced from fetal jejunal explants in response to insulin (68). Indeed, Federico et al observed cellular changes in the insulin receptor signaling pathway, as well as a lack of response to insulin-induced downregulation of CM secretion in a fructose-fed hamster model of insulin resistance (69). Several additional changes accompanying CM over secretion may occur at the level of the enterocyte through altered CM assembly. CM assembly involves lipidation of apoB-48, mediated by MTP, which subsequently prevents apoB-48 degradation. Insulin resistant hamsters exhibited increased intracellular apoB-48 stability, enhanced intestinal *de novo* lipogenesis, and increased MTP mass, compared to chow-fed controls (70). Diacylglycerol acyltransferase (DGAT) is another enzyme involved in TG synthesis in the enterocyte, and thus CM assembly. Activity and expression of DGAT has also been shown to be elevated in insulin resistant hamster models (71). Enhanced dietary lipid absorption may also be upregulated in insulin resistant states through upregulation of various FA transporters to provide increased substrates for CM assembly (72). Taken together, intestinal lipoprotein production is a highly regulated process that becomes significantly altered in insulin resistant states.

CLINICAL ASSESSMENT OF POSTPRANDIAL LIPIDS IN HUMANS

Non-fasting lipid measurement is a simple approach to assess postprandial lipids, however it does not allow for a complete functional assessment of postprandial lipid excursion and potential abnormalities in insulin resistant states. An oral glucose tolerance test (OGTT) is a well-established method used clinically to assess glucose intolerance in pre-diabetic and diabetic states (73). A similar method to assess lipid parameters at fixed time points following ingestion

of a high-fat meal (i.e. oral fat tolerance test (OFTT)) to examine the efficiency of lipid metabolism is not currently performed routinely in the clinic. This is mainly due to a lack of standardized methodology and reference values for result interpretation. Nevertheless, postprandial lipid responses to fat-containing meals have been examined in research settings in human subjects for the past 30 years (74). Assessing postprandial lipid metabolism provides indications of an individual's capacity to process dietary lipids from digestion and absorption of lipids through secretion and clearance of lipoproteins. A wide range of methodologies have been used, varying in pre-test meal conditions, the size, ingredients, nutrient composition, and frequency of the meal, time of blood collection, and selection of circulating markers to measure (74). Additionally methodology will differ depending on the research question. For example, OFTT studies can be used to compare postprandial lipid responses between different nutrient mixtures, between subjects with different habitual diets, between subjects with and without disease, or even to assess the relationship between postprandial response and other markers of disease risk (e.g. fasting lipids, inflammatory markers). Several factors affect TG response to a fat-containing meal, including the amount of fat consumed, consumption of alcohol before or during the meal, fiber content, contents of other macronutrients, and physical activity (reviewed in (74) and (75)).

Postprandial lipemia studies in adults using OFTT

Previous studies have analyzed the healthy postprandial lipid profile following ingestion of a fat-containing meal. For example, Tanaka et al performed an OFTT study in 19 healthy adults to observe the normal postprandial profile of RLPs, which were found to remain elevated even after 8 hours of OFTT and display a similar profile to

total serum TG (76). Others have assessed differences in the postprandial profile between healthy and diseased populations. For example, OFTT cream (Jomo Food Industry) was provided to T2D patients (normoinsulinemic and hyperinsulinemic) and healthy volunteers at a dose of 17g fat/m² body surface area and blood samples were collected at fasting, 2 and 4 hours following the oral fat load (66). Although TG levels did not differ between the three groups, RLP-TG and RLP-C were higher in hyperinsulinemic T2D patients compared to the other two groups, with no difference between normoinsulinemic T2D and healthy subjects. This suggests that hyperinsulinemia, rather than the presence of T2D, may be the causal link between postprandial dyslipidemia and CVD risk. More recently, Larsen et al found high peak and delayed clearance of serum and CM-TG in obese compared to healthy adults 6 hours after consumption of a weight-adjusted (1g fat per kg body weight) high-fat meal (70% calories from fat) (77).

Postprandial lipemia studies in pediatrics using OFTT

Although limited, studies have assessed postprandial lipemia in the pediatric population (78–82) (Table 1). The rate of pediatric obesity is increasing significantly worldwide and one in five children with a BMI above the 95th percentile is hypertriglyceridemic, which is a 7-fold higher rate than for nonobese children (83). Furthermore, vascular pathophysiological changes begin soon after birth and accelerate in adolescence (84). Thus, examining abnormalities in postprandial lipid metabolism in children may allow for early identification of those at risk for additional metabolic co-morbidities. Recent studies found no significant difference in postprandial TG between overweight and normal weight adolescents at fasting, 2 and 4 hours following a high-fat meal (78,79). However, when Sahade et al examined central

Table 1 Studies assessing postprandial lipemia after ingestion of a fat-containing meal in adolescents with obesity and/or associated co-morbidities

Reference	Population	Fat-containing meal	Time points	Blood parameters measured	Key findings
Couch et al Am J Clin Nutr 2000 (82)	60 adolescents (M/F: 27/33, mean age: 14.0y) from families with or without history of premature CHD	52.5g fat, 24g carbohydrates, 16g protein per m ² body surface area	0 (fasting), 3, 6, 8 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, HDL-C, LDL-C, retinyl palmitate, apoE genotyping	Delayed postprandial TG was associated with the combination of high fasting TG and low HDL-C.
Moreno et al J Pediatr Endocrinol Metab 2001 (80)	12 obese adolescents (M/F: 5/7, mean age: 12.8y) 12 normal weight adolescents (M/F: 5/7, mean age: 12.7y)	39.0g fat, 47.7g carbohydrates, 18.5g protein	0 (fasting), 2, 4, 6 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, HDL-C, LDL-C, apoAI, apoB <u>Other:</u> glucose, insulin	Postprandial TG positively correlated with central obesity.
Umpaichitra et al J Pediatr Endocrinol Metab 2004 (81)	12 T2D obese adolescents (M/F: 5/7, mean age: 14.0y) 15 non-diabetic obese adolescents (M/F: 9/6, mean age: 13.2y) 12 healthy adolescents (M/F: 5/7, mean age: 14.9y)	117g fat, 41.5g carbohydrates, 0.5g protein	0 (fasting), 2, 4, 6 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, HDL-C, LDL-C <u>Other:</u> glucose, insulin, C-peptide, HbA1c	Postprandial TG in T2D obese adolescents was associated with the presence of insulin resistance.

Sahade et al Lipids Health Dis 2013 (78)	49 overweight adolescents (M/F: 20/29, med(IQR) age: 12.0 (11.0-14.0)y) 34 normal weight adolescents (M/F: 17/17, med age: 13.0 (11.0-15.3)y)	25g fat, 25g carbohydrates, 0g protein	0 (fasting), 2, 4 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, HDL-C, LDL-C <u>Other:</u> glucose, insulin	Only overweight adolescents with insulin resistance and fasting hyper- triglyceridemia had higher post- prandial TG. Waist circumference positively correlated with 4h TG.
Schauren et al J Dev Orig Health Dis 2014 (79)	38 overweight adolescents (M/F:18/20, mean age: 14.2y) 24 normal weight adolescents (M/F: 9/15, mean age: 14.2y)	64g fat, 69g carbohydrates, 37g protein	0 (fasting), 4, 6 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, HDL-C, LDL-C <u>Other:</u> glucose, insulin, fibrinogen, leukocyte count, hsCRP	BMI z-score significantly correlated with postprandial TG.
Vine et al J Clin Endocrinol Metab 2017 (85)	12 obese- PCOS female adolescents (mean age: 15.3y) 18 obese female adolescents (mean age: 14.9y) 10 normal weight female adolescents (mean age: 15.6)	0.61g fat, 0.66g carbohydrates, 0.16g protein per kg of body weight	0 (fasting), 2, 4, 6, 8 hours	<u>Lipids/</u> <u>Lipoproteins:</u> TC, TG, apoB48, apoB100 <u>Other:</u> hormone profile	Obese female adolescents with and without PCOS have exacerbated postprandial TG and apoB-48 compared to normal weight controls.

*apoA-I: apolipoprotein A-I; apoB-48: apolipoprotein B-48; apoB-100: apolipoprotein B-100;
apoE: apolipoprotein E; BMI: body mass index; CHD: coronary heart disease; HbA1c: haemoglobin A1c;
HDL-C: high-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein; LDL-C: low-density
lipoprotein cholesterol; PCOS: polycystic ovary syndrome; TC: total cholesterol; TG: triglycerides

obesity specifically, 4 hour TG levels were significantly higher in subjects with central obesity (78). Similarly, Moreno et al reported that adolescents with central obesity had higher postprandial TG levels compared with subjects with a peripheral pattern of fat distribution (80). Umpaichitra et al examined postprandial TG (fasting, 2, 4, and 6 hours after a high-fat meal) in obese subjects with and without T2D, as well as non-diabetic, non-obese controls (81). They found that the degree of insulin resistance, rather than the presence of T2D, determines the degree of postprandial lipemia. However, additional studies in adolescents are warranted which measure markers more indicative of intestinal lipoprotein metabolism (e.g. apoB-48, RLP-C), as well as potential relationships with regulators of lipid metabolism (e.g. GLP-1, GLP-2). One recent study measured postprandial apoB-48 in adolescents following an OFTT (0.61g fat/kg body weight) in obese females with and without polycystic ovary syndrome (i.e. PCOS; a metabolic-endocrine disorder associated with obesity and insulin resistance), as well as normal weight controls (85). Obese adolescent females with and without PCOS were found to have elevated postprandial TG and apoB-48 compared to controls, and these levels were associated with indices of adiposity and insulin resistance (85). These data suggest apoB-48 may be a good marker of metabolic disturbances associated with insulin resistance in adolescents, necessitating further studies to confirm these results, as well as investigate relationships with fasting parameters and hormones involved in intestinal lipoprotein regulation.

Postprandial biomarkers to measure in OFTT

In addition to the traditional lipid profile (i.e. TC, LDL-C, HDL-C, TG), it is important for OFTT studies to measure laboratory markers that are more reflective of postprandial dyslipidemia and intestinal lipid metabolism. For example,

measuring apoB-48 (i.e. a measure of CM particle number) and TRL remnants can provide a more complete indication of pro-atherogenic remnant accumulation (34). ApoB-48 measurement allows a direct measure of CMs, as this protein is specific to intestinally-derived lipoproteins in humans. ApoB-48 is indicative of CM particle number, with each CM or CM remnant particle containing one apoB-48 molecule that does not transfer to other lipoproteins (86). ApoB-48 can be determined through commercially available sandwich ELISA kits (87). Remnant-like particles can also be measured by commercially available kits. Japan Immunoresearch Laboratories produce a kit to measure remnant-like particles that uses an immunoaffinity gel with immobilized anti-apoAI monoclonal antibodies and a unique anti-apoB-100 monoclonal antibody that does not bind apoE-rich apoB-100 lipoproteins (88). Lipoproteins that do not bind to the immunoaffinity gel resemble apoE and cholesteryl ester-enriched CM and VLDL remnants, called remnant-like particles, from which cholesterol can be quantified (88). A more recent assay for serum remnant lipoprotein cholesterol (RemL-C) directly solubilizes and degrades remnants using surfactant and phospholipase-D. This assay can be performed on an automated analyzer in only 10 minutes (89). Although RLP-C measurements detect remnants of both intestinal and hepatic origin, examining postprandial RLP-C in populations with various cardiometabolic conditions can provide an indication of the magnitude and duration of RLP-C in the circulation following a meal.

Furthermore, measuring markers secreted following meal ingestion, which are involved in regulation of intestinal lipoprotein metabolism, may aid in early detection of postprandial dyslipidemia and CVD risk. For example, it has been suggested that postprandial GLP-1 may be an early biomarker of obesity-associated metabolic dysfunction. After consuming a mixed-meal

(34% energy from fat), T2D patients had significantly reduced active GLP-1 at 75, 90, and 120 minutes compared to healthy controls (90). Furthermore, following ingestion of a mixed meal (42% energy from fat), fasting and postprandial (60-150min) GLP-1 concentration was significantly lower in T2D compared with healthy controls (91). However, others found no difference in postprandial GLP-1 between subjects with and without the metabolic syndrome (i.e. a cluster of CVD risk factors including central obesity, fasting dyslipidemia, impaired glucose tolerance/impaired fasting glucose, and hypertension) (92). Overall, pathophysiological changes in endogenous GLP-1 concentration in obese/insulin resistant states remains controversial, with some studies indicating an increase (93,94), decrease (95,96), or unaltered (97) GLP-1 concentration in obese/insulin resistant subjects. Although studies have measured postprandial GLP-1, they are often in the context of abnormal glucose metabolism, rather than in relation to postprandial dyslipidemia. Further studies are warranted to assess changes in endogenous GLP-1 concentration in obese and insulin resistant subjects, and examine associations with postprandial dyslipidemia.

FUTURE DIRECTIONS

A paradigm shift towards measuring postprandial, as opposed to fasting lipids has occurred in recent decades. Some countries have already adopted nonfasting lipid testing (i.e. measured on a random blood sample irrespective of time since last meal) in routine practice, including Denmark in 2009 (3), the UK in 2014 (9), as well as Europe (7) and Canada (10) in 2016. Although several advances have been made in understanding dysregulation in intestinal lipid metabolism in insulin resistant states and its association with CVD, several limitations remain. Assessment of the functional postprandial lipid profile (i.e. lipid measurements at specified

time points following a standardized meal) is the preferred methodology to ensure optimal comparability between test subjects. However, OFTT methodology remains largely unstandardized, and thus more studies are required to develop standard procedures which are able to distinguish between healthy and at-risk populations, including population-specific meal sizes, nutrient composition, blood sampling time points, and markers to measure. Additionally, robust reference values, which are critical to interpret postprandial parameters, remain to be accurately established. However, these must be specific to the methodology of the OFTT used. These limitations become exacerbated in the pediatric population even though the genesis of these metabolic disturbances often occur early in life. As quantifiable outcome measures are often harder to detect early in life, it is also difficult to assess predictive ability of biomarkers in children and adolescents at risk early enough to prevent disease development later in life.

