Prevalence of Type 2 diabetes in patients admitted with acute coronary syndrome: The role of easily reproducible non-invasive screening methods

A thesis submitted to the

Birmingham University for the degree of DOCTOR OF MEDICINE (RESEARCH) by

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Abstract:

Background:

Diabetes mellitus is one of the major risk factors for cardiovascular disease and associated mortality. There is increasing recognition of the need to assess glucose tolerance in all patients with cardiovascular disease but less agreement about the most appropriate screening methodology in all patient groups. Until recently the diagnosis of diabetes mellitus was based on WHO 1998 criteria. However an International Expert Committee (IEC) comprising experts from ADA, W.H.O and I.D.F came together and recommended the use of an HbA1c cut-off of 6.5% for the diagnosis of diabetes mellitus. Recently we developed and published a simple T2DM Screening Algorithm (T2DSA) based on the FPG and HbA_{1c}.

Aims:

Our aims were

1. To determine the prevalence of undiagnosed diabetes and impaired glycaemic state (IGS) and compare the WHO 1998 and IEC criteria for diagnosis of T2DM in patients admitted to hospital with acute coronary syndrome

2. To investigate the role of screening algorithm that includes fasting plasma glucose (<7.0 mmol/l) and HbA_{1c} (>6.0%) to accurately define glucose tolerance in patients admitted with an acute coronary syndrome (ACS)

3. To explore the potential of a panel of biomarkers to enhance the predictive power of our screening tests.

Hypothesis:

In patients with acute coronary syndrome, long term glycaemic status can be determined on hospital admission using reproducible and easily obtainable measures other than the oral glucose tolerance test.

Methods:

A prospective 3 year study carried out in two large inner city hospitals in United Kingdom. The participants were all admitted to hospital with ACS and underwent an initial OGTT within 7 days of hospital admission which was followed up by glycaemic stratification at 3 months.

Results:

Patients (n=118) were included in the analysis. The prevalence of diabetes mellitus was 20% and 16% respectively according to the W.H.O and IEC criteria at baseline. The prevalence of diabetes remained similar at 3 months at 21%. However two thirds of participants with IGS and a third of those with DM changed their glycaemic status at 3 months. This could be possibly due to stress hyperglycaemia as urinary cortisol creatinine ratio was elevated in patients who had T2DM at baseline compared to NGT and IGS.

The two diagnostic criteria appeared to identify different cohort of patients. Our screening algorithm had sensitivity of over 85% at baseline in comparison with W.H.O 1998 criteria. We also designed a diabetes predictor score based on age, fasting plasma glucose and HbA1c and it had an excellent sensitivity of over 80% and negative predictive value of over 90%. These novel formulae have a clear advantage over IEC criteria with better sensitivity.

At baseline mean C-peptide, glucagon, intact pro-insulin and HOMA IR were higher in the diabetic group compared to normal and IGS groups. HOMA IS was lower in the diabetic and IGS groups compared to normal cohort At 3 months mean C-peptide, IL 1 RA, TIMP 2 and intact pro-insulin were higher in the diabetic group compared to NGT and IGS groups.

Conclusion:

The W.H.O and IEC diagnostic criteria identify different populations with diabetes at baseline as well as 3 months. This is clinically relevant as we are basing screening in a high risk population on these criteria. The IEC criteria do not identify patients with IGS which is known to be associated with increased cardiovascular morbidity and mortality. Our screening algorithm can reduce the number of OGTTs and detect half of the participants with IGS; however it cannot be used on its own to detect diabetes mellitus. A Diabetes Predictor Score demonstrated potential to diagnose diabetes and has an excellent sensitivity, specificity and negative predictive value.

Acknowledgment:

I am indebted to a large number of people, and without their individual contributions this work would have not been possible.

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I would like to thank my family, specially my wife for her endless support and patience and my parents for their prayers.

Finally, I have to thank all the patients (and their relatives) who were very supportive and kept coming back whenever asked to provide us with more data; without their help this project would have not been possible, and I hope that we have benefited some of them.

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1. INTRODUCTION

1.1 Diabetes Mellitus:

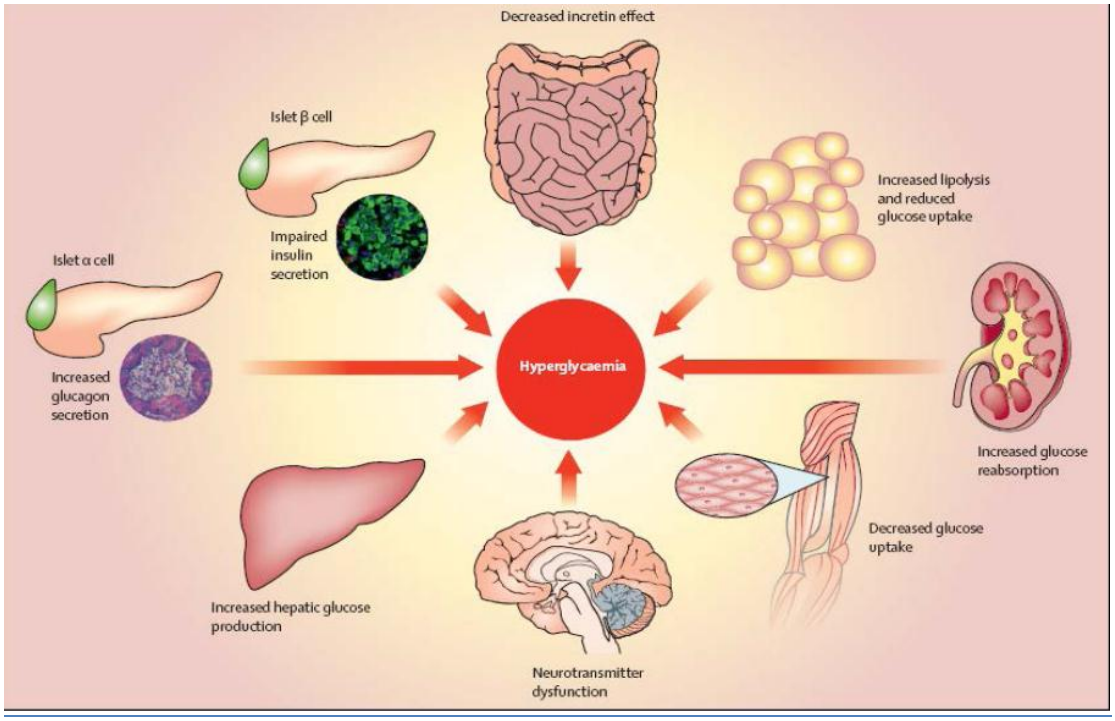
Type 2 diabetes mellitus (T2DM) is an extremely common lifelong health condition. The latest figures from Diabetes U.K suggest 2.9 million people are known to suffer from diabetes in U.K while another 850,000 remain undiagnosed. By 2025 there will be more than 4 million people with diabetes in the U.K. In England the current prevalence of diabetes is estimated at around 5.5% (1).

T2DM is a global epidemic with an estimated worldwide prevalence of 6.4% (285 million) in 2010 that is forecast to rise to 7.7% (438 million) in 2030 (2). In addition 344 million people have impaired glucose tolerance (IGT) that is forecast to increase to 472 million by 2030 (2).

The World Health Organisation (WHO) defines diabetes mellitus (DM) as: "a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of DM include long-term damage, dysfunction and failure of various organs such as retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease."

The health, economic and social burden of T2DM is immense. The U.K. spends 10% of total NHS spending on treating diabetes and its complications (3). World wide it amounts for 12% of the total health budget (1). At the same time the rising prevalence of obesity presents a massive challenge to the world wide health services.