With the obesity and diabetes epidemics upon us, it is becoming imperative to develop more effective approaches to assess metabolic abnormalities that increase CVD risk. Recent studies have clearly highlighted the importance of intestinal lipid dysfunction in pathogenesis of insulin resistant and diabetic states. Translating important new findings from basic research studies into the clinic is essential to improve clinical assessment of postprandial dyslipidemia, increasingly recognized as a major contributor to development of atherosclerosis and future CVD. Further studies are also warranted to elucidate mechanisms of postprandial dyslipidemia associated with insulin resistant states. A more complete understanding of the underlying pathobiology will allow subsequent development of standardized methodologies and biomarker profiles to be used in clinical practice for early and accurate identification of those at risk for CVD.

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e-Learning: a model to support ongoing education

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OPINION

INTRODUCTION

Education is fundamental for creating opportunities, empowering individuals and stimulating long-lasting change in communities. It is internationally recognised that education is the right of every child and must be equally accessible to girls and boys and be free and fair [1]. Access to education relates to all children attending and completing primary school, secondary school and tertiary or vocational education. In this process great teachers have the power to instil a love of learning, leading students to embrace education as a vital tool that provides opportunities to foster growth and success for themselves and the wider community. Many of these fundamental concepts surrounding education can be equally applied to adult learners. (Figure 1)

The opportunity for on-going education for professionals in medical science is important for ensuring that the quality of patient care remains optimal. International guidelines and standards, such as ISO15189:2012, include requirements for on-going education in their documents to ensure employers and employees are held accountable for this activity [2]. However, the reality is that developed urban

Figure 1 Article 28 of the Convention on the Rights of the Child

1. States Parties recognize the right of the child to education, and with a view to achieving this right progressively and on the basis of equal opportunity, they shall, in particular:
 - (a) Make primary education compulsory and available free to all;
 - (b) Encourage the development of different forms of secondary education, including general and vocational education, make them available and accessible to every child, and take appropriate measures such as the introduction of free education and offering financial assistance in case of need;
 - (c) Make higher education accessible to all on the basis of capacity by every appropriate means;
 - (d) Make educational and vocational information and guidance available and accessible to all children;
 - (e) Take measures to encourage regular attendance at schools and the reduction of drop-out rates.
2. States Parties shall take all appropriate measures to ensure that school discipline is administered in a manner consistent with the child's human dignity and in conformity with the present Convention.
3. "States Parties shall promote and encourage international cooperation in matters relating to education, in particular with a view to contributing to the elimination of ignorance and illiteracy throughout the world and facilitating access to scientific and technical knowledge and modern teaching methods. In this regard, particular account shall be taken of the needs of developing countries" [1].

Article 28 of the Convention on the Rights of the Child which was adopted and opened for signature, ratification and accession by General Assembly resolution 44/25 of 20 November 1989 entry into force 2 September 1990, in accordance with article 49 of the United Nations. Many of these principles, particularly 28.1c and 28.3, can be applied to the direction of efforts for ongoing education in medical science (Figure 1).

communities have greater access to on-going education compared to rural, remote and developing communities. Now we possess the information communication technology (ICT) capability to make significant steps in addressing this disparity. As such, e-Learning can be implemented to reach out to health professionals who have previously not had the same access to opportunities for professional development.

This manuscript is an outcome of a presentation which aimed to discuss the development of an e-Learning strategy for the medical science

community in Myanmar. To achieve this aim, some definitions, examples, recommendations and personal thoughts related to e-Learning are provided.

LEARNING STYLES AND STRATEGIES

On-going education for health professionals is important for optimising the quality of patient care. The preferred method for providing professional development activities remains debated, and this is not surprising given that individuals can have different styles of learning [3].

The main learning styles are: visual learners (divided further into two sub-channels of linguistic and spatial); auditory learners; and kinaesthetic learners (divided further into two sub-channels of movement and tactile i.e. touch). Ideally ongoing education activities should encompass aspects that incorporate all of these learning styles.

There are seven main learning strategies for on-going education; these being lectures, reading, audio-visual, demonstration, discussion, practical doing and teaching others. Lectures, reading and audio-visual strategies are key activities that can be provided face-to-face and delivered equally well remotely through ICT. Demonstrations can also be provided as part of face-to-face training or incorporated into audio-visual e-Learning. Discussions and practicals are often best delivered face-to-face, although there are some examples of applying these on-line. Finally, “teaching others” by any of the other six strategies provides an effective learning approach; hence learners should be encouraged to deliver professional development activities. Each of these learning strategies has been applied with varying success to e-Learning.

A simple and clear definition of e-Learning is “learning conducted via electronic media, typically on the Internet” [4]. E-Learning’s greatest advantage over many other forms of education delivery is that it can be accessed twenty four hours a day seven days per week from almost anywhere and is cost effective. As such, e-Learning activities have increased significantly in recent years, and many industries worldwide have actively embraced this approach for compliance management and on-going professional development. The breadth of activities has led to a variety of terms used synonymously with e-Learning (including blended learning, on-line learning and distance education) and the definition can vary as the result of adapting the term

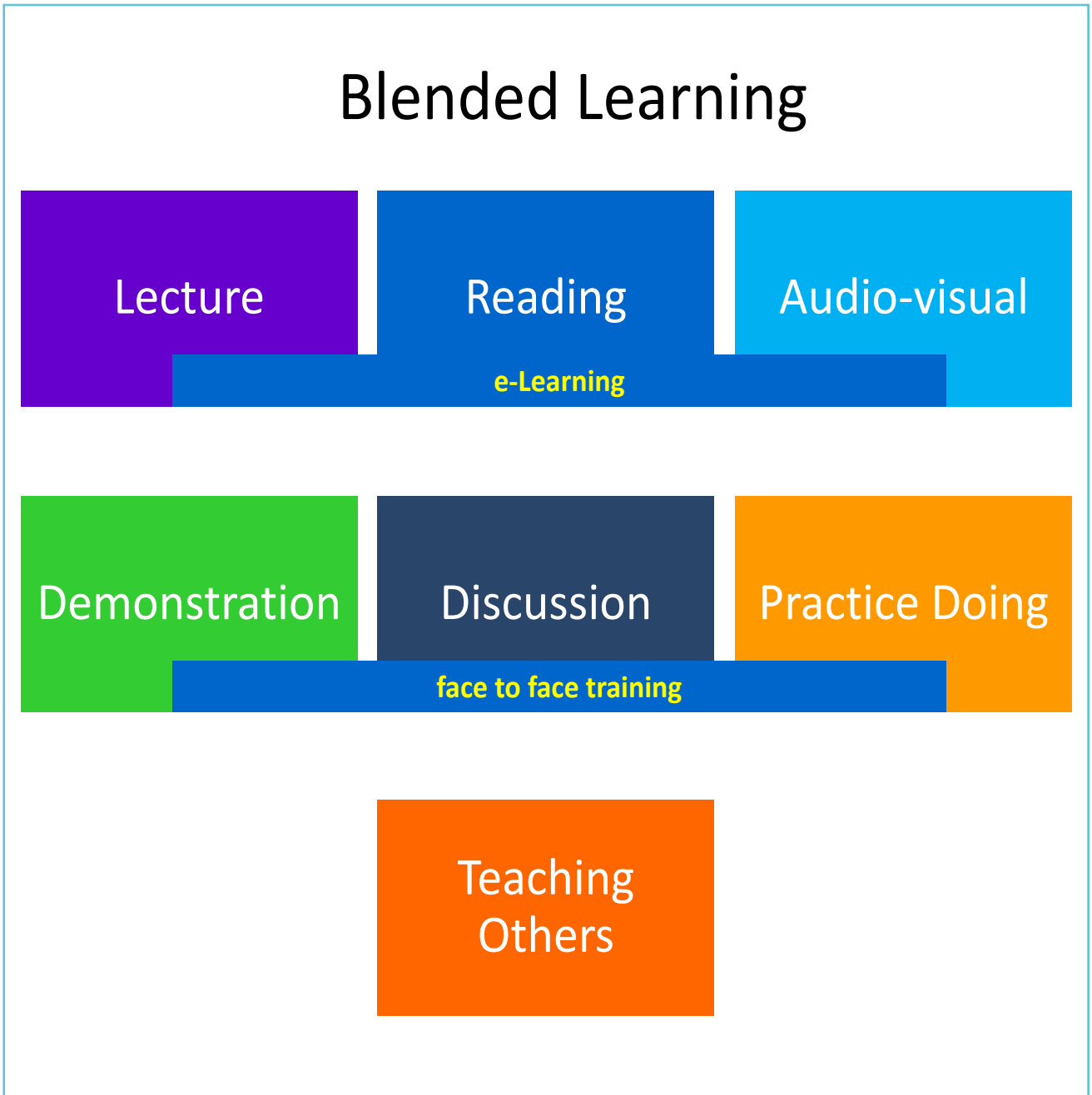
for an organisation’s specific purpose [5]. Whilst e-Learning offers many advantages, the computer based approach cannot currently achieve all goals, especially related to hands on practical experience. Hence, universities in particular are now embracing the concept of “blended learning” which combines e-Learning with face-to-face workshops/practicals. Figure 2 demonstrates the potential approaches for blended learning, demonstrating the main role of e-Learning.

PROFESSIONAL SOCIETIES’ ROLE IN e-LEARNING

Significant professional and personal benefits arise from individuals engaging with their relevant professional society. Engagement provides a network for individuals to exchange ideas and remain up to date with wider developments in the field. Often, a primary goal of such societies is to promote on-going education activities which are often valued by industry for their perceived relevance and commercial independence. Whilst traditional face-to-face education may provide greater networking opportunities, it may also potentially limit the access and participation of professionals working outside of major cities. With the consistent improvements in ICT there are now options to supplement or replace these face-to-face activities with on-line learning i.e. e-Learning. Online learning activities, such as live streaming and recording of webinars, enable societies to reach out to colleagues who otherwise may not be able to participate. In addition, an important aspect of on-line education is its potential to eliminate or minimise the duplication of activities and provide a broader and more comprehensive approach to continuing professional development.

“The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is a worldwide, non-political organisation for clinical chemistry and laboratory medicine”. In 2012 the IFCC endorsed

Figure 2 Seven learning strategies for ongoing education in the blended learning model



Lectures, reading and audio-visual are key activities that can be incorporated into e-Learning.

Demonstrations are categorised as face to face training, however they may also be incorporated into audio-visual e-Learning.

Discussions and practicals are often best delivered face to face, although there are some examples of applying these to e-Learning.

Finally, teaching by any of these six methods provides an effective learning approach; hence learners should be encouraged to contribute to professional development activities.

two committees (one to develop education material and the other to implement the ICT strategy) to work collaboratively for the new IFCC e-Academy. The “IFCC e-Academy is an open educational resource created and/or reviewed by IFCC experts for the continuous professional development of members of IFCC member organisations” globally [6]. This e-Learning resource has a focus to support the national professional societies that do not already have an established on-going education framework; this equates to focused support for developing economies.

The timeline and overall development of the IFCC e-Academy can generally be surmised as the following: 2011 plan by the IFCC and development of the terms of reference; 2012 committees were formed and first meetings conducted; 2013 was the development, distribution to member societies and review of a questionnaire; 2014 material was sourced, and website, copyrights and gaps reviewed; 2015 the pilot e-Academy module was launched [7]; 2016 saw the review, improvement and expansion of e-Academy content; and 2017 has involved finalising the curriculum and mapping content to the curriculum. Although there is still work to be done, there are already significant demonstrated advantages of the e-Academy, including that the material is free open access and obtained from resources worldwide. In addition, this resource is not static and will provide interactive links in the medium term.

RECOMMENDATIONS

Continuing professional development is important for medical quality. Edwards Deming is a globally recognised champion of quality who implemented Deming’s management improvement cycle to provide a mechanism to move forward within a quality framework. Deming’s simple “Plan-Do-Check-Act” (PDCA), also known as “Plan-Do-Study-Act”, approach has been used and updated since its inception, but the underlying approach is

still applicable to project management today [8]. The PDCA cycle process can be directly related to the concept of continuous improvement, which in turn can be related to continuous learning throughout our careers. As such the PDCA cycle can be directly applied to learning and to the development of an e-Learning strategy.

Plan-do-check-act is considered a cyclical process that provides a mechanism to efficiently utilise resources and implement in stages. It is akin to the lean start-up methodology whereby a minimum viable product (MVP) is developed then subjected to testing to validate its usefulness. The learning’s from this process are then utilised to improve its effectiveness and develop other products or features of value. Unwanted features and items not aligned to the mission statement are to be discarded or at least “parked” for future review. In applying this methodology to e-Learning the focus must remain on the establishment of a valued and credible point of learning.

The initial PDCA cycle to apply to an e-Learning strategy could involve the following:

Plan

Create a committee that engages both local and regional stakeholders, ensuring the people nominated comprise a variety of skill sets. Establish a communication system for the committee to discuss activities between official meetings. The committee and/or people forming the committee should develop a terms of reference (TOR) to guide the committee’s activities. It is important to understand and work within any ICT limitations – these may be different for various parts of the country. Once these are understood, then proceed to investigate what is already available; i.e. there is no need to “reinvent the wheel”. From this a vision can be synthesized and framed into a mission statement as an established point of reference for ongoing planning and decisions.

Do

Find and utilise resources to build the e-Learning platform. A website, establishes the connection to the intended customers of the endeavour. The website should target only the main objectives and provide only the minimum content consistent with the mission statement yet allow for expansion or evolution to a new platform at a later date. This activity establishes the fundamental building blocks.

Check

All stakeholders engaged in the development of the terms of reference should be encouraged to interact with the website as a process of validating the result of the initial endeavour against the mission statement. This engagement process should stimulate great contribution and discussion as to the content and priority which should be established.