T2DM is a complex disorder. Genetic and environmental factors play a major part in the pathogenesis of T2DM (Fig 1) (4). Mechanistically it is related to insulin resistance (IR) and beta cell dysfunction. Obesity leads to development of IR and impaired glucose tolerance (IGT) (4-6). IGT may progresses to T2DM. There are several pathways leading to the development of IR including hormonal imbalance (e.g. increased leptin, reduced adiponectin and elevated glucagon) and increased cytokines (e.g. tumour necrosis factor-α, interleukin-6) (4,6,7-10). Impaired glycaemic state (IGS) progresses to T2DM when the amount of insulin released cannot overcome IR. The decline in pancreatic beta cell function is related to damage caused by chronic hyperglycaemia (glucotoxicity), oxidative stress (OS) and inflammation (11-14). Some of these will be covered in more detail in the upcoming chapters.

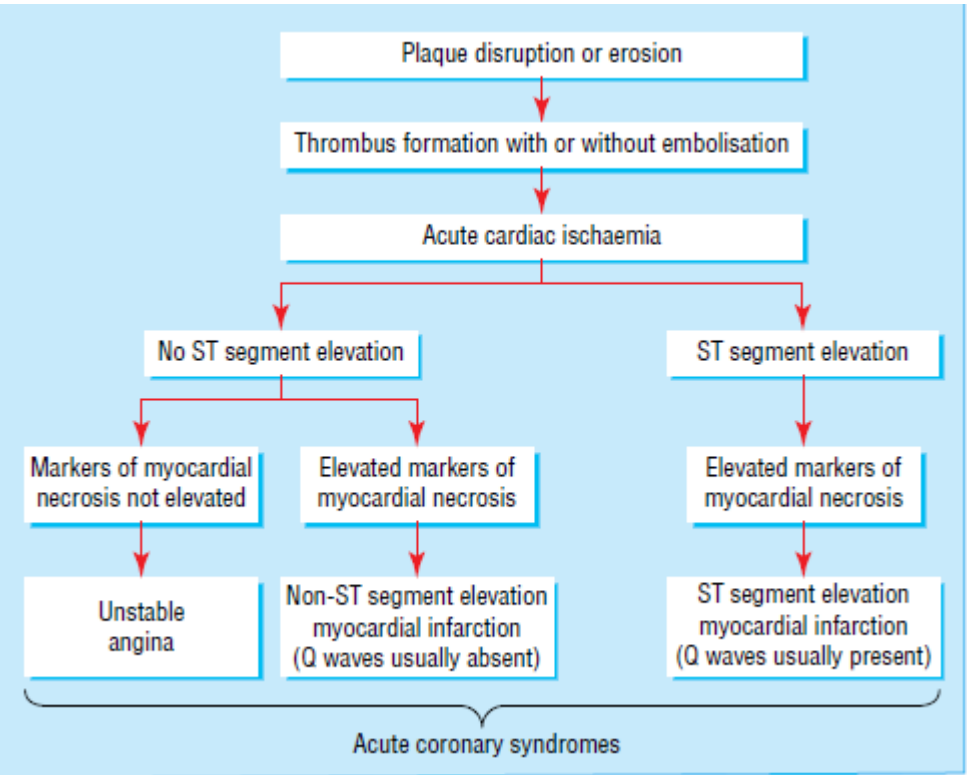


1-1: Pathogenesis of Type 2 Diabetes Mellitus (Adapted with permission from 15)

1.2 Acute Coronary Syndrome:

The term acute coronary syndrome (ACS) includes a wide spectrum of cardiovascular diseases ranging from unstable angina (UA) and non-ST segment elevation myocardial infarction (NSTEMI) to ST segment elevation myocardial infarction (STEMI) (16, 17). 3 million people are admitted with a STEMI and 4 million with NSTEMI worldwide (17, 18).

Short term mortality is higher among patients admitted with STEMI as compared to long term which is higher with NSTEMI (17, 19). The main distinction among these conditions is based on the underlying severity of the disease and the resulting myocardial damage. UA and NSTEMI is associated with partially occlusive while STEMI with stable occlusive thrombus (16) Fig 1.2.

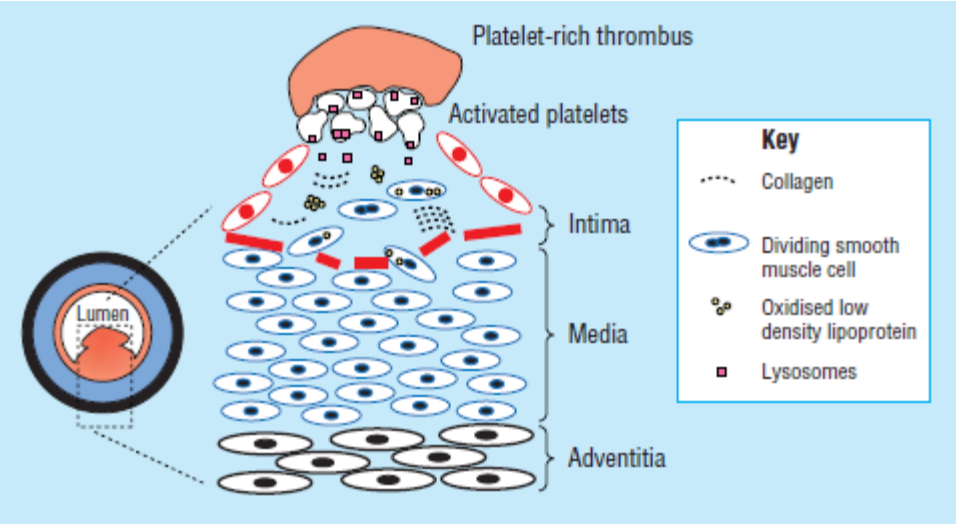


1-2: Spectrums of Acute Coronary Syndromes (Adapted with permission from 16)

Inflammation plays a major part in the pathogenesis of atherosclerosis. Any insult to the endothelium e.g. hyperglycaemia, dyslipidaemia, smoking, hypertension or obesity can lead to adhesion of monocytes to the arterial lumen (20, 21). Monocytes become macrophages and ingest modified lipoproteins to transform into foam cells. The core of the plaque consists of foam cells, modified lipoproteins, apoptotic debris, collagen and von-Willebrand factor (20, 22). Plaque formation starts from childhood however clinical manifestations of ACS happen when they become large enough to cause circulatory blockage (20, 23, and 24).

Plaques can become symptomatic in three ways (20, 25). Firstly endothelial disruption causes exposure of collagen and von Willebrand factors that lead to thrombus formation (20, 26). Secondly angiogenesis (new vessel formation) is promoted by factors within the plaque (20, 27).Upon rupture production of thrombin leads to release of platelet derived growth factor (PDGF) and transforming growth factor beta (TGF beta) which stimulate smooth muscle production (20, 28). Thirdly fibrous cap of plaques can be weakened by mediators like interferon gamma which inhibit collagen production (20, 22). In addition existing collagen is also left weakened which leads to micro tear in the fibrous plaque (20, 29). Plaque disruption leads to contact between blood and collagen which leads to platelet activation (20, 30). This in turn leads to transformation of glycoprotein IIb/IIIa receptors on

platelets (20, 31). Fibrinogen connects to these leading to platelet aggregation. Factors such as plasminogen activator inhibitor (PAI) that inhibit fibrinolysis promote clot formation. These are raised in conditions like type 2 diabetes mellitus (20, 32).