In addition, the development and distribution of a questionnaire of needs should be a priority; later review questionnaires can also be used to see what people think about the success of the activity. The de-identified results of the questionnaire/s should be reviewed by the committee to:

1. look for opportunities for improvement; and
2. summarise and share back to stakeholders.

This primary information can be used to develop a pilot education strategy, e.g. one topic, should be developed and trialled. Recording (i.e. documenting) and analysis of this feedback should guide the next steps.

Act

The “act” will be to roll-out the e-Learning strategy. In preparation for the “role out” of the e-Learning approach, ideally the following should also be in place:

1. web page that is low tech;

2. central office;
3. regular management meetings; and
4. on-going planning.

Laying out the curriculum, prioritising the content development and establishing key roles for the ongoing maintenance and updating the web page should suffice for the initial iteration of the PDCA cycle for establishing e-learning.

Individual learning styles and strategies should be considered throughout the cycles to ensure the developed e-Learning model for on-going education is effective.

After the launch of your e-Learning initiative it is still necessary to perform an on-going review and have or develop a plan to expand to other regions. In these later cycles activities can be reviewed by:

1. asking people what worked well and what needs improvements;
2. drilling down to the root cause of any problems;
3. reviewing and refining the plan;
4. identifying gaps; and
5. deciding if more pilots are needed.

Finally, the success of the e-Learning project will also depend on the self-motivation of individuals to study effectively and therefore the creation of a reward system should be considered to support participant motivation.

There are some important general points to avoid in the development of the-Learning strategy:

1. Do not make the plan overly complicated;
2. Don't be afraid to receive feedback;
3. Do not limit it to one type of activity;
4. Don't make the committee too large and ensure members are activity participating - this can usually be achieved through the

development of committee rules related to structure, expectations and length of membership;

5. The final don't is – do not reinvent the wheel i.e. if the relevant e-Learning information is already freely available make use of it e.g. the IFCC e-Academy.

Relationship to WHO eHealth

In relation to the World Health Organization's e-Health plan there are many synergies that could be adapted across to e-Learning [9]. An outline of the WHO strategy adapted to e-Learning is generally concordant with the suggested PDCA approach as it includes:

1. identifying and engaging with the key stakeholders who will need to be involved in the development of the e-Learning vision and plan and its subsequent implementation;
2. establishing a governance mechanism to provide improved visibility, coordination and control;
3. establishing the strategic context to enable the Ministry of Health and Sport to assess and make informed decisions on whether to pursue opportunities that present themselves from the ICT industry and other stakeholders; and
4. assessing the current e-Learning environment in terms of the components that already exist as well as existing programmes or projects that will deliver on-going education.

Summary

In summary, the development and implementation of an e-Learning program will take planning and courage: clear communication, observation via pilot studies, working within the uncertainty of ICT, reviewing strategies, asking questions of the intended students, and development of guidelines or terms of reference and evaluation of the success against the criteria developed.

In these respects, the Myanmar Academy of Medical Science is well placed to lead the development and implementation of e-Learning.

FINAL THOUGHTS

Education is about creating opportunities and a significant driver for on-going education is passion. In the opening address for the Myanmar National Education Strategic Plan 2016 – 2021, Aung Sun Suu Kyi stated “all the leading implementers who set up policies and teachers must encourage the desire to learn mindset, and they themselves need to have **the desire to learn** in order to be able to spread the sentiment widely” [10]. Supporting the implementation and overall success of e-Learning for ongoing professional development in Medical Science requires this commitment and desire for life-long learning at all levels.



Acknowledgements

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About the “Compendium of Terminology and Nomenclature of Properties in Clinical Laboratory Sciences”

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BOOK REVIEW

Since the publication of the “Compendium of Terminology and Nomenclature of Properties in Clinical Laboratory Sciences” (Silver Book) in 1995, significant development in metrological concepts and terminology has occurred. The second edition of the Silver Book [1], published in 2017 with the joint support of IFCC and IUPAC updates recommendations and technical reports for the clinical laboratory sciences. The book describes a common structure and language for a reliable exchange of person examination data. This format, developed by the IFCC-IUPAC Committee-Subcommittee on Nomenclature for Properties and Units (NPU), applies to multiple disciplines, including clinical allergology, clinical chemistry, clinical haematology, clinical immunology and blood banking, clinical microbiology, clinical pharmacology, molecular biology and genetics, reproduction and fertility, thrombosis and haemostasis, and toxicology. It is adapted both to quantitative and qualitative information (where no magnitude is involved). Concepts and rules in the communication of clinical laboratory information are explained and numerous examples are given to ensure interoperability between the various participants in clinical laboratory sciences.

CONTENTS OF THE REVISED SILVER BOOK

Section 1 - History of recommendations on properties in clinical laboratory sciences

Over the period 1954-2016 section 1 describes the steps to implement in clinical chemistry the recommendations of the IUPAC Commission on Symbols and Physicochemical Terminology and of ISO Technical Committee 12 on Quantities and Units. Preferences were affirmed for amount-of-substance with the acceptance of the mole as a base unit, for the liter in the expression of concentration, for the katal in expressing catalytic activity, for the adoption of the concept 'kind-of-property'. A unified format (NPU format) to express a property of a system was developed under the auspices of IFCC and IUPAC. The listing of dedicated kinds-of-property in an IFCC-IUPAC data bank was another step.

Section 2 - Definitions of some disciplines applied in the clinical laboratory

Forty-five disciplines related to clinical laboratory sciences are defined. They are hierarchically related in a generic concept diagram.

Section 3 - Conventions and instructions for use

Details are given to understand the structure and wording of the text. The primary purpose of this Silver Book is to be a guide towards a structured and uniform way of reporting on examinations from clinical laboratories ensuring interoperability, i.e. the ability of information systems to communicate successfully across organisational and systems boundaries [2].

Section 4 - Fundamental concepts in communication of clinical laboratory information

A set of rules based on the IFCC-IUPAC NPU format is given to obtain and communicate information on the chemical, biochemical, physiological, pathological, and sometimes physical properties

of people. It is based on three main items structured as follows:

System—Component; kind-of-property

The concept of 'property' comprises several types, i.e.: *nominal property, ordinal property, linear differential property, logarithmic differential property, and rational property*. The concept of 'kind-of-property' relates to both quantities and properties of a classificatory nature that are devoid of magnitude. Thus, a NPU format is not limited to measurements, but is generalized to dedicated kinds-of-property without dimensions of the International System of Quantities (ISQ).

Section 5 - Principles and practice of kinds-of-quantity and units

The International System of Units or SI, based on the ISQ, is described with their terms and symbols, including a series of prefixes and their terms and symbols, together with rules for their use. Corresponding examples taken from clinical laboratory practice are given.

Section 6 - Requesting, generating, and transmitting clinical laboratory information

This part is mainly devoted to the recommendations regarding the clinical laboratory report. It is based on the recommendations elaborated by several international organizations. Proposed English-language abbreviations are given for systems in the human body and for kinds-of-property.

Section 7 - Choice and use of kinds-of-property for different examination purposes

This part presents recommendations for the expression of compositional and material kinds-of-quantity. Terminology for kinds-of-quantity related to different measurement principles (optical spectroscopy, centrifugation, electrophoresis and enzymology) are listed with examples.

Section 8 - Kinds-of-quantity of dimension one; SI Unit 1

Ten subsections list numerous types of number, fraction, ratio, relative kinds-of-quantity, derivative kinds-of-quantity, activity, and factors.

Section 9 - Kinds-of-quantity of dimension other than one

Kinds-of-quantity are classified in 110 subsections according to their dimension. Recommendations regarding terms, abbreviation, symbol(s), definition, relevant information, and often example(s) are given.

Section 10 - Kinds-of-property without dimensions of the ISQ

The text concerns nominal and arbitrary kinds-of-quantity that have no dimension and are outside

the ISQ. It presents nominal and arbitrary kinds-of-property with definition, explanatory notes, and examples.

An extensive list of Symbols, Terms and SI Units for Kinds-of-quantity is presented. References to ISO, IEC, CGPM, WHO, and EN standards to IUPAC and IFCC recommendations and technical reports, as well as to some secondary sources are given at the end of each section.

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Correlation of HbA_{1c} levels with body mass index in newly diagnosed polycystic ovary syndrome

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Key words:

HbA_{1c}, body mass index, PCOS

ABSTRACT

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous, multisystem endocrinopathy in women of reproductive age manifested with various metabolic disturbances and a wide spectrum of clinical features such as obesity, menstrual abnormalities and hyperandrogenism. Hyperinsulinemia was noted in 50% to 70% of PCOS patients and plays a central role in the development of further complications. The prevalence of obesity in PCOS ranges from 38% to 87%. It has been reported in previous studies that the prevalence of insulin resistance (IR) is higher in obese PCOS women than obesity without PCOS.

Objective

To correlate HbA_{1c} levels with body mass index (BMI) in newly diagnosed polycystic ovary syndrome (PCOS) women.

Material and methods

This case-control study performed at Pt. B. D. Sharma PGIMS, Rohtak includes 30 newly diagnosed PCOS patients and 30 age matched healthy controls of any age. Fasting venous blood samples were obtained for

analysis of fasting blood glucose, haemoglobin and HbA_{1c} after obtaining written consent.

Results

HbA_{1c} concentration was higher in cases (6.03±0.19%) than in controls (5.35± 0.08%) with p = 0.002. 33.3% were in pre diabetic range (5.7- 6.4%), 36.7% in diabetic range (≥6.5%) and 30% in non diabetic range (<5.7%). BMI of cases (26.09±4.2 kg/m²) was significantly higher than controls (22.9±3.3 kg/m²) with p=0.002. HbA_{1c} showed positive correlation with BMI (p= 0.001).

Conclusion

In conclusion, BMI is associated with glycemic control in PCOS.



INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous, multisystem endocrinopathy in women of reproductive age manifested with various metabolic disturbances and a wide spectrum of clinical features such as obesity, menstrual abnormalities and hyperandrogenism. Current incidence of PCOS (5-6%) in women is related to change in lifestyle and stress [1].

According to the World Health Organization, it was estimated that 116 million women (3.4%) have PCOS with prevalence ranging from 2.2% to 26% globally [2]. Hyperinsulinemia was noted in 50% to 70% of PCOS patients and played a central role in the development of further complications. In spite of hyperinsulinemia, there is an increased prevalence of insulin resistance (IR) in PCOS patients which can lead to increased glucose tolerance and type 2 diabetic mellitus (T2DM). Increased insulin leads to increased androgen production from the ovarian thecal cells and this hyperandrogenemia is responsible for androgenic obesity.

The prevalence of obesity in PCOS ranges from 38% to 87%. It was reported that in obese PCOS women, the prevalence of IR was higher than obese women without PCOS in the control group [3].

MATERIAL AND METHODS

The present case control study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynaecology, Pt. B. D. Sharma PGIMS, Rohtak. After getting written consent from the cases and controls, detailed history were obtained and recorded in their respective proforma. They were subjected to physical examination and anthropometric measurements as per protocol followed by systemic examination, urine pregnancy test was done to rule out pregnancy, ultrasonography of abdomen and pelvis were performed in all subjects of this study.

Inclusion criteria

Newly diagnosed cases of PCOS as per Rotterdam definition - the existence of the following three criteria to make the diagnosis of PCOS:

1. oligo-ovulation or anovulation;
2. clinical or biochemical signs of hyperandrogenism; and
3. polycystic ovaries by Ultrasonography [4].

Exclusion criteria

Any history suggestive of other potential causes of hyperandrogenism/oligo/amenorrhoea [congenital adrenal hyperplasia, androgen secreting tumor], hypothyroidism, cushing's syndrome, hyperprolactinemia, other pituitary/adrenal disorders, other insulin resistance conditions (acromegaly), history of any drug intake and pregnancy [3].

Thirty age matched healthy females with regular menstrual cycle and not on any treatment were enrolled as controls.

Anthropometric measurements

The weight and standing height of all study subjects were measured twice by using calibrated weighing scale and stadiometer with a fixed vertical backboard and an adjustable head piece respectively by two different examiners to avoid subjective error. BMI is expressed in the units of kg/m². BMI can be calculated by the present weight in kg divided by square of height in metres (Quetelet index). According to the World Health Organisation, BMI can be graded into the categories listed in Table 1.

SAMPLE COLLECTION

Six mL of venous blood sample after fasting (10 - 12 hours) was taken from the antecubital vein aseptically on the second day of menstruation, out of which 2 mL of blood in EDTA anti-coagulant vacutainer, 2 mL in sodium fluoride vacutainer and 2 mL in plain vacutainer. Serum from sodium fluoride and plain vacutainer were separated by centrifugation at 2000 rpm for 5 minutes and fasting plasma glucose (FBG) and hormones were determined. EDTA anticoagulant sample was used for hemoglobin and HbA_{1c} determination.

The FBG was performed by enzymatic method (Glucose oxidase method) [6], HbA_{1c} by latex agglutination inhibition assay [7] on the RANDOX autoanalyser. Prolactin, total testosterone, luteinising hormone (LH) & follicular stimulating hormone (FSH) were estimated on the Advia Centaur' CP immunoassay system by chemiluminescence method [8]. Thyroid stimulating hormone (TSH) was quantitated by immunoradiometric assay by IRMA kit (IRMAK - 9) [9]. Hemoglobin estimation was done by acid haematin method using Sahli's hemoglobinometer [10].

STATISTICAL ANALYSIS

Unpaired 't' test and two-tailed Pearson's correlation were done between variables of PCOS cases and controls using the IBM SPSS version 20 statistical package. Data were considered to be significant if $p < 0.05$ and highly significant with $p < 0.001$.