1-3 Pathophysiology of atherosclerosis (Adapted with permission from 16)

Medical management includes bed rest, oxygen, morphine and the use of antithrombotic (aspirin, clopidogrel, low molecular weight heparin, warfarin and glycoprotein IIb/IIIa inhibitors) and anti-angina (nitrates and beta and calcium channel blockers) medications. More recent studies demonstrated that an early interventional therapy (percutaneous angioplasty or coronary artery bypass grafting) provide a clear benefit over conservative management (16). Definitive management is always based on risk stratification (20).

1.3 Diabetes Mellitus and Acute Coronary Syndrome:

T2DM is associated with an increased risk of cardiovascular disease (CVD) and mortality (33). Despite advances in the management of CVD and risk factors we have only achieved limited success in terms of reduction in mortality when compared to patients without diabetes (33, 34). Some of the excess risk is related to already established risk factors like obesity, hypertension and dyslipidaemia (33, 35). However these factors do not fully explain the increased risk (33, 36). Therefore it has been suggested that there are additional factors some of which may be the following (33) (Table 1).

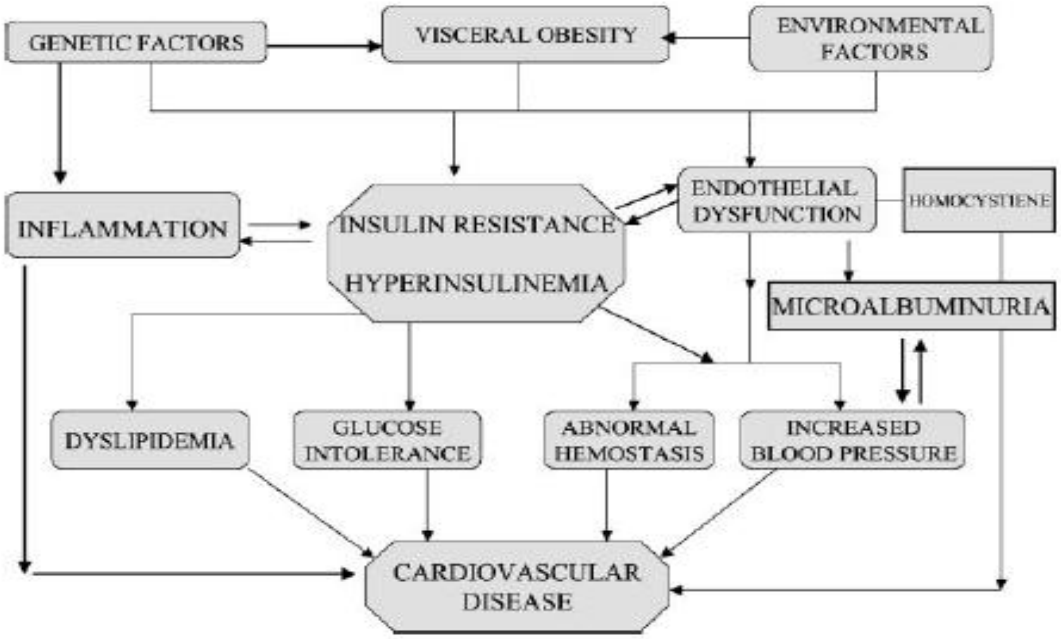
1-1 Traditional and Non-traditional risk factors for CVD in diabetes (Adapted with permission from 33)

TABLE 1. Risk factors for CVD in diabetes

Traditional	Nontraditional
Hypertension	IR
Dyslipidemia	Endothelial dysfunction
Family history of premature CVD	↓ Vascular reactivity, ↓ NO
Cigarette smoking	↓ ADMA
	Impaired fibrinolysis
	↑ PAI-1
	Inflammation
	↑ hs-CRP, ↑ WBC
	↑ Adhesion molecules, ↑ MMP-9
	Microalbuminuria
	Hyperhomocysteinemia
	Postprandial abnormalities
	Vascular wall abnormalities
	↑ IMT, calcification, ↓ compliance

↑, Increased; ↓, decreased; WBC, white blood cells; MMP-9, matrix metalloproteinase 9.

Pathogenesis of CVD in diabetes is related to a complex interaction between many of these factors as they rarely exist in isolation. These include factors such as insulin resistance (IR) and inflammation (33, 37). Inflammation, endothelial and clotting abnormalities are all associated with IR and may play a role in the pathogenesis of diabetes as well as CVD (33, 38).The following figure is an illustration of some of the interaction between the non-traditional risk factors of CVD in diabetes mellitus (33). I will discuss some of these factors in more detail next as they form an important part of our project.



1-4: Interaction between non-traditional risk factors of CVD. (Adapted with permission from 33)

1.3.1 Insulin Resistance:

Insulin resistance syndrome or metabolic syndrome was first described as association between obesity, T2DM, IR, high triglycerides (TG) and low high density lipoprotein (HDL) cholesterol (33, 39 and 40). More recently it has been shown to be associated with inflammation, CVD, endothelial and clotting abnormalities (33, 41) as well as obstructive sleep apnoea, non-alcoholic fatty liver disease and polycystic ovary syndrome. Traditionally research studies have identified insulin sensitivity, plasma insulin levels or formulas based on plasma insulin and glucose (homeostatic model assessment or popularly known as HOMA) to define insulin resistance (33). All of these do however have their limitations. Whatever method is used there is a clear association between insulin resistance and the incidence of ischaemic heart disease as well as all cause cardiovascular mortality (33).

Obesity is one of the major associations of IR. Central deposition of fat appears to have a close relationship with DM, IR and high blood pressure (33, 42). Adipose tissue also produces a number of biomarkers which are responsible for some of the consequences of IR as well as being predictors of CVD. Some of these include C-reactive protein (CRP), Interleukin 6(IL-6) and tumour necrosis factor alpha (TNF@). Patients with IRS have been shown to have high levels of CRP in the circulation (33, 43). TNF@ expression is increased in obesity. It inhibits lipoprotein lipase and endothelial nitric oxide synthase (NOS) and promotes adhesion of monocytes as well as having effects at insulin receptor level. IL-6 is similarly shown to effect endothelial function (33).

The term "diabetic dyslipidaemia" refers to high TGs and/or low HDL-cholesterol and is suggestive of IR (33, 44 and 45). Hormone sensitive lipase activity is increased and there is also higher breakdown of stored TGs (33, 46). The levels of low density lipoprotein cholesterol (LDL-cholesterol) may be similar between subjects with and without IR (33, 45).

Hypertension is another association of IR. Obese people with IR are known to have higher blood pressure (33). Even slight reduction in weight is associated with lower fasting plasma insulin levels and a reduction in blood pressure (33, 47). The mechanisms responsible for this association are multifactorial as is the underlying complex disorder of hypertension (33, 48).

1.3.2 Endothelial Dysfunction:

Endothelial dysfunction plays a major role in the pathogenesis of CVD in diabetes (33, 49). Nitric oxide and prostacyclin have vasodilator properties and are protective to the endothelium. These actions are opposed by vasoconstrictor substances like endothelin 1. Biochemical markers of endothelial dysfunction include von Willebrand factor (vWF), thrombomodulin and adhesion molecules (33, 50).

Endothelial dysfunction starts early in life and progresses with the passage of time (33, 51-53). At a cellular level insulin induces NOS which regulates nitric oxide synthesis (33, 54). Abnormalities of insulin signalling through the phosphatidylinositol-3 kinase pathway also accounts for some of the higher risk (33, 55-57). Both these features lead to abnormalities of nitric oxide which acts as protector of the endothelium (33).