RESULTS AND OBSERVATIONS

In the present study, we found that 8 newly diagnosed PCOS cases (26.7%) had history of sudden weight gain. Based on categories of BMI, 16

Table 1 WHO classification of BMI grading [5]

BMI (kg/m ²)	Classification
< 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0–34.9	Class I obesity
35.0–39.9	Class II obesity
≥ 40.0	Class III obesity

Table 2 Descriptive data of the study groups

S.No.	Descriptive data	Cases (n = 30) Mean ± SD	Controls (n = 30) Mean ± SD	't' value	p value
1.	Age (years)	23.26 ± 5.65	23.6 ± 6.45	-0.213	0.832
2.	BMI (kg/m ²)	26.1 ± 4.2	22.9 ± 3.3	3.245	0.002*

* - significant difference, ** - highly significant difference

cases and 7 controls were overweight and obese (≥ 25 kg/m²) with statistically significant difference between cases and controls ($p=0.002$). Thirteen cases were within the normal range of BMI and 1 case was underweight.

We observed that 7 cases (89.2 ± 13.9 mg/dL) and 2 controls (88.5 ± 2.7 mg/dL) had FBG ≥ 100 mg/dL with non significant p value > 0.05 . We found that HbA_{1c} showed statistical significant difference ($p=0.002$) between cases (mean $6.03 \pm 1.03\%$) and controls (mean $5.35 \pm 0.43\%$) (Tables 2 & 3).

We noted from our HbA_{1c} data, 10 cases (33.3%) were in pre diabetic range (5.7 - 6.4%), 11 cases (36.7%) were in diabetic range ($\geq 6.5\%$) and 9 cases (30%) were in non diabetic range ($< 5.7\%$) according to ADA criteria (Figure 1).

We observed the prevalence of T2DM and prediabetes were 13.3% (4 cases) and 6.6% (2 cases) in obese PCOS women, respectively. But the prevalence of T2DM and prediabetes were 10% (3 cases) and 16.6% (5 cases) in overweight PCOS women, respectively.

We found that 7 cases (23.3%) were prediabetes with FG 100-126 mg/dL. But among controls, we noticed only one case (3.3%) had prediabetes (Figure 2).

TWO-TAILED PEARSON'S CORRELATION BETWEEN PARAMETERS

In the present study, it was found that HbA_{1c} had positive correlation with BMI ($r=0.439$, $p=.001$) (Figure 3).

Table 3 Analytical data of the study groups

S.No.	Analytical data	Cases (n = 30) Mean ± SE	Controls (n = 30) Mean ± SE	't' value	p value
1.	FG (mg/dL)	89.23 ± 2.5	88.47 ± 1.4	0.264	0.792
2.	Hb (g/dL)	14.16 ± 3.24	11.5 ± 0.17	0.929	0.357
3.	HbA _{1c} (%)	6.03 ± 0.19	5.35 ± 0.08	3.364	0.002*

* - significant difference, ** - highly significant difference

Figure 1 The mean value \pm SE of HbA_{1c} of the cases and controls

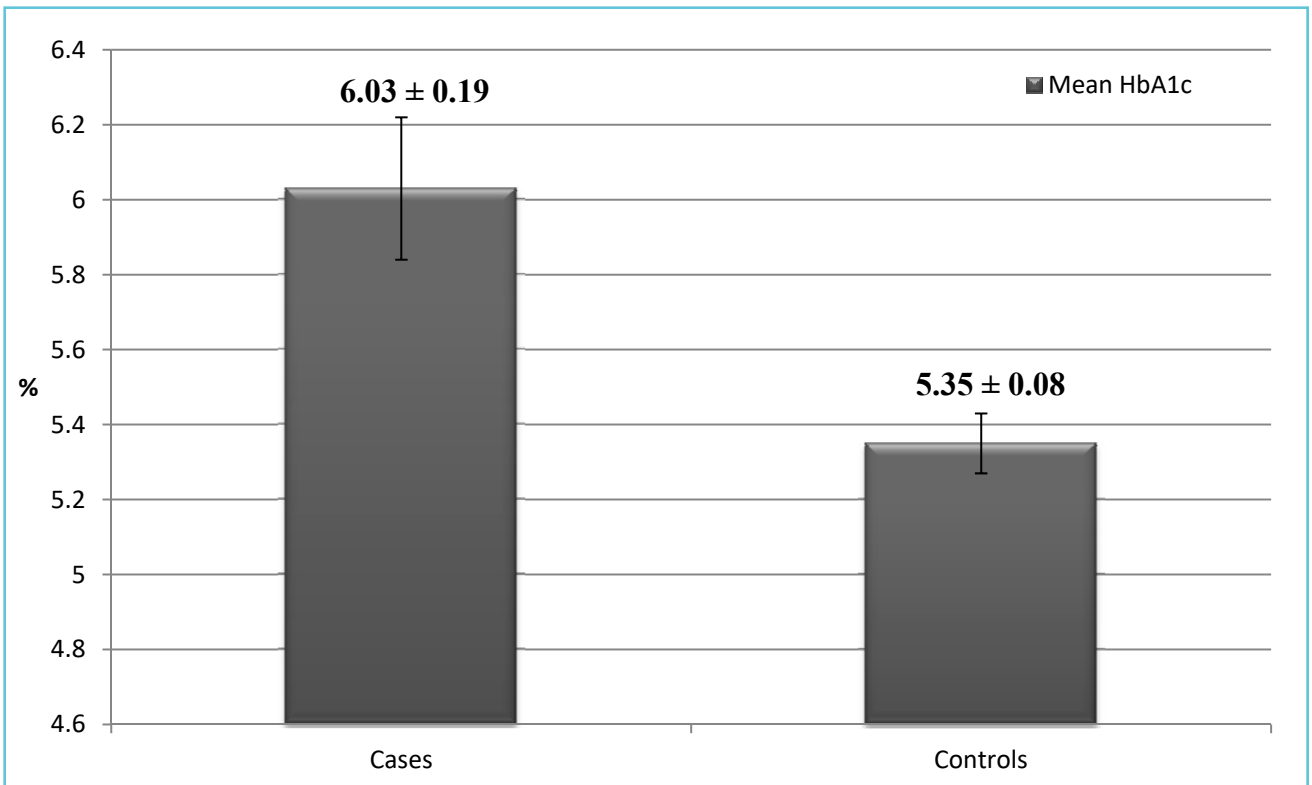


Figure 2 Bar diagram representing various categories based on HbA_{1c} level in the cases and controls

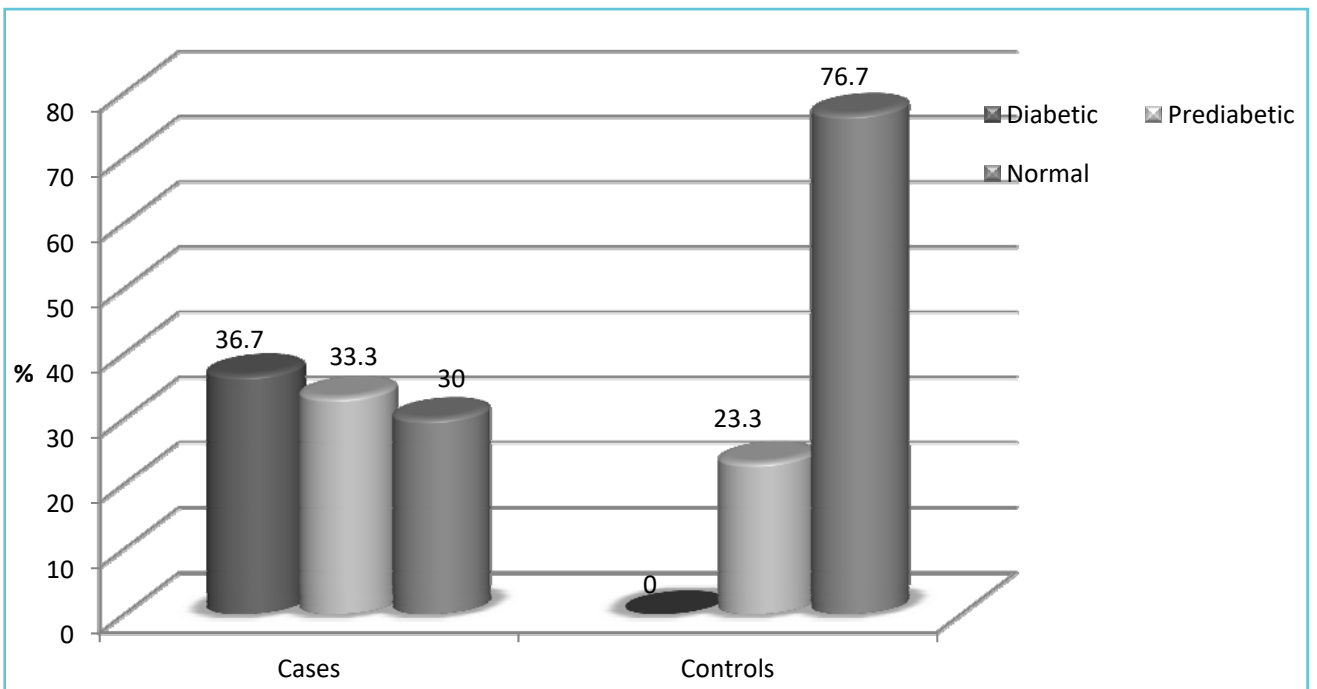
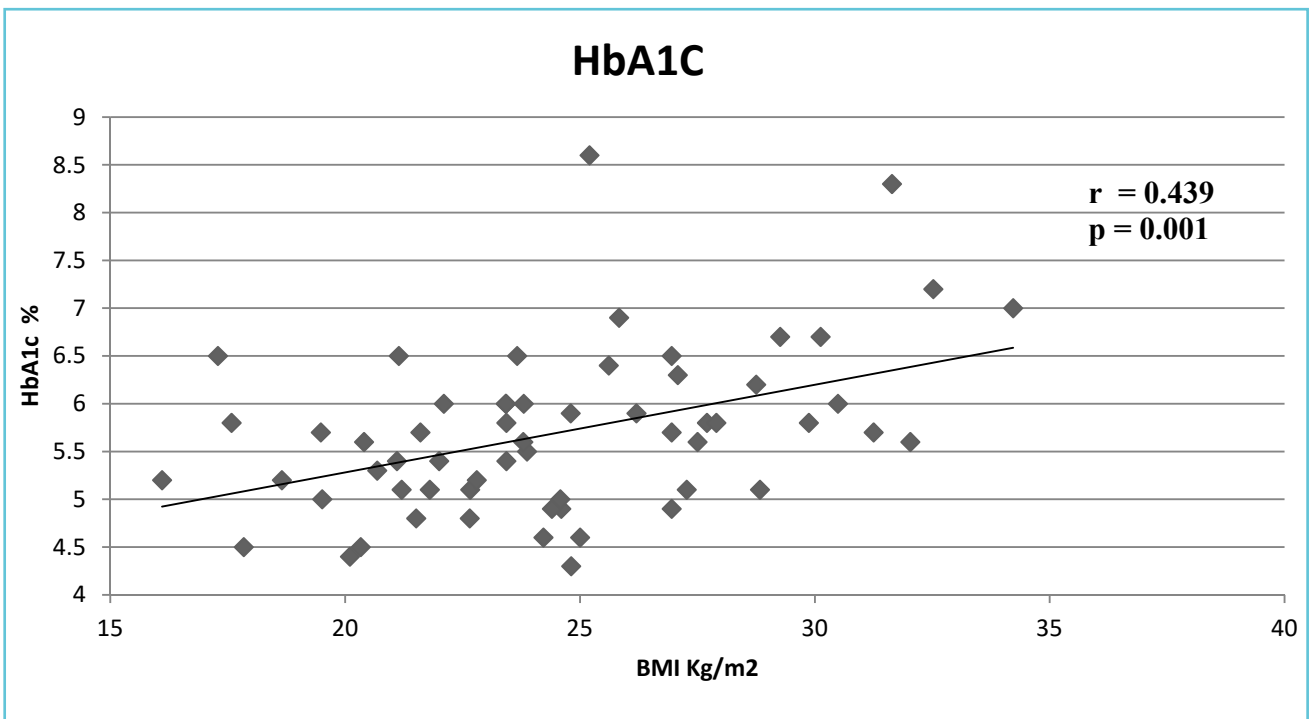


Figure 3 Graph showing the correlation between BMI & HbA_{1c}



DISCUSSION

When the hemoglobin is exposed to plasma glucose, there is a spontaneous non-enzymatic glycation of hemoglobin resulting in HbA_{1c} formation. The rate of formation of HbA_{1c} is directly proportional to the concentration of the glucose in the blood and represents integrated values for the glucose over the preceding 8 to 12 weeks. HbA_{1c} is formed by the condensation of glucose with the N-end of each beta chain to form an unstable schiff base. The schiff base may dissociate or may undergo an amadori rearrangement to form a stable ketomine called as HbA_{1c}. It has been established that HbA_{1c} is an index of long term blood glucose concentrations and as a measure of the risk for the development of microvascular complications in patients with diabetes mellitus. The formation of HbA_{1c} is an irreversible process and depends on lifespan of RBC and glucose concentration in the plasma. Severe anemia can show false high HbA_{1c} value as the

old red blood cell (RBC) population is higher than non anaemic conditions. In haemolytic anaemia, HbA_{1c} value is falsely low as life span of RBC is less. Therefore, it is mandatory to rule out anaemia in our study to avoid false results [11].

Lerchbaum et al reported that the prevalence of obesity was 24.8% and overweight was 21.8% among PCOS women [12]. Gomathi et al found that 54% of the women with PCOS were overweight or obese with respect to WHO classification of BMI grading [13]. Randeve et al reviewed that excess triglycerides enter into cells and activate proteins kinase C- ϵ and C- θ , ultimately reducing the glucose uptake. This leads to compensatory hyperinsulinemia which can stimulate excess fat deposition by hypertrophy and hyperplasia of adipose cells in the excess calorie environment. This is further aggravating IR by increasing obesity as a vicious cycle. This abnormal fat accumulation increases IR causing glucose intolerance and T2DM [14].

The prevalence of obesity in the PCOS population ranges from 38% to 87%. It has been reported that in obese PCOS women, the prevalence of IR was higher than obese women with normal menstrual cycle in the control group [3].

Medeiros et al concluded from their study, among amazonian PCOS women, that HbA_{1c} was elevated in nearly 40% of PCOS patients and had positive correlation with several anthropometric, metabolic factors and androgen levels [15].

While evaluating PCOS women, along with FBG estimation, they are needed to have oral glucose tolerance test (OGTT) or HbA_{1c} also to assess their metabolic status and can be categorised as per the American Diabetic Association (ADA) guidelines 2015 by the following any one of the criteria listed in Table 4.

As OGTT is time consuming and cumbersome test, HbA_{1c} and FBG have been suggested as screening tools for prediabetes and T2DM.

They also recommended HbA_{1c} as the superior screening tool for dysglycemia assessment in PCOS women [12].

Increased LH leads to increased stromal growth accounts for increased ovarian volume. Increased circulating LH stimulates ovarian thecal cells to produce more androgens leading to hyperandrogenemia in turn leads to increased male pattern (top / apple shaped upper abdomen) of fat accumulation both subcutaneously and around viscera which in turn leads to obesity, increased BMI.

High saturated fat, trans fat diet, less poly unsaturated fatty acid (PUFA) intake, high fructose/sucrose intake, low fat and high carbohydrate diet, sedentary life style, leptin resistance can contribute high triglycerides and VLDL-C level in the circulation which can alter plasma membrane composition resulting in decreased tyrosine acetylphosphorylation of insulin receptor [17-26].

Table 4 The ADA guidelines 2015 for diagnosis of diabetes & prediabetes [16]	
Criteria for diabetes diagnosis	
1.	HbA _{1c} ≥ 6.5%
2.	FBG ≥ 126 mg/dL (7.0 mmol/L)
3.	2-hr plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during OGTT (75g)
4.	Random plasma glucose ≥ 200mg/dL (11.1mmol/L)
Criteria for prediabetes diagnosis	
1.	HbA _{1c} 5.7%-6.4%
2.	FBG 100-125 mg/dL (5.6-6.9 mmol/L)
3.	2-hr plasma glucose 140-199 mg/dL (7.8-11.0 mmol/L) during OGTT (75g)

Kumar et al also observed higher BMI in women with PCOS than in controls [27]. The present study observations showed that 14 cases (46%) had increased BMI (≥ 25 Kg/m²) and increased HbA_{1c} and 16% of cases had normal BMI with increased HbA_{1c} levels. Among controls who had increased HbA_{1c}, we found that 6.7% of them had increased BMI and 3.3% had normal BMI. The prevalence of IR was found to be increased in obese PCOS women than in normally menstruating obese women. IR plays a central role in the pathogenesis of PCOS. Obesity is a co-morbid condition which can increase its risk. Though obesity is more prevalent among PCOS women, about 20 - 30% of PCOS women are not obese. Hyperinsulinemia causes direct hypothalamic effects which lead to abnormal appetite and gonadotropin secretion resulting in increased LH secretion in PCOS. This increased level of LH causes excessive androgen production in the ovaries [3].

CONCLUSION

In conclusion, BMI is associated with glycemic control in PCOS.

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Factors affecting quality of laboratory services in public and private health facilities in Addis Ababa, Ethiopia

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ABSTRACT

Background

Quality laboratory service is an essential component of health care system but in Sub-Saharan Africa such as Ethiopia, laboratories quality system remains weak due to several factors and it needs more attention to strengthen its capacity and quality system.

Methodology

A cross sectional study was conducted using a questionnaire to assess factors affecting the quality of laboratory service at private and public health institutions in Addis Ababa.

Results

A total of 213 laboratory professionals participated in the study and 131 (61.5%) participants had bachelor degree. Majority, 133 (62.4%), of the professionals did not attend any work related training. Seventy five (35.2%) respondents believed that their laboratories did not provide quality laboratory services and

the major reported factors affecting provision of quality services were shortage of resources (64.3%), poor management support (57.3%), poor equipment quality (53.4%), high workload (41.1%), lack of equipment calibration (38.3%) and lack of knowledge (23.3%). Moreover logistic regression analysis showed that provision of quality laboratory service was significantly associated with result verification (AOR=9.21, 95% CI=2.26, 37.48), internal quality control (AOR= 6.11, 95% CI=2.11, 17.70), turnaround time (AOR=5.11, 95% CI=1.94, 13.46), shortage of equipment (AOR=7.76, 95% CI=2.55, 23.66), communication with clinicians (AOR=3.24, 95% CI=1.25, 8.41) and lack of job description (AOR=3.67, 95% CI=1.319, 10.22).

Conclusion

In conclusion, the major factors that affecting the quality of laboratory service were associated with poor human resource management, poor resources provision, poor management commitment, ineffective communication system and lack of well-established quality management system.



INTRODUCTION

In Sub-Saharan Africa, the major challenge for delivering quality health service is the lack of reliability of medical laboratory services¹. Quality laboratory service is essential for a wide range of diagnosis, treatment and monitoring in health care delivery. But due to lack of awareness on the laboratory service role in many developing countries, laboratory services have shortage of resources, poor management system²⁻³, lack of quality assurance program, shortages of equipment, shortage of training and poor staff motivation system⁴.

Poor quality laboratory services lead to unnecessary expenditures, misery in human lives and suffering, and producing wrong data in disease prevalence due to misdiagnosis. The results are over-treatment and overuse of antibiotics for inappropriate clinical circumstances which leads to the emergence of drug resistant microorganisms include multi-drug resistant TB⁵. Likewise patient safety is also influenced by the frequency and seriousness of errors that occur in the health care system⁶.

It is well known that quality of laboratory service is dependent on technical skills, quality management systems and the motivation of human resources⁷. However, the first barrier for quality improvement at health care system is human capacity development, which continues to be a gap in implementing health programs including laboratory services⁸, in addition several barrier are identified in laboratory services, including lack of laboratory supplies, poor quality management system, absence of laboratory standards and policy⁹. Besides lack of access to reliable diagnostic services and under-resourced laboratory infrastructure in developing countries are another challenges for quality of diagnoses which lead to inadequate treatment, increased morbidity, and inaccurate determination of the burden of disease¹⁰.

Moreover, studies from Sub-Saharan Africa revealed that major factors affecting the laboratory services were staff shortages, poor communication system, inadequate equipment, low motivation, lack of training¹¹, lack of internal quality control (IQC), power supply interruption, equipment failure, and poor infrastructure. Furthermore, another limitation for establishing an efficient laboratory system in developing countries was a lack of resources and a system for implementing, managing, and monitoring laboratory activities⁹.

To tackle these factors, several efforts are continued in sub-Saharan Africa however there are still numerous challenges hindering the quality of laboratory services as well as health care system. Moreover as Ethiopia is one of Sub-Saharan Africa country, laboratory infrastructure and quality assurance activities remain weak¹² and there is little information available on factors affecting quality of medical laboratory services. Therefore, this study was conducted to assess factors affecting the provision of quality of laboratory services and provide baseline information to policy and decision makers.

MATERIALS AND METHODS

Study design

A descriptive cross sectional study was conducted using questionnaire to assess factors that affecting the provision of quality of medical laboratory services in public (owned by government) and private (owned by private and non-government organization) health facilities from December 2013 and February 2014 in Addis Ababa, Ethiopia. The estimated population size of Addis Ababa was 2.74 million¹³ and according to 2011 Health and Health Related Indicators report the city had 42 public and private hospitals, 37 health centers and 394 laboratory professionals¹⁴. Health institution that have functional laboratories and willing to participate were included in the study.

Sampling procedures

A single population proportion formula was used for determination of the sample size considering the following assumptions: proportion of 50% taken due to absence of similar previous study, level of significance = 0.05, Marginal of error (d) = 5%, $Z(\alpha/2)$ = Z-score at 95% confidence interval = 1.96.

$$n = [Z_{\alpha/2}^2 \times p(1-p)] / d^2$$

$$n = [1.96^2 \times 0.5(1-0.5)] / 0.05^2 = 384$$

$$n = 384$$

According to Health and Health Related Indicators there were 394 laboratory professionals in Addis Ababa¹⁴, therefore, the correction factor was done using the finite population formula (nf) from a target population (N) and the sample size was reduced according to the following formula:

$$nf = n / (1 + n/N)$$

$$nf = 384 / (1 + 384/394) = 194$$

$$nf = 194$$

Considering 10% of non-response rate, the sample size for laboratory professionals was 213. Finally sample size was allocated proportionately for 30 voluntary and eligible health facilities according to number of laboratory professionals working in their laboratories and laboratory professionals were selected for the interview using random sampling method.

Data collection procedures

Structured questionnaire was used for data collection. It included different questions, such as socio-demography, education background, work experience, motivation, communication, training, quality assurance activities, and factors affecting quality of laboratory service.

All laboratory professionals having more than a years experience and willing to participate were included in the study. Trained and experience laboratory technologists interviewed laboratory professionals using questionnaire for data collection. Principal investigator involved in overall controlling activities of data collections and assisting data collectors during the process of data collection.

Data management and statistical analysis

All data were coded and fed into SPSS version 16 statistical software and data were checked for completeness and consistency of variables. Descriptive statistics were computed to calculate the frequency and percentage, and bivariate analysis were also conducted to assess the presence of associations between dependent variable and the independent variables. Dependent variable is provision of quality laboratory services; defined as the ability of a laboratory service to satisfy stated or implied needs of a specific customer or fulfils requirements, and the independent variables are socio demography variables, education background, work experience, motivation, communication, training, workload and quality assurance practices (*Job descriptions, supplies & reagents management, equipment calibration & maintenance, result verification, Internal quality control activities practice and turnaround time customer management and services interruption*).

Moreover multiple logistic regressions were carried out to control the confounding factors, and variables which had a p-value less or equal to 0.05 in bivariate analysis were included in the multivariate logistic regression model. Odds ratio with 95% confidence interval were used to measure the strength of association between

potential affecting factors and provision of Quality laboratory services.

Ethical consideration

Before any attempt to collect data, ethical clearance was obtained from Institutional Review Board (IRB) of School of Medicine, Addis Abba University. Each participant was informed about the purpose of the study, the right to refuse to participate in the study, and anonymity and confidentiality of the information gathered.

RESULTS

Socio-demographic characteristics

A total of 213 laboratory professionals participated in this study from 13 public and 17 private health institutions in Addis Ababa, and 130 (61%) of the respondents were employed in public health institutions and majority 135 (63.4%) of participants were male. One hundred and twenty-one (56.8%) of respondents were between 20-30 years old with an average age of 32 years. One hundred thirty one (61.5%) were Medical Laboratory Technologist (Bachelor Degree) referred to as highly trained laboratory professionals and 82 (38.5%) were Medical Laboratory Technicians (Diploma) referred to as mid-level trained laboratory professionals.

In terms of work experience, 179 (84.0%) the respondents had 3 and above years of experience

Table 1 Socio-demographic characteristics of laboratory professionals working in public and private health institutions in Addis Ababa Ethiopia (n=213)

Variable	Frequency (%)
Sex	
Male	135 (63.4%)
Female	78 (36.6%)

Age group	
20-30 Years	121 (56.8%)
31-40 Years	59 (27.7%)
41-50 Years	18 (8.5%)
51-60 Years	15 (7.0%)
Educational level (profession)	
Diploma (Laboratory Technician)	82 (38.5%)
Bachelor Degree (Laboratory Technologist)	13 (161.5%)
Working organization	
Public	130 (61.0%)
Private	83 (39.0%)
Working experience in laboratory fields	
1-2 Years	34 (16.0%)
3-5 Years	61 (28.6%)
6-10 Years	48 (22.5%)
>10 Years	70 (32.9%)
Position	
Laboratory head	20 (9.4%)
Supervisor	33 (15.5%)
Expert	152 (71.3%)
Quality Officer	8 (3.8%)

Laboratory discipline	
General laboratory	127 (59.6%)
Clinical chemistry	28 (13.1%)
Hematology	23 (10.8%)
Parasitology	15 (7.0%)
Microbiology	11 (5.2%)
Immunology/Serology	9 (4.2%)

and they worked as head, supervisor, quality officer and expertise and 127 (59.6%) of the professionals worked at general laboratory which perform basic chemistry, hematology, parasitology, microbiology and serology tests and the remaining professionals work at Clinical Chemistry, Hematology, Parasitology, Microbiology and Immunology laboratories (Table 1).

Motivation and communication

Most 187 (87.8%) of the laboratory professionals were not satisfied with their salary and 178 (83.6%) respondents indicated that there was no system for staff recognition, and 133 (62.4%) of the laboratory professionals did not attend any task specific training and 128 (60.1%) continuing education program. A total of 150 (70.4%) of the laboratory professionals had high workload while 125 (58.7%) of the respondents indicated a shortage of human resources in their laboratories. In addition 110 (51.6%) of the laboratory professionals did not have job descriptions for the task assigned. Concerning communication, the laboratory professionals indicated that there were no systems to communicate with laboratory staff, upper management, and clinicians with respondents 126 (59.2%), 120 (56.3%) and 75 (35.2%) respectively (Table 2).

Quality assurance practices

In terms of quality assurance practice, findings from our study revealed that 85 (39.9%) laboratory professionals did not perform equipment calibration & maintenance as per instruction and 91 (42.7%) of the respondents also indicated that there was supplies and reagents interruption and the available supplies and reagents had also poor quality. Likewise, internal quality control (IQC) was not conducted regularly as stated by more than 33% of the respondents besides 34 (16.0%) and 55 (25.8%) of the laboratory professionals did not participate on external quality assessment (EQA) and quality improvement activities respectively, is shown in Table 3.

Table 3 shows that 33 (15.5%) laboratory professionals did not verify laboratory results and 70 (32.9%) of respondents claimed that laboratory results were not released within predefined turnaround time. Laboratory documentation (documents and records) system were not practiced as per standard by 45 (21.1%) the laboratory professionals. One hundred twenty-one (56.8%) laboratory professionals also indicated that customer services management system was poor in their respective laboratories.