Asymmetric dimethylarginine (ADMA) is an inhibitor of NOS and its levels are directly related to abnormalities of the insulin mediated glucose mechanism in IR. Therefore elevated levels of ADMA may also explain some of the elevated risk of CVD in diabetes (33, 58 and 59). Other features such as reduced activity of the enzyme that produces tetrahydrobiopterin(BP4) and higher expression of adhesion molecules also contribute to risk of IR (33, 60 and 61).

1.3.3 Impaired fibrinolysis and prothrombotic state:

The fibrinolytic system is maintained by a balance between factors which promote and inhibit plasminogen. These include tissue type plasminogen activator and plasminogen activator inhibitor type 1 (PAI-1) (33, 62). This balance is disturbed in diabetes and IR and is another explanation for the higher incidence of CVD in diabetics as well as non-diabetics (33, 63-67). Studies have shown an increased activity of PAI-1 in IR states like obesity and polycystic ovary syndrome (33, 68 and 69).

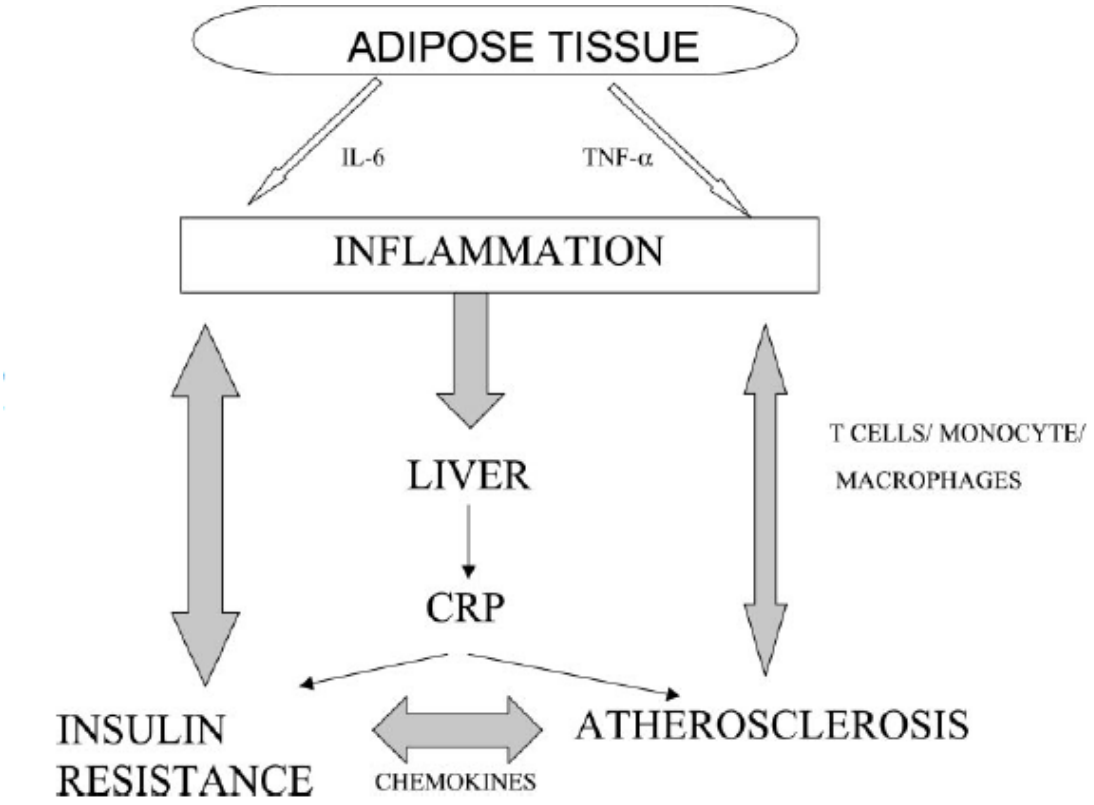
Platelet aggregation and the number of glycoprotein receptors are increased in diabetes. A number of other non-specific abnormalities of coagulation are also suggested in diabetes and can partly explain the increased risk for CVD in diabetes (33, 70 and 71).

The production and release of PAI-1 is controlled by insulin as well as its precursors and other biomarkers (33, 72). Levels of PAI-1 are clearly shown to have a positive correlation with metabolic (IR) syndrome (33, 72). PAI-1 levels are increased in

atherosclerotic plaques with T2DM (33, 73). A combination of actions of insulin on PAI-1 and the clotting cascade can also partly explain the elevated risk of CVD (33).

1.3.4 Inflammation:

Following is a schematic representation of the interaction between inflammation, circulatory factors released by adipose tissue and CVD in diabetes.



1-5: Interplay between inflammation, I.R and atherosclerosis (Adapted with permission from 33)

Inflammation has been shown to play a part in the pathogenesis of both CVD and T2DM (33). Factors such as infectious agents, adipokines and oxidized lipids promote the release of IL-6 which in turn leads to the release of CRP from the liver (33, 74).

Elevated levels of CRP have been shown in the setting of obesity as well as other features of metabolic syndrome (33, 75). Thus inflammation plays an important and complex role in pathogenesis of both T2DM and CVD.

1.4 Screening for Diabetes Mellitus and Impaired Glycaemic Status in Acute Coronary Syndrome:

There is increasing recognition of the need to assess glucose tolerance in all patients admitted to a coronary care unit (CCU) with acute coronary syndrome (ACS). Studies have shown an association between abnormal glycaemic status and long term mortality and morbidity in such groups as well as immediate outcome (76-81, 94). It is also quite clear that people admitted to hospital with ACS have a higher incidence of impaired glycaemic status (IGS) and T2DM (81-85, 94). Evidence also clearly supports that early detection of diabetes plays an important role in preventing complications (86-88). In addition lifestyle measures and/or metformin (outside license) therapy may be useful in those with IGS (89).

Despite all of this, most centres in the UK have not implemented a screening strategy in these patients, which partly reflects the lack of consensus on a screening modality (i.e. fasting plasma glucose, HbA1c and oral glucose tolerance test).

The European Association for the study of Diabetes (EASD) recommends the use of an oral glucose tolerance test to investigate glycaemic abnormalities in patients with CVD but without a known diagnosis of diabetes mellitus (90, 94). To the contrary the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity and Metabolism does not support the use of OGTT (91, 94). This is partly due to the absence of conclusive evidence supporting early intensive glycaemic control improves cardiovascular outcome. It is also unclear whether high blood glucose is a cause or effect and whether it should be treated or just taken as a marker due to stress (92, 93).

A survey carried out in Holland suggests 76% of cardiologists do not check HbA1c in patients with ACS before discharge. Therefore, it is unlikely that a more impractical test like OGTT would be used more often than HbA1c (93, 95).

The rapid transit of patients through coronary care units makes it very difficult to arrange an investigation like OGTT which needs logistical support and prior organization to arrange fasting the night before as well as the appropriate glucose drink etc. being available at the right time. In addition patients are usually being discharged within 3 days locally so asking them to fast on one of those days when they are also having a therapeutic procedure like angioplasty can be difficult.

Reports have suggested OGTT at the time of discharge in patients with ACS is reliable in predicting glycaemic status at 3 and 12 months (81). However, closer examination of these data suggest that less than 50% of patients diagnosed with diabetes at discharge have T2DM on OGTT at 12 months (81). More recently people have been looking at comparison of the different diagnostic criteria in this cohort (93).