Table 2 Knowledge & laboratory management activities reported by laboratory professionals working in public and private health institutions in Addis Ababa, Ethiopia (n=213)

Variable	Frequency (%)
Knowledge on laboratory quality system essentials	
Yes	197 (92.5%)
No	16 (7.5%)
Laboratory communication with Clinicians	
Yes	138 (64.8%)
No	75 (35.2%)
Laboratory communication with upper management	
Yes	93 (43.7%)
No	120 (56.3%)
Laboratory communication among laboratory staff	
Yes	126 (59.2%)
No	87 (40.8%)
Laboratory staff satisfied with their salary	
Yes	26 (12.2%)
No	187 (87.8%)
System for employees recognition	
Yes	35 (16.4%)
No	178 (83.6%)

Attending of continuing education program	
Yes	85 (39.9%)
No	128 (60.1%)
Attending of laboratory refreshment training	
Yes	80 (37.6%)
No	133 (62.4%)
Job descriptions for assigned task	
Yes	103 (48.4%)
No	110 (51.6%)
Availability of quality and adequate equipment in laboratory	
Yes	93 (43.7%)
No	120 (56.1%)
Availability of quality and adequate supplies & reagents	
Yes	122 (57.3%)
No	91 (42.7%)
Adequate number of staff for laboratory services	
Yes	88 (41.3%)
No	125 (58.7%)
Laboratory workload	
High	150 (70.4%)
Fair	63 (29.6%)

Table 3 Quality assurance practices and provision of laboratory services reported by laboratory professionals working in public and private health institutions in Addis Ababa, Ethiopia (n=213)

Variable	Frequency (%)
Laboratory documentation (documents and records)	
Yes	168 (78.9%)
No	45 (21.1%)
Adherence to the standard operating procedures	
Yes	143 (67.1%)
No	70 (32.9%)
Customer services management	
Yes	92 (43.2%)
No	121 (56.8%)
Equipment calibration & maintenance	
Yes	128 (60.1%)
No	85 (39.9%)
Laboratory quality improvement activities	
Yes	158 (74.2%)
No	55 (25.8%)
External quality assessment activities	
Yes	179 (84.0%)
No	34 (16.0%)

Internal quality control activities	
Yes	141 (66.2%)
No	72 (33.8%)
Providing diagnostic services for all requested tests	
Yes	139 (65.3%)
No	74 (34.7%)
Providing uninterrupted laboratory services	
Yes	92 (43.2%)
No	111 (52.1%)
No information	10 (4.7%)
Laboratory result verification	
Yes	180 (84.5%)
No	33 (15.5%)
Laboratory results reported within turnaround time	
Yes	143 (67.1%)
No	70 (32.9%)
Laboratory safety practices	
Yes	140 (65.7%)
No	73 (34.3%)
Utilization of personal protective equipment	
Yes	164 (77.0%)
No	49 (23.0%)

Provision of quality laboratory services	
Yes	138 (64.8%)
No	75 (35.2%)

Seventy-three (34.3%) laboratory professionals did not monitor laboratory safety practices and 69 (23%) the professionals did not use personal protective equipment (PPE) during working in laboratory.

Factors affecting provision of quality laboratory services

Regarding to factors that affecting the quality assurance practice, our finding discovered that high workload, shortage of resource, poor management supports, poor staff motivation, lack of knowledge, high workload equipment failure, shortage of supplies and reagents were the major factors that affecting the quality assurance practice at the laboratories, is shown in Table 4.

As regard to laboratory services, 75 (35.2%) laboratory professionals believed that their laboratories did not provide quality of laboratory services as per the standards and 74 (34.7%) respondents indicated that their laboratories did not provide diagnostic services for all requested tests, in addition to this, 111 (52.1%) of respondents also reported that there were laboratory services interruption in their respective laboratories due to several reasons.

Concerning to factors that affecting the provision of quality laboratory services, our finding showed that shortage of resources was rated highest factor influenced the provision of quality laboratory services to a great extent by 64.3% and the second and third highest ranked factors were lack of management supports and poor equipment quality and rated by 57.4% and

53.4% respectively. Moreover high workload, lack of equipment calibration, lack of knowledge and skills and poor staff motivation were the major factors that affecting the provision of quality of laboratory services in this study, is shown in Figure 1.

Logistic regression analysis result

Logistic regression analysis showed that provision of quality laboratory services was significantly associated with laboratory result verification (adjusted odds ratio (AOR)=9.21, 95% CI=2.26, 37.48), shortage of equipment (AOR= 7.76, 95% CI= 2.55, 23.66), internal quality control (AOR= 6.11, 95% CI=2.11, 17.70), results turnaround time (AOR=5.11, 95% CI=1.94, 13.46), communication with clinicians (AOR= 3.24, 95% CI= 1.25, 8.41) and lack of job description (AOR= 3.67, 95% CI= 1.319, 10.22).

Laboratory professionals, who did not verify laboratory results were found to be 9.2 times more likely to provide poor quality laboratory services when compared with those who verify laboratory results.

Laboratory professionals who did not practice internal quality control activities were 6 times more likely to provide poor quality laboratory services compared to regular practice. Moreover laboratory professionals who did not report results within defined turnaround time were 5 times more likely to provide poor quality laboratory services when compared with those who report results within turnaround time, is shown in Table 5.

Table 4 Factors affecting quality assurance practices reported by laboratory professionals working in public and private health institutions in Addis Ababa, Ethiopia (n=213)

Variable	Shortage of resources	Lack of knowledge	Poor staff motivation	High workload	Poor management support	Equipment failure	Factors unknown
Documents and records (n=45)	21 (46.7 %)	6 (13.3 %)	20 (44.4 %)	24 (53.3 %)	21 (46.7 %)	-	5 (11.1 %)
Customer services management (n= 121)	39 (32.2 %)	22 (18.2 %)	39 (32.2 %)	34 (28.1 %)	39 (32.2 %)	-	5 (4.1 %)
Equipment calibration & preventive maintenance (n=85)	36 (42.4 %)	29 (34.1 %)	33 (38.8 %)	23 (27.1 %)	35 (41.2 %),	-	-
Laboratory quality improvement activities (n=55)	12 (21.8 %)	8 (14.5 %)	39 (70.9 %)	24 (43.6 %)	39 (70.9 %)	-	5 (9.1 %)
Internal quality control activities (n=72)	35 (48.6 %)	12 (16.7 %)	30 (41.7 %)	18 (25%)	-	-	-
External quality assessment activities (n=34)	11 (32.4 %)	10 (29.4 %)	4 (11.8 %)	-	11 (32.4 %)	-	9 (26.5 %)
Laboratory result verification (n=33)	14 (42 %)	3 (9.1 %)	11 (33.3 %)	7 (21.2 %)	-	-	3 (9.1 %)

Laboratory results reported within turnaround time (n=70)	43 (61.4 %)	-	30 (42.9 %)	49 (70 %)	-	48 (68.6 %)	-
Laboratory safety practice (n=73)	30 (41.1 %)	16 (21.9 %)	18 (24.7 %)	21 (28.8 %)	30 (41.1 %)	-	4 (5.5 %)
Utilization of personal protective equipment (n=49)	46 (93.8 %)	3 (6.2 %)	-	-	-	-	-

Note: Since respondents provided more than one answer, the total percentage of responses was more than 100%.

Figure 1 Factors affecting the provision of quality laboratory services in public and private health institutions in Addis Ababa, Ethiopia



Note: Since respondents provided more than one answer, the total percentage of responses was more than 100%.

Table 5 Logistic regression analysis showing the association between provision of quality laboratory services and covariates in public and private health institution laboratories in Addis Ababa, Ethiopia

Variable	Provision of quality laboratory services		Crude odds ratio (95 % CI)	Adjusted odd ratio (95 % CI)
	Yes	No		
Profession				
Lab Technician	63	20	1	1
Lab Technologist	75	55	2.310 (1.253, 4.258)	0.701 (0.255, 1.925)
Work Experience				
1-2 Years	27	7	1	1
3-5 Years	39	22	0.611 (0.195, 1.911)	1.114 (0.242, 5.122)
6-10 Years	24	24	0.636 (0.248, 1.631)	1.558 (0.325, 7.481)
>10 Years	48	22	0.733 (0.137, 3.938)	0.947 (0.204, 4.409)
Satisfaction with salary				
Yes	24	2	1	1
No	114	73	7.684 (1.763, 33.491)	5.926 (0.719, 48.81)
Providing uninterrupted services				
Yes	76	15	1	1
No	62	60	4.903 (2.540, 9.465)	1.938 (0.677, 5.549)
Communication with clients				
Yes	105	33	1	1
No	33	42	4.050 (2.221, 7.384)	3.238 (1.246, 8.414)*

Communication with upper management				
Yes	69	24	1	1
No	69	51	4.050 (2.221, 7.384)	0.320 (0.106, 0.961)
Job descriptions				
Yes	85	18	1	1
No	53	57	5.079 (2.701, 9.548)	3.672 (1.319, 10.22)*
Enough equipment				
Yes	82	11	1	1
No	56	64	8.519 (4.129, 17.579)	7.76 (2.548, 23.659)*
Enough supplies & reagents				
Yes	88	34	1	1
No	50	41	2.122 (1.198, 3.760)	1.113 (0.458, 2.701)
Adherence to SOP				
Yes	107	36	1	1
No	31	39	3.739 (2.044, 6.842)	1.028 (0.364, 2.905)
Clients' satisfactions assessment				
Yes	81	11	1	1
No	57	64	8.268 (4.009, 17.053)	2.261 (0.851, 6.007)
Equipment calibration & maintenance				
Yes	99	29	1	1
No	39	46	4.027 (2.222, 7.296)	0.605 (0.207, 1.767)

Laboratory results verification				
Yes	131	49	1	1
No	7	26	9.930 (4.050, 24.346)	9.21, (2.263, 37.482)*
Internal quality control activities practice				
Yes	111	30	1	1
No	27	45	6.167 (3.302, 11.518)	6.105, (2.105, 17.70)*
Results reported within turnaround time				
Yes	114	29	1	1
No	24	46	7.534 (3.972, 14.290)	5.111 (1.94, 13.464)*

* The odds ratio indicated that there was significant association between provision of quality laboratory service and independent variables.

DISCUSSION

The majority of laboratory professionals work under high workload without job descriptions, continuing education and training. However Baidoun and Zairi pointed out that education, training and motivation are major factors for implementation of quality system¹⁵ and non-trained professionals can be costly to the laboratory system due to inaccurate test results. In addition to this, more than 83% of the professionals were not satisfied with their salary and staff recognition system, as well as poor communication system, this is in agreement with studies done by Lyons et al¹⁶ and Al-Enezi et al¹⁷. However it is well understood that motivation could be brought with simple letter of recognition¹⁸ and effective communication also contributes to quality of services¹⁹.

Moreover it is well documented that implementation of laboratory standards helps laboratories to demonstrate a well-functioning quality

management system, technical competence, and customer-focused services that contribute to health care services²⁰. But this study found out that documentation system, result verification & reporting system, equipment calibration & maintenance, quality control activities, customer management and laboratory safety were not implemented as per the standards. So poor quality management system directly affects the provision of quality laboratory services as well as patients and health care services at large.

Besides this, our study discovered that the major factors that affecting provision of quality laboratory services were high workload, shortage of resource, poor management supports, poor staff motivation, lack of knowledge and skills, high workload, equipment failure and lack of calibration, shortage of supplies and reagents. It was comparable with previous studies done by Wanjau et al., Alash'le et al., Birs D et al., and Bates et al. who found that major factors

affecting laboratory services were lack of lack of resource^{9,19}, equipment failure, poor management system⁹, shortage of staff, low staff motivation, lack of knowledge^{19,21} and lack of training¹¹. Furthermore, another study done in Ethiopia revealed that lack of equipment maintenance, shortage of reagents and supplies, poor laboratory management and lack of follow-up were identified as factors²².

Additionally, the study also revealed that provision of quality laboratory services had statistically significant association with internal quality control, result verification, result report time, communication, equipment and job description. This clearly indicates that there existed a negative relationship between factors affecting quality laboratory service and provision of quality laboratory service and it implied that the variables affect delivery of quality laboratory service to health sector programs and patient satisfaction.

In general the major findings from this study were factors associated with human resource, resources, infrastructure, quality management system, and policies, and these challenges facing laboratory systems in resource-poor settings like Ethiopia. Together, these factors compromise the provision of quality laboratory services and quality health care services delivery as well as public health provision. So improving laboratory quality systems requires political commitment, financial support, and strong support and follow up from the health system leaders, and competent and motivated laboratory staff.

CONCLUSION

In conclusion, most of the laboratories did not have well established laboratory quality management system, system for staff recognition, and continuing education/training program. The major factors affecting the provision of quality

laboratory services were shortage of resources, lack of management supports, poor equipment management system, high workload, lack of competent staff, low staff motivation, ineffective communication system and lack of well-established quality management system. In addition poor internal quality control practice, absence of result verification system, delay of result reporting time, and lack of job description were affected the provision of quality laboratory services.

As laboratory services is an essential component of the health care system, laboratory capacity building and quality management system implementation will enable to provide quality and reliable services for disease treatment and prevention. Therefore, government and stakeholders should understand and address the factors affecting the provision quality laboratory service and they should work together for strengthening laboratory quality assurance and accreditation program.



Authors' contributions

Eyob Abera Mesfin conceived and designed the study and collected data, performed analysis, interpretation of data and draft the manuscript. Binyam Taye, Getachew Belay, Aytenev Ashenafi and Veronica Girma assisted with the design, performed analysis, interpretation of data and the critical review of the manuscript. All authors read and approved the final manuscript. All authors participated in critical appraisal and revision of the manuscript.

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What is known about this topic?

- Laboratory service is an essential component of health care system with a wide range of diseases diagnosis, treatment and monitoring services.
- There are many factors affecting provision of quality laboratory services in developing countries especially in sub-Saharan Africa.
- Efforts to improve laboratory capacity and quality system in resource-limited countries are very limited and access to reliable laboratory testing remains limited in many countries. This results in delayed diagnosis, misdiagnosis, & inappropriate treatment and leads to increase morbidity and mortality.