1.5 Diagnostic Criteria for Diabetes Mellitus:

Until recently diagnosis of diabetes mellitus was based on WHO 1998 criteria. Patients with venous FPG≥7.0 mmol/l were classified with diabetes mellitus, if 6.1 to 6.9 mmol/l with impaired fasting glycaemia (IFG) and ≤6.0 mmol/l with normal fasting glucose. Patients with a 2 hour plasma glucose (2hPG) >7.8 and <11.1 mmol/l obtained from venous blood were classified as having impaired glucose tolerance (IGT) and those with 2 hour plasma glucose >11.1 mmol/l with DM (96).

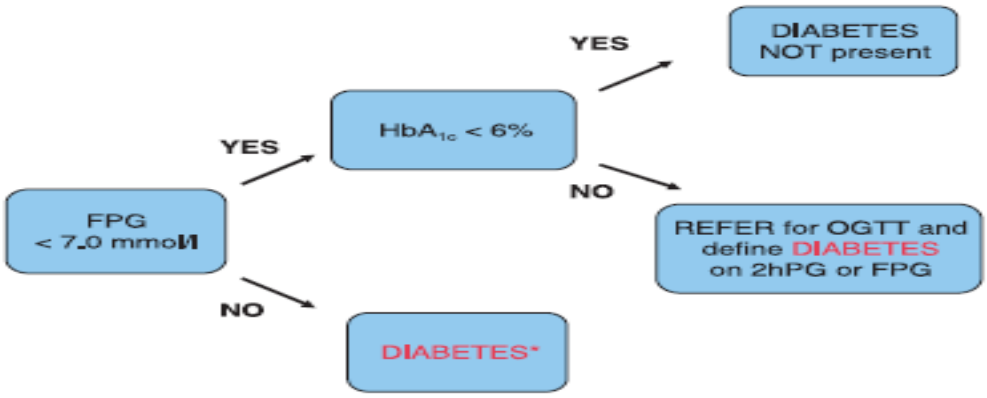
Representatives of European Association for Study of Diabetes (EASD), American Diabetes Association (ADA) and International Diabetes Federation (IDF) came together in 2009 and in a consensus statement recommended the use of an HbA1c cut-off of 6.5% as a diagnostic marker for diabetes mellitus provided the method used for testing HbA1c is standardized and subjected to quality assurance protocols (97).More recently World Health Organization (WHO) has also adopted it in the following statement

"HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement. An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests." (98)

This has led to some debate in the field of diabetes and biochemistry. On the one hand HbA1c provides a clear advantage over OGTT. OGTT is expensive, time consuming unpleasant and unsuitable for large scale screening (97). Added to the poor reproducibility, because of the high coefficient of variation of the 2 hour value, alternative screening methods like FPG and HbA1c were needed. By contrast HbA1c testing can be costly, needs to be standardized and may not be readily available in some areas of the world (97). In addition HbA1c can be dependent on race/ethnicity (97, 99 and 100). It will also be inaccurate with certain anaemia's and hemoglobinopathies. There is no validation for HbA1c testing in children and only partial correlation between average glucose levels and HbA1c.

HbA1c testing may also not be suitable in other conditions such as pregnancy, recent blood loss and recent blood transfusions (97).My aim was to explore the role of alternative screening methods which are more reproducible, easier to perform, less expensive and suitable for large scale screening in our cohort of patients admitted to hospital with acute coronary syndrome.

The Diabetes team at Selly Oak Hospital, Birmingham developed and published a T2DM screening algorithm based on the FPG and HbA_{1c}. (101). This algorithm was derived from oral glucose tolerance test (OGTT) capillary samples in 500 consecutive UK patients referred with IFG according to World Health Organization criteria. It was validated in 500 UK patients as well as venous specimens in 1175 unselected Australian patients (101). In the derivation cohort median age was 61 years (50-69) with 52% male and 12% South Asian. Median HbA1c was 6.2% (5.8-6.6%) and FPG 6.7 mmol/l (6.3-7.2 mmol/l). The FPG identified 36% of patients with diabetes mellitus while OGTT identified a further 12%. The derived algorithm, (HbA1c greater than or equal to 6.0% with FPG < 7.0 mmol/l) was utilized to identify patients requiring an OGTT to diagnose diabetes. When applied to the UK validation cohort, sensitivity was 97% and specificity 100%. The algorithm was equally effective in the unselected group, aged 59 years (49-68 years) with sensitivity 93% and specificity 100%. HbA1c was 6.0% (5.6-6.6%) and FPG 6.0 mmol/l (5.3-6.8 mmol/l), with 26% having IFG. Use of the algorithm would have reduced the number of OGTTs performed in the UK validation cohort by 33% and in the Australian cohort by 66%. This suggested that use of this algorithm could simplify procedures for diagnosis of diabetes and could also be used for monitoring pre-diabetes. Validation was still needed in other populations and other patient groups (101).



1-6: Algorithm combining HbA1c and FPG (Adapted with permission from 101)

My aim was to look at the role of this algorithm in reducing the need to perform an OGTT in this cohort. In addition we decided to consider comparing the two diagnostic criteria (WHO 1998 and Expert Committee 2009).

1.6 Dysglycaemia and Cardiovascular disease and mortality:

It has been shown that diabetic patients who have never had myocardial infarction have a risk similar to non-diabetic patients who have had a prior myocardial infarction. Therefore it is suggested that all patients with diabetes should be considered to have the same risk as if they have already had a myocardial infarct (102).

In the previous section I have shown the diagnostic criteria for IFG and IGT according to WHO diagnostic criteria. Both these groups are high risk of developing T2DM as well as increased risk of cardiovascular disease (103). The progression of IGT to T2DM typically varies from 1.5 to 4% annually (104). Both IFG and IGT are supposed to be intermediary disorders of carbohydrate metabolism (103). The worldwide prevalence of IGT is much higher compared to IFG. It has been suggested that this may be due to age, sex and ethnicity (103,105). Similarly IGT is also suggested to lead to a higher risk of cardiovascular disease as compared to IFG (103). Some of the factors associated with increased risk of dysglycaemia include raised body mass index, advancing age, family history of diabetes, selected ethnicities, history of IGT or gestational diabetes mellitus, and lipid abnormalities [106-110]. Although many were shown to be associated with T2DM, most of them were also shown to be associated with IGT in an analysis of data from the Second National Health and Nutrition Examination Survey (NHANES II).(111)

The Honolulu heart programme examined over 8000 participants over 23 years and showed a clear association of higher incidence of total mortality cardiovascular disease and mortality with dysglycaemia (112). The Funagata diabetes study in 1999 examined over 2500 citizens of Funagata over 7 years and showed significantly reduced survival from cardiovascular disease and stroke among those with IGT and T2DM (113).In this study the IFG group were not shown to have reduced survival from stroke or cardiovascular mortality compared to those with IGT (113). The Framingham offspring study also showed similar findings and suggested the examining of 2 hour glucose levels is important as an independent risk factor for cardiovascular disease (114). In a combined analysis of 6 prospective studies body mass index (BMI), fasting and 2 hour glucose concentrations were the two parameters shown to be the best independent predictors of progression to T2DM (115).

In a meta-analysis of 15 studies dysglycaemia was clearly shown to be associated with increased cardiovascular mortality. Patients with glucose concentrations more than or equal to 6.1-8.0 mmol/l without previously known T2DM had a 3.9-fold higher risk of dying than patients without diabetes who had lower glucose concentrations. Glucose concentrations higher than 8.0-10.0 mmol/l on admission were associated with increased risk of congestive heart failure or cardiogenic shock in patients without diabetes (77). Another study has demonstrated that each 18 mg/dl (1 mmol/l) rise in blood glucose in patients admitted to hospital with ACS appears to lead to a 4 fold rise in mortality (116).

In a separate study by Hofsten et al, the impact of dysglycaemia was studied on cardiovascular outcome. The investigators went on to correlate the glucose levels with echocardiographic markers of both systolic and diastolic dysfunction. They were able to show a linear relationship between dysglycaemia and indices of systolic and diastolic function. Similarities were shown in the relationship between pro-B type natriuretic peptide and glycaemic abnormalities. A clear association was shown in this study between the glycaemic abnormalities and cardiovascular outcomes as well as readmission. The impact on cardiovascular outcome remained even after correcting for the echocardiographic abnormalities. The follow-up duration in this study varied from 12 to 44 months (117).

Earlier in the section I mentioned the W.H.O definition of IFG and IGT i.e. the groups which are supposed to be at increased risk of developing T2DM and in the case of IGT also at elevated risk of cardiovascular disease and mortality (96). The American Diabetes Association have also examined this and issued separate guidelines. In addition to 6.5% as the diagnostic cut-off for diabetes, the ADA recommends the use of an HbA1c value of 5.7-6.4% (39-46 mmol/mol) to identify patients with an increased risk of future diabetes (118). However studies which have looked at comparing these two groups using W.H.O and ADA diagnostic criteria in the setting of acute admissions from cardiac disease, have shown clear differences in the populations detected by these two guidelines (93).

Other studies have also looked at therapeutic intervention in people with IGT to prevent or delay progression to T2DM. One such programme examined lifestyle-intervention and administration of metformin to see if it would prevent or delay the development of diabetes (89). The life style intervention reduced the incidence by 58% while metformin reduced it by 31% as compared to non-intervention group. The average numbers needed to treat was 6.9 in the life style intervention and 13.9 in the metformin group (89).

I examined the incidence of IFG as well as IGT in this acute setting in our patient group. We also went on to analyse the comparison of those with IGS according to

W.H.O and ADA criteria in our population group. A unique feature of my work was that this comparison was carried out at baseline as well as follow-up at 3 months.

1.7 Stress Hyperglycaemia:

Stress hyperglycaemia represents an increased blood glucose level as a result of activation of neurohormonal processes when exposed to stress (119). Increased glucose during stress is result of sympathetic nervous system activation and raised production of catecholamines (adrenaline and noradrenaline) and cortisol that stimulate processes of gluconeogenesis, glycogenolysis and lipolysis. These hormones are responsible for insulin resistance, at the receptor and post receptor level (120-122). In addition other hormones like glucagon and growth hormone are also considered part of the counter regulatory response to stress. However it has not been clearly established whether the role of growth hormone is cause or effect.

Recent studies have shown a high incidence of glycaemic abnormalities in patients being admitted to hospital with ACS (81). Further sub analysis has been carried out to look at the reasons behind this abnormality.

Circulating catecholamine levels did not appear to be directly related to this abnormality. However a possible role for them at sympathetic nerve endings has not been established (119, 123).

On the other hand circulating cortisol levels (glucocorticoids) appear to have a bigger role to play in the glycaemic regulation (119).

Insulin levels appear to be increased in the acute setting of ACS (124), hence suggesting high incidence of insulin resistance. Insulin increase may be related to the high glucose levels which in turn are likely to be caused by cortisol release (119).

The relationship between insulin levels and the size of the infarct has been explored in a number of studies with conflicting findings (119, 123).

Other studies have however suggested stress hyperglycaemia plays a more important role in the pathogenesis of ACS. However there was a difference in the timings at which samples were collected and most of the patients did not undergo coronary intervention which is now considered the mainstay of management of ACS (119, 125).

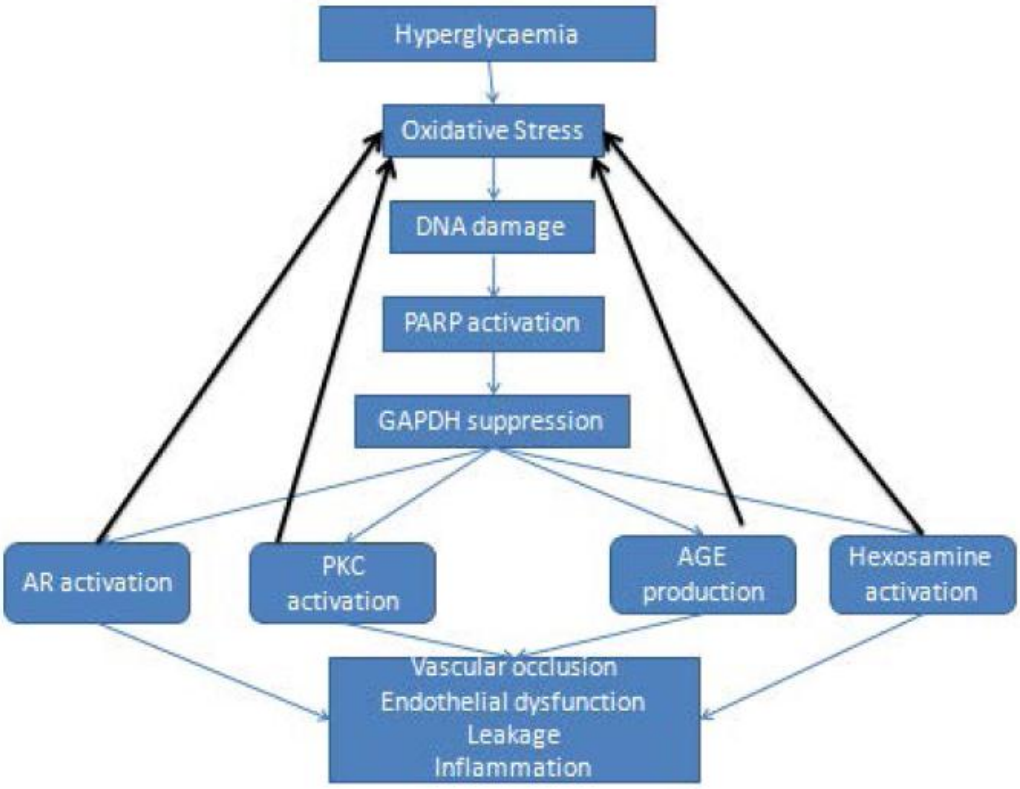
I planned to determine the utility of urinary cortisol creatinine ratio as a marker of glucocorticoid response to stress in our participants. These were collected within 7 days of hospital admission and then repeated at the second visit at 3 months. I wanted to examine if there was any difference between baseline and 3 month ratios

and whether they correlated with the glycaemic stratification. We also studied glucagon levels as part of the pancreatic biomarkers.

1.8 Oxidative Stress:

The term oxidative stress (OS) refers to an imbalance between the production of free radicals and the defence mechanisms that prevent cell damage (126). Free radical injury has been implicated in the aetiology and progression of many chronic diseases, including type 2 diabetes mellitus (127-130).