What this study adds

- There are several factors affecting provision of quality laboratory services in public and private health institution in Addis Ababa Ethiopia.
- There is need for a government and stakeholders to be supporting and strengthening laboratory quality system since it is an essential component of health care system.
- Additionally this study adds knowledge and information on factors affecting the provision of quality of laboratory services in health care system to health policy and decision makers and provides useful baseline information for all efforts that will be made to improve quality laboratory services in futures.

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Retrospective approach to evaluate interferences in immunoassay

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heterophilic- antibody, immunoassay,
interferences, cross reactive

ABSTRACT

Background

Despite the increase in sensitivity and specificity of immunoassay technique over years, analytical interference remains to be major area of concern.

The interfering substances are endogenous substances that are natural, polyreactive antibodies as heterophilic or auto antibodies, or human anti-animal antibodies together with other unsuspected binding proteins that are unique to the individual. Interfering substances can interfere with the reaction between analyte and reagent antibodies in immunoassay resulting in false positive or negative values. This ultimately results in misinterpretation of patients reports and finally to wrong course of treatment.

Objective

In our study, we used a retrospective approach to find out the extent of interferences and type of interferences in some cases during our routine practice.

Method

The immunoassay reports which were clinically not correlating were retrospectively evaluated after discussion with the clinician. Over a period of six month a total of 42 samples were evaluated for interference for

different immunoassay parameters such as Beta HCG, Estradiol, CA 125, AFP, prolactin, Hepatitis B Surface antigen (HbSAg) and troponin I. The samples were treated with commercially available antibody blocking agents and were reanalyzed. Commercially available diluents were used in some cases to evaluate high dose hook effect. Different platform, methodology and reagents were used for re-analysis.

Results

Out of 42 samples, 19 were found to be affected by interferences. The data obtained for interferences was as follows: beta HCG - 6 samples (2 positive and 4 negative interference); estradiol - 3 samples (2 positive and 1 negative interference); CA-125 - 3 samples (2 positive and 1 negative interference); Alfa Feto Protein - 2 samples (2 positive interference); prolactin - 1 sample (positive interference); Hepatitis B Surface antigen - 1 sample (negative interference); troponin I - 2 samples (positive interference).

Conclusion

Despite the use of state of the art laboratory equipments, chances of interference in immunoassay analysis resulting from endogenous substances could not be ruled out. In conclusion, thorough evaluation of all immunoassay reports should be carried out in cases of suspected interference.



INTRODUCTION

All laboratory assays are subject to interferences. The effects of hemolysis, lipemia, and bilirubinemia (i.e., icterus) on laboratory methods are well documented. Each of these may affect the analytical measurement. Despite the analytical sensitivity of immunoassays and measurements often being made without the need

for prior extraction, immunoassays may lack adequate specificity and accuracy (1).

Developing immunoassays for the quantification of an analyte in a buffer solution has its own challenges, nonetheless quantification of the same analyte in a biological matrix (usually serum or plasma) bears additional complexities. The challenges include background assay signal changes, biological variability (between matrix samples) exceeding analytical imprecision and recovery of the spiked reference standard can be challenging. Despite the increase in sensitivity and specificity of the immunoassay techniques over years, analytical interference remains to be a major area of concern.

The interfering substances change the measurable concentration of the analyte or the altered antibody binding can potentially result in immunoassay interference. These are endogenous substances that are natural, polyreactive antibodies with other unsuspected binding proteins that are unique to the individual. These substances can interfere with the reaction between analytes and reagent antibodies in immunoassay resulting in false positive or false negative values (2,3,4,5,6). This ultimately results in misinterpretation of patients reports and finally to wrong course of treatment.

Heterophile antibodies accounts for large amount of interference in immunoassay. The presence of a heterophile antibody is characterized by broad reactivity with antibodies of other animal species (which are often the source of the assay antibodies). Such antibodies are commonly referred to as human anti-animal antibodies (HAAA). Human anti-mouse antibodies (HAMA) belong to this category. These can result in both false positive and false negative results (7). These are endogenous antibodies produced against poorly defined antigens. Both IgG and IgM heterophilic antibodies have been reported (8). These antibodies react

with various antigens and the variable region of other antibodies (anti-idiotypic antibodies). In most of the cases there is no history of medical treatment with animal immunoglobulin or other well-defined immunogens, these are characteristically multi-specific (reacts with immunoglobulin from two or more species) or exhibit rheumatoid activity. So-called 'sandwich' immunoassays are particularly susceptible to this interference.

High dose hook effect is one of the cause of analytical interference in immunoassay. The hook effect or the prozone effect is a type of interference which plagues certain immunoassays and nephelometric assays, resulting in false negatives or inaccurately low results (9). The effect can also occur because of antigen excess, when both the capture and detection antibodies become saturated by the high analyte concentration. In most case no sandwich can be formed

by the capturing antibody, the antigen and the detection antibody (10).

Analytical interference may also be due to cross reactivity. It is the most common interference - mostly in competitive assay. Cross reacting substance compete for binding site of antibody, resulting in over - or underestimation of analyte concentration (11). Cross reactivity is a major problem in diagnostic immunoassays. Cross reactivity can occur where endogenous molecules with a similar epitopes to the measured analyte exist in the sample, these may be metabolites of the analyte, or structurally similar pharmaceutical agents. (12)

Considering these interferences in immunoassay we have done follow up of non clinically correlating results. After excluding the pre analytical and post analytical factors root cause analysis of analytical interferences is done and presented here.

Table 1 Details of various platforms, methodologies, reagents used in study

Sr. No.	Equipment model & make	Methodology	Reagent used
1.	Access 2 Beckman Coulter	Chemiluminescence Immunoassay	Dedicated reagent
2.	Dxl600 Beckman Coulter	Chemiluminescence Immunoassay	Dedicated reagent
3.	Vitros Eci Ortho Clinical Diagnostic	Particle Enhanced Chemiluminescence Immunoassay	Dedicated reagent
4.	Vitros 3600 Ortho Clinical Diagnostic	Particle Enhanced Chemiluminescence Immunoassay	Dedicated reagent
5.	Mindray ELISA Reader	Enzyme Linked Immuno absorbent Assay	DRG Diagnostics ELISA kits for beta HCG, estradiol, Ca 125, AFP, Prolactin

Table 2 Details of antibody blocking agent used in study

Sr. No.	Test parameter	Antibody blocking agent	Specification
1.	Estradiol	True Block	Active HA/HAMA blocker - goat anti-human IgG (GAH IgG) Fc fragment
2.	CA 125	True Block	Active HA/HAMA blocker - goat anti-human IgG (GAH IgG) Fc fragment
3.	AFP	True Block	Active HA/HAMA blocker - goat anti-human IgG (GAH IgG) Fc fragment
4.	Prolactin	True Block	Active HA/HAMA blocker - goat anti-human IgG (GAH IgG) Fc fragment
5.	Troponin I	True Block	Active HA/HAMA blocker - goat anti-human IgG (GAH IgG) Fc fragment

OBJECTIVE

In our study, we have used a retrospective approach to evaluate the extent and type of interferences in immunoassays during our routine practice laboratory.

METHOD

All the immunoassay reports which were clinically non correlating were retrospectively evaluated following consultation with the clinicians. Over a period of six month nearly 87,780 immunoassays were performed of which 42 samples were evaluated for interference - Beta HCG, Estradiol, CA 125, AFP, prolactin, HbSAg and troponin I results were scrutinized. Thorough patient history was collected to evaluate exposure to animal antibody and recent immunoglobulin inoculation. The samples were treated with commercially available antibody blocking agents and were reanalyzed (Table No. 2). Commercially available diluents were used to evaluate high dose hook effect (Table No. 3).

Different platform, methodology, reagents were used for re-analysis (Table No. 1). Strict quality control measures were followed throughout the analysis process.

RESULTS

Out of 42 samples 19 were found to be affected by analytical interferences. The pre-analytical interferences was found in 20 cases and were attributed to wrong time of collection, wrong patient identity and wrong dilution protocols. Post analytical errors were found in 3 cases and were mainly due to wrong transcription in manual entry and wrong calculation in case of manual dilutions.

The present study focused on analytical interference. The data obtained for analytical interferences was as follows: beta HCG - 6 samples (2 positive and 4 negative interference); estradiol - 3 samples (2 positive and 1 negative interference); CA-125 - 3 samples (2 positive and 1 negative interference), Alfa Feto Protein - 2 samples

Table 3 Details of diluents used in study

Sr. No.	Test parameter	Antibody blocking agent	Specification
1.	Beta HCG	Access Total β hCG Calibrator S0 (zero)	S0 calibrator with beta HCG concentration 0 mIU/mL.
2.	Ca 125	Access Sample Diluent A	On board sample diluents available ready to use from
3.	AFP	Access AFP sample Diluent	Ready to use
4.	Troponin I	Access Sample Diluent A	On board sample diluent available ready to use from
5.	Estradiol	VITROS High Sample Diluent A Reagent	On board sample diluent available ready to use from

Table 4 Result before and after evaluation with probable interference

Sample number	First report	Report after evaluation	Interference reason
Beta HCG			
1.	12586 mIU/mL	6875 mIU/mL (Alternate Platform)	Antibody Interference/ Cross Reactivity
2.	1856 mIU/mL	15 mIU/mL (Antibody Blocking)	Antibody Interference
3.	23 mIU/mL	7584 mIU/mL(1:10 Dilution)	High Dose Hook Effect
4.	115 mIU/mL	23584mIU/mL (1:20 Dilution)	High Dose Hook Effect
5.	85 mIU/mL	2846 mIU/mL (1:5 Dilution)	High Dose Hook Effect
6.	8 mIU/mL	3589 mIU/mL (1:5 Dilution)	High Dose Hook Effect
Estradiol			
1.	4285 pg/mL	2865 pg/mL (Alternate Platform)	Antibody Interference/ Cross Reactivity

2.	58 pg/mL	3421 pg/mL (Antibody Blocking)	Antibody Interference
3.	1884 pg/mL	758 pg/mL (Alternate Platform)	Antibody Interference/ Cross Reactivity
CA 125			
1.	58 U/ml	87564 U/ml (1:100 Dilution)	High Dose Hook Effect
2.	13 U/ml	158964 U/ml (1:500 Dilution)	High Dose Hook Effect
3.	1852 U/ml	58 U/ml (Antibody Blocking)	Antibody Interference
AFP			
1.	86 ng/mL	86945 ng/mL (1:50 Dilution)	High Dose Hook Effect
2.	25 ng/mL	148697 ng/mL (Antibody Blocking)	Antibody Interference
Prolactin			
1.	984 ng/mL	28 ng/mL (Antibody Blocking)	Antibody Interference
Hbs Ag			
1.	Negative	Positive (Alternate Platform)	Antibody Interference/ Cross Reactivity
Troponin I			
1.	0.02 ng/ml	>150 ng/ml (1:3 Dilution)	High Dose Hook Effect
2.	0.02 ng/ml	87 ng/ml (Antibody Blocking)	Antibody Interference

(2 positive interference); prolactin - 1 sample (positive interference); HBSAg - 1 samples (negative interference); troponin I - 2 samples (positive interference).

In our study, the cases wherever cross reactivity or antibody interference were suspected an alternate platform was used, alternately commercial antibody blocking agents were used. Two

different chemiluminescence (CLIA and CMIA) platforms were used. Cases where high dose hook effect was suspected serial dilutions were performed with commercially available diluents.

The serial dilutions were performed in the following order; 1:1, 1:2, 1:4, 1:10, 1:20, 1:50, 1:100, 1:500. The final results obtained in specific dilutions are shown in Table No. 4.

DISCUSSION

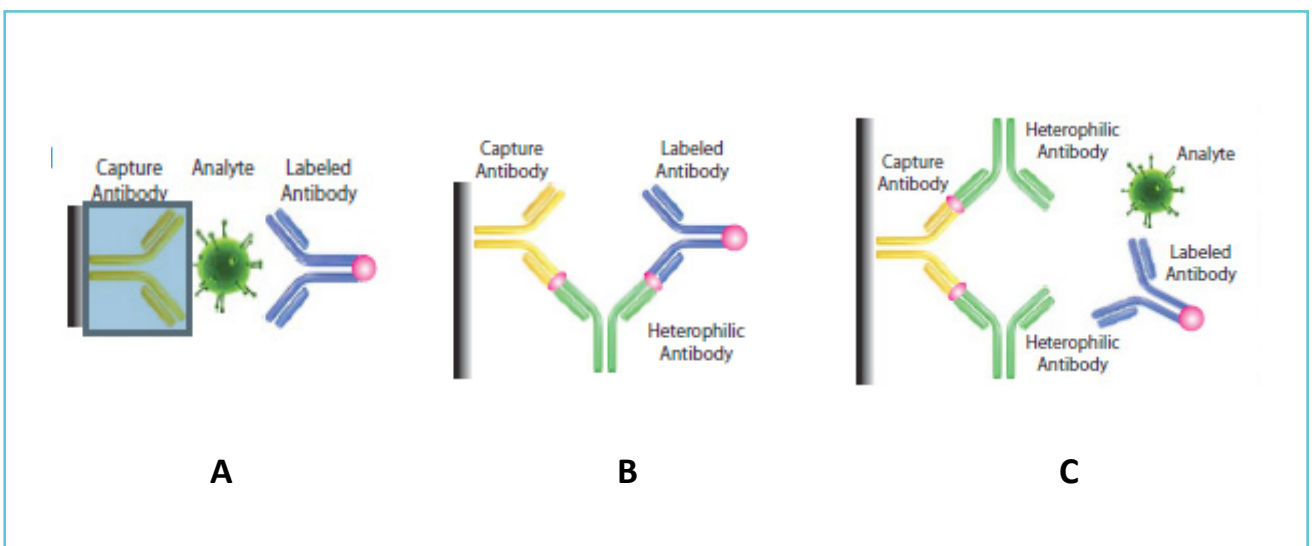
Analytical errors arising from the presence of antibodies to mouse (monoclonal) immunoglobulins in the patient's plasma or serum have received the most attention but are just one of the many causes of interference in immunoassays. Reproducibility within a laboratory or among laboratories using the same or different analytical systems is no guarantee of the validity or correctness of the results (13). Possibly the most important of the idiosyncratic interfering substances found in patient samples are those that are either autoantibodies against the analyte itself (e.g., insulin autoantibodies) or heterophilic (including antianimal) antibodies that react with one or more of the assay reagents. Both types of antibodies can produce false high or false low results (13).