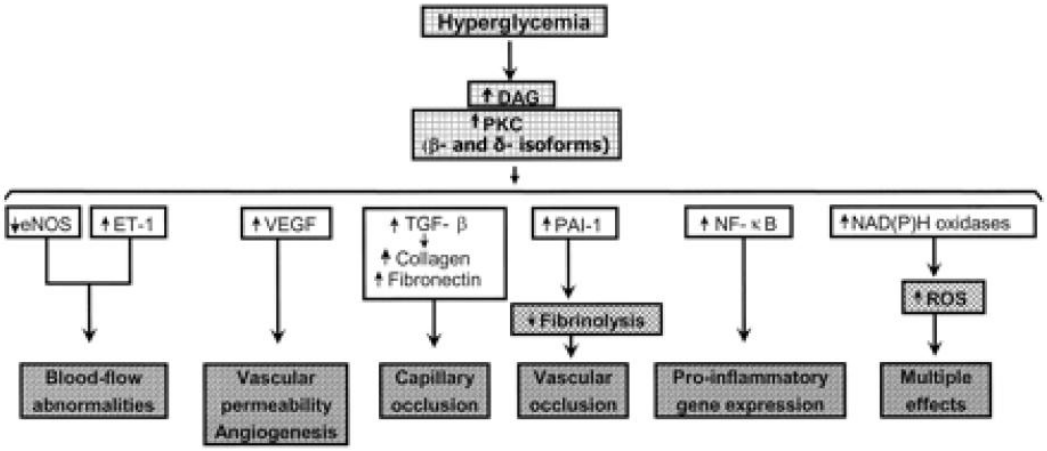
Free radical species are a variety of highly reactive molecules that can be divided into different reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chlorine species (RCS). A common feature of cells that are damaged by hyperglycaemia is the presence of ROS/RNS causing OS (132,133).



1-7: Mechanism of hyperglycaemia related complications. (Adapted with permission from 131)

AR: Aldose Reductase, PK: Protein Kinase, AGE: Advanced Glycation End-products, PAPRP: poly(ADP-ribose) polymerase , GAPDH: glyceraldehyde-3 phosphate dehydrogenase.

Intracellular hyperglycaemia results in an increased synthesis of diacylglycerol (DAG), which is a critical activating cofactor, for Protein Kinase C (PKC) (133, 134). PKC activation results in a variety of effects on gene expression resulting in decreased production of endothelial nitric oxide synthase (eNOS), increased endothelin-1, increased TGF- β, Vascular endothelial Growth Factor (VEGF) and increased plasminogen activator inhibitor-1 (**Figure 1-8**) (133). These changes are associated with vascular occlusion and increased endothelial permeability resulting in tissue damage.



1-8: Consequences of activation of PKC by hyperglycaemia. (Adapted with permission from 133)

It is now being recognized that oxidative stress (OS) is the main mechanism for hyperglycaemia induced micro and macro vascular complications in diabetes. Furthermore oxidative stress leads to complications even in patients with metabolic syndrome without diabetes. However the role of oxidative stress in predicting glycaemic status in patients with macro vascular disease i.e. ACS has not been examined before.

1.9 Matrix Metalloproteinases (MMPs):

These belong to a group of zinc binding proteolytic enzymes which have been proposed as playing a major part in the pathogenesis of atherosclerosis. Several conditions like T2DM, CVD and rheumatoid arthritis are associated with an increased activity of MMPs (135,136). MMPs 2 and 9 are two of the common species in the myocardium as well as the vasculature (135, 137). MMP 9 plays a major role in the vasculature and myocardium remodelling (135, 138). The unstable areas of the atherosclerotic plaques have been shown to have higher expression of MMP 9 (135, 139). Its levels as well as those of MMP 2 are also raised in the circulation in acute coronary syndromes (135, 140). There is still considerable debate about the interplay between MMPs and their inhibitors (tissue inhibitor of matrix metalloproteinase's or TIMP) and their role in diabetic vascular disease. TIMP 2 is the main inhibitor of MMP-2 (135).

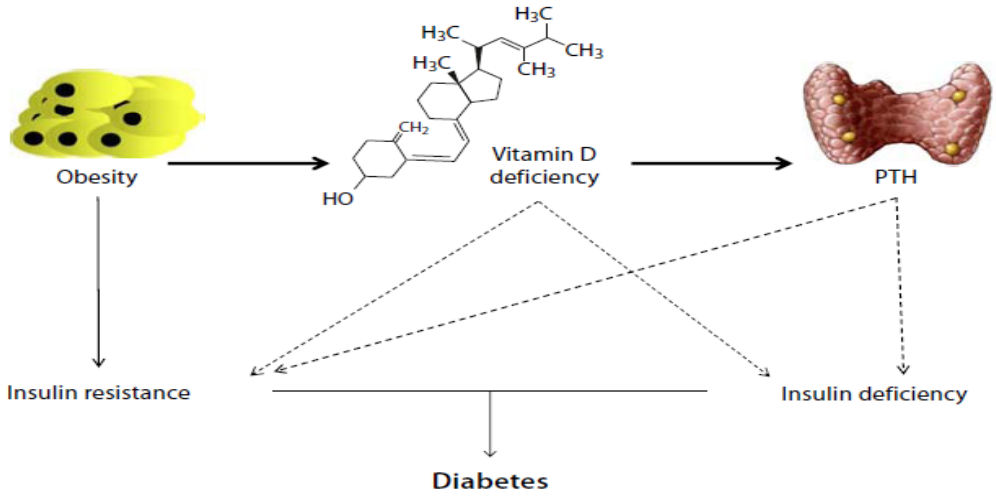
Diabetes is associated with a high incidence of mortality from CVD. It has been postulated that part of this could be related to abnormalities in the synthesis and function of MMPs (135, 141). Low activity of MMPs has been associated with diabetic nephropathy (135, 142 and 143). Some studies have shown elevated and others lower levels of MMPs 2 and 9 in diabetic subjects. Others have shown no difference as well (135, 144-147). Some of the postulation suggests it could be related to the use of different medications as well as coronary intervention (135, 148-150).

Chronic hyperglycaemia has been shown as associated with reduced levels of MMP 9 in circulation (135). The effects of acute elevations in glucose appear a little more complicated. Studies have shown no change in MMP 9 at 90 minutes following an OGTT (147) but a reduction at 120 minutes (135). On the other hand no change was reported in MMP 2 (135).

I decided to look at the activity of MMP-9 in our participants to compare among those with normal, impaired glucose tolerance and T2DM. In addition to MMP-9 we also measured TIMP 1 and TIMP 2 levels at baseline to examine if it had any link with the glycaemic stratification at baseline and again at follow-up.

1.10 Vitamin D:

Deficiency of vitamin D is associated with a number of medical conditions including T2DM (151). A recent meta-analysis looked at the effects of high vitamin D levels. The risk of diabetes mellitus was reduced by 55%, cardiovascular disease by 33% and metabolic syndrome by 51% (151, 152). Vitamin D has been shown to play an important part in the pathogenesis of T2DM. It is proposed that some of these effects are via vitamin D actions on insulin sensitivity and beta cells of pancreas (151, 153-155).



1-9: Role of Vitamin D in the pathogenesis of insulin resistance (Adapted from 156). Solid and bold arrows indicate cause and effect relationships; dotted ones indicate not firmly established evidence

Vitamin D has been shown to exert its effects by improving the expression of insulin receptors and therefore improving insulin glucose sensitivity (156, 157). Vitamin D can also play direct and indirect effects on the beta cells of the pancreas (156, 158). Vitamin D receptors are expressed on macrophages, thus suggesting Vitamin D can also act on the cytokines and mediate the inflammatory response that plays a major role in the pathogenesis of IR and T2DM (151, 159) .

I decided to examine vitamin D status in our cohort to look for an association of vitamin D levels in the circulation and glycaemic status as well as the kind of cardiac event.

1.11 Serum Fructosamine and HbA1c Standardization:

Blood glucose binds to serum protein by glycation to produce serum fructosamine. Its levels in serum are therefore directly related to the degree of glycaemic control. Serum fructosamine as a marker has been used clinically as a monitoring tool to help patients with diabetes control their blood sugar (160).