Specificity of an immunoassay depends on the binding property of the antibody, composition of the antigen and the matrix. Substances that alter the measurable concentration of an analyte in the sample or alter antibody binding

can potentially result in assay interference (14, 15,16). These interfering substances may be unique to individuals and their concentration may change over a period of time. The interfering substances may have low or high affinity and its concentration determines the extent of interference and may affect one or more analytes. Antibody blocking agents may not be sufficient to overcome all types of interferences (14, 15,16).

In immunoassays the heterophile antibody (or any other cross reacting substance) bridges the capture and detection antibodies to mimic analyte binding as such resulting in false high values. In contrast even in the presence of the analyte, heterophile antibody (or any other cross reacting substance) bind to the capture antibody preventing the analyte binding with the capture antibody resulting in falsely low values (negative interference). This is the basic mechanism for false high or low values resulting from the presence of heterophile antibody (Figure 1).

Figure 1 Showing mechanism for: (A) Intended Antigen - Antibody Bridge falsely; (B) high; and (C) low values in presence of heterophile (or any other cross reacting substance) antibody



Heterophile antibodies may be present in all patients (17). The frequency of immunoassay interferences resulting from these antibodies depends on the magnitude of bias in the analytical method that contributes to significant interference (13). The prevalence of potentially interfering antibodies has been reported to be as high as 40%, the incidence of immunoassay interference is estimated to be less than 2%.

They probably arise from mundane activities such as keeping pets, ingesting animal antigens, vaccination, infection, or even blood transfusion. High concentration of interfering proteins, which may be measurable in grams per litre, or proteins with high binding affinity can, however, overwhelm the analytical system, leading to assay interference and erroneous results (17).

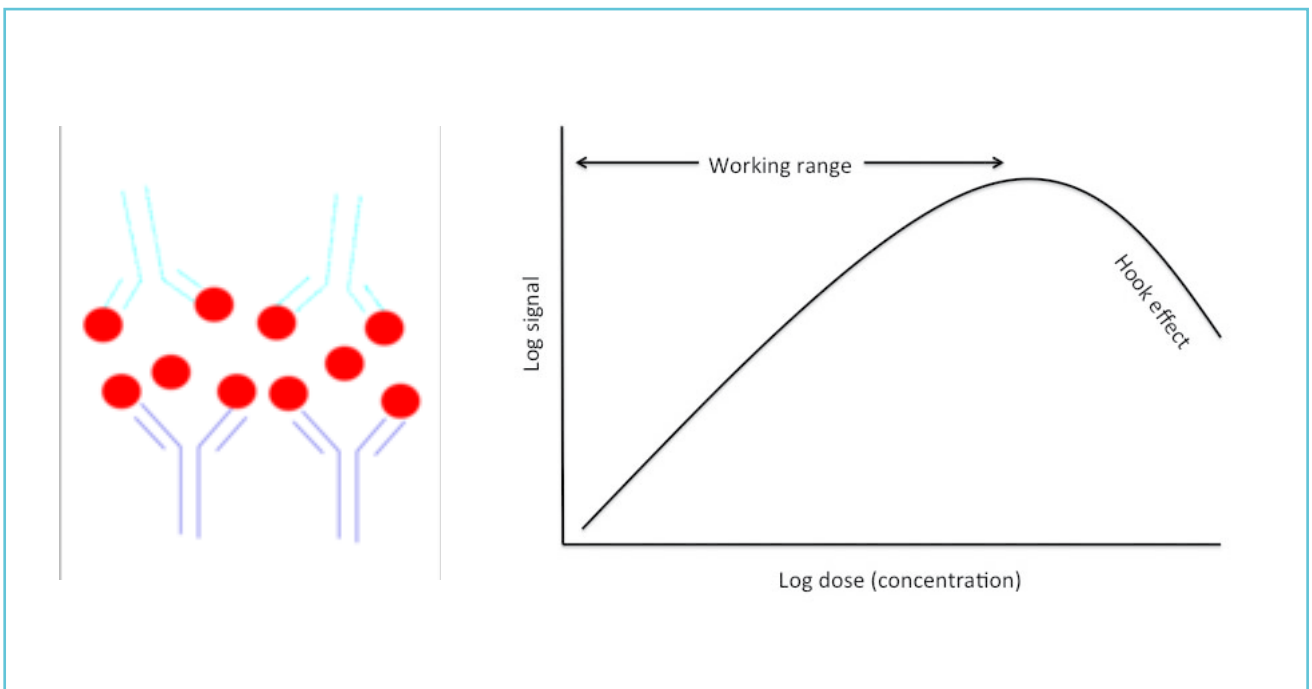
The interference from heterophile antibodies can be avoided by combining different assay antibodies from different species with little cross

reactivity (18) or by using antibody blocking agent which contains IgM and IgG antibodies with high affinity for the anti-animal antibody.

In high dose hook effect, all the available binding site from capture as well as detection antibodies are occupied by the analyte creating a stage of hyper saturation preventing formation of true antigen-antibody bridge. This can be prevented by diluting the sample as such decreasing analyte concentration and allowing improved antigen - antibody complex formation, eventually and final concentration is obtained by multiplying with the dilution fraction (Figure 2).

Limitation of our study is our retrospective approach, where evaluation was indicated only following feedback from clinicians. The screening for interfering antibody was done only after suspecting interference and was done only for the related subjects. The screening was not

Figure 2 Analyte is binding to both capture as well as detection antibody leading to a reduction in formation of antibody-antigen-antibody complexes and a decrease in signal at higher concentrations of analyte



done actively for all the patients visiting the laboratory. As such, some of the interferences may have been missed during this period.

CONCLUSION

Despite use of state of the art laboratory equipment, chances of interference in immunoassay analysis resulting from endogenous substances cannot be ruled out. In conclusion, thorough evaluation of all non clinically correlating immunoassay results is advised.

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Case report: biliary pancreatitis with acute cholangitis in a patient under anticoagulant treatment with dabigatran

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ABSTRACT

We report a case of acute onset of a biliary pancreatitis with cholangitis presented in our emergency department. The patient was under anticoagulant therapy with dabigatran due to persistent atrial fibrillation. Pancreatic enzymes including lipase were elevated above the linear measuring range and bilirubin together with cholestasis enzymes was also highly elevated. An ERCP with papillotomy was urgently indicated because postponing could lead to further deterioration of the patient's condition. Coagulation testing showed a prolonged thrombin time above 160sec which was followed by a diluted thrombin time (Haemoclot Test) resulted in a peak-level of dabigatran thus confirming full anticoagulation. Therefore, idarucizumab (Praxbind®) was administered pre-procedural of ERCP, the patient underwent uneventful ERCP without any bleeding complications, a full recovery was achieved and the patient was scheduled for elective cholecystectomy.

Table 1 Laboratory testing of coagulation parameters

Prothrombin time (PT)	10 sec	[9-12 sec]
Activated partial thromboplastin time (aPTT)	54 sec	[26-36 sec]
Thrombin time (TT)	150 sec	[-22 sec]
Drug level dabigatran (diluted TT; Haemoclot test)	54 ng/ml	[50-300 ng/ml] Peak level

INTRODUCTION

Non-VKA oral anticoagulants (NOACs) have been approved for various thromboembolic indications. In the past, the biggest disadvantage of these drugs has been the lack of specific antidotes to reverse the anticoagulant effect in emergency situations. Idarucizumab represents the first novel antidote against the direct thrombin inhibitor dabigatran and was approved by US FDA in October 2015 (1).

In our case report we used idarucizumab for dabigatran reversal in a patient with mechanical cholestasis and cholangitis needing urgent endoscopic retrograde cholangiopancreatography (ERCP).

CLINICAL-DIAGNOSTIC CASE

We report the case of a 75 year old man who was admitted to the emergency department because of right sided upper abdominal pain with

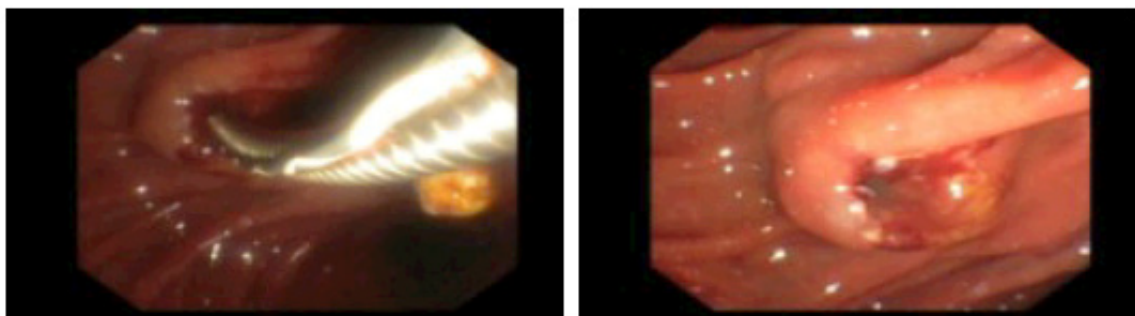
a duration of a few hours. Besides he described a clay-coloured stool and brown urine in the last days. He denied vomiting, fever or chills.

About 6 weeks before, he was hospitalized in another clinic because of an acute cholecystitis with multiple gallstones. He was taking dabigatran 150mg twice a day for non-valvular atrial fibrillation (A-fib).

He had a CHA2DS2-VASc score of 3 and no history of bleeding. He complained about pressure pain in the epigastrium and right upper quadrant, with no peritoneal signs on examination.

Laboratory testing showed a white cell count of $17.5 \times 10^6/L$, with 88% neutrophils, 6.2 U/L bilirubin, (normal range: 0.1-1.2), 131 U/L ALT (0-35), 230 U/L AST (0-45), 164 U/L ALP (40-130), 410 U/L GGT (0-55), 22.000 U/L lipase(0-60) and 37 CRP (0-5). Calculated creatinine clearance (Cockcroft and Gault) was 61 mL/min.

Figure 1 Sphincterotomy during the ERCP showing an oozing bleeding.



The abdominal ultrasound revealed dilated intrahepatic bile ducts in both liver lobes. The common bile duct (CBD) and pancreas were not visible due to intestinal gas overlay. Indication of urgent ERCP due to acute cholangitis was made. As the patient has taken dabigatran only a few hours before, laboratory testing of coagulation parameters were ordered including thrombin time (TT) and dabigatran drug level (Table 1). During the ERCP a small sphincterotomy was performed with an oozing bleeding (Figure 1). Consequently, 2.5 g idarucizumab (Praxbind®) was injected intravenously and the bleeding stopped immediately. Afterwards one impacted concrement was removed from the distal CBD with the aid of a dormia basket. After the intervention the patient received the second dose of idarucizumab (2.5g) and no further bleeding occurred throughout hospital stay.

DISCUSSION

Bleeding is a rare complication of Non-VKA oral anticoagulants potentially associated with high mortality rates (2). The humanized monoclonal antibody fragment idarucizumab is the first available specific antidote for the NOAC dabigatran. It binds dabigatran with 350-fold higher affinity compared to dabigatran for thrombin (3). The application of idarucizumab completely reversed the anticoagulant effect of dabigatran within minutes and this effect is maintained for up to 24 hours (4).

The recently published ISTH (International Society on Thrombosis and Haemostasis) guideline (3) recommends to use this antidote in the case of life-threatening bleeding, bleeding into a critical organ or closed space, prolonged bleeding despite local hemostatic measures, high risk of recurrent bleeding because of overdose or delayed clearance of NOACs, and need for an urgent intervention associated with a high risk of bleeding (3).

In our case report, the mechanical cholestasis with cholangitis represents an urgent intervention. ERCP with sphincterotomy is considered as higher risk procedure for bleeding, and according to the European Society of Gastrointestinal Endoscopy (ESGE) guidelines the last dose of NOACs should be taken at least 48 hours before the intervention (5).

The thrombin time (TT) and dabigatran drug level represents useful parameters to determine the anticoagulation effect and bleeding risk of dabigatran (3). The TT determines the conversion of fibrinogen to fibrin, which is the final step in the coagulation cascade. It is prolonged in the presence of thrombin inhibitors such as dabigatran and this prolongation is directly proportional to the dabigatran concentration (6).

When analysing dabigatran drug concentration, it is important to consider when the last dose of the NOAC was taken to determine whether the levels are likely to raise or decline over time (3).

According to the ISTH recommendations, antidote administration should be considered if the drug concentration exceeds 30 ng/mL and the patient requires an urgent intervention associated with a high risk of bleeding (3).

In conclusion, idarucizumab can safely normalize clotting times within a few minutes. According to the ISTH guidelines, this antidote is indicated in patients with life-threatening bleeding and/or urgent interventions with a high bleeding risk (3).

At the moment, several antidotes for NOACs are under various stages of development: for example andexanet alfa, an antidote for the oral factor Xa inhibitors or ciraparantag, an antidote of all NOACs (3). Further studies are however needed to refine the use of antidotes for in different situations and patient groups.

TAKE HOME MESSAGES/LEARNING POINTS

- With the use of idarucizumab urgent, vital interventions can be performed safely and quickly despite patients are on fully effective therapy with dabigatran.
- To determine a substantial bleeding risk in patients with urgent interventions in patients on dabigatran, determination of thrombin time (TT) and dabigatran drug level using the Haemoclot® test are useful to verify dabigatran effectiveness. This is even more indicated, if clear history of medication intake cannot be obtained.

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