Half-life of albumin (14-20 days) and other proteins (2-23 days) compares rather unfavourably with haemoglobin (60 days). Therefore fructosamine will only reflect glycaemic control over 2 to 3 weeks as opposed to HbA1c of 6-8 week period (160).

It is however quite a useful marker in situations where the HbA1c cannot be reliably measured. For example the HbA1C test will not be accurate when a patient has a condition that affects the average age of red blood cells (RBCs) (i.e. haemolytic anaemia or blood loss).

In these situations checking the full blood count and reticulocyte count can also provide quite useful information. Similarly the presence of some haemoglobin variants may also affect certain methods for measuring HbA1c (97-99). In these cases, fructosamine can be used to monitor glucose control.

HbA1c was first described as abnormal haemoglobin in diabetics in 1969 (98, 161).

Later on it was recognized that it is as a result of chemical glycation of N-terminal lysine and valines of haemoglobin A (162- 164). A number of small studies confirmed the relationship between HbA1c and average glucose and then a larger study involving around 643 participants established a validated relationship (98, 165).

In view of a possible linear relationship between HbA1c and average blood glucose in adults and children it has been suggested that it can be reported as an estimated average glucose (98).

As it is dependent upon red cell turn over it reflects average glycaemic status over the preceding 2 to 3 months (98, 164,166). It has long been recognized as the best marker for estimating clinical evidence of glycaemic control. However until recently there were major issues with regards to different assays which were being used to measure HbA1c levels (97, 98, and 164).

Standardization of HbA1c was first proposed in 1984; however international standardization was suggested in the D.C.C.T study in 1993 (88, 98).

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) created a Working group which developed a reference measurement for international standardization (98, 167, and 168).

Closer to home in United Kingdom this process has started and currently a number of laboratories are moving towards reporting HbA1c according to IFCC method and mmol/mol. Some are currently doing dual reporting in percentage (as shown in DCCT) as well as mmol/mol while clinicians working in the field of diabetes get used to the new reporting method.

There were concerns raised about using HbA1c as a diagnostic tool in the past in view of the imprecision of some of the assays as well as concerns regarding sensitivity. However with the standardization of the assays a lot of these concerns have been allayed (98, 164).

The HbA1c cut-off value recommended for diagnosis is 6.5%. It has been shown to have a specificity of 99.6% and sensitivity of 42-44% (164, 169).

1.12Role of novel biomarkers in diagnosing dysglycaemia:

T2DM can affect patients in a number of ways. Similarly all people with T2DM are not similar and have different characteristics. While markers such as FPG, OGTT and HbA1c can illustrate the glycaemic status of patients they do not completely demonstrate the functioning of the pancreas or the way insulin is working peripherally (170).

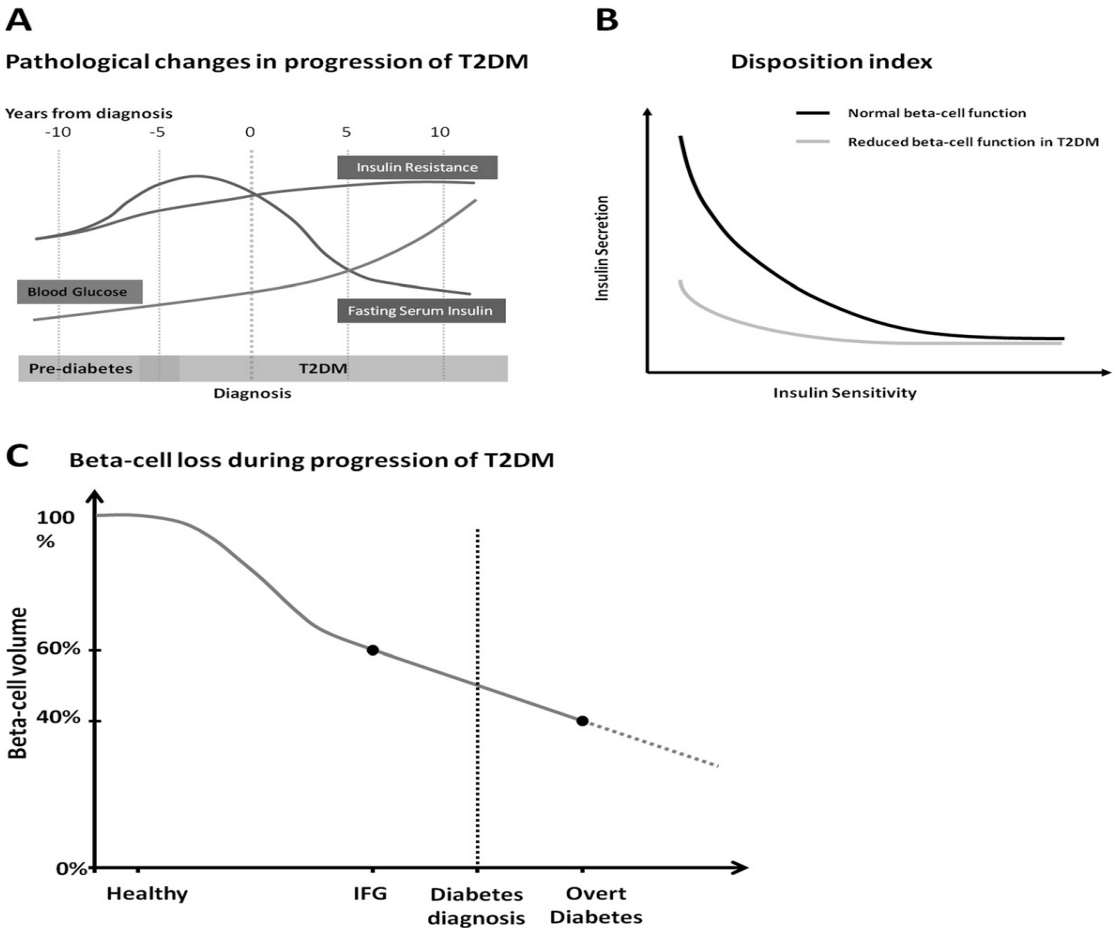
An obvious target in the pancreas would be to look at the functioning of β cells of the pancreas. Studies suggest a 40% reduction in β cell mass in obese patients with IFG and 60% in those with T2DM (170-172).

The pancreas in humans is both an endocrine as well as exocrine organ. 1-2% of the pancreas consists of structures called Islet of Langerhans which have at least 5 different cell types. (α-cells; β-cells; δ-cells; PP cells and ε-cells) The α-cells and β-cells produce glucagon and insulin respectively which have a dominant role in glucose regulation (170). β-cells constitute around 70-80% of the Islets and release pro-insulin on exposure to different stimuli such as glucose and amino acids which is in turn broken down to release insulin and C-peptide (170, 173).

It has been almost impossible to discriminate between changes in insulin sensitivity and secretion so that there is no explanation at this time for the development and progression of T2DM (170, 174).

Small changes in insulin can lead to small changes in glucose and vice versa. Fasting hyperinsulinaemia is associated with a reduction in β-cell mass and function (170, 175).

The following figure demonstrates possible sequence of events in the pathogenesis of T2DM.



1-10: Characteristics of T2DM development and progression.

A) Changes in blood glucose, fasting serum insulin and I.R during initiation and progression of T2DM. B) Disposition index showing relationship of insulin sensitivity and insulin secretion in β-cells of normal individuals and T2DM. C) B-cell loss during disease progression in T2DM. Graph based on findings by Butler et al. and Holman et al. [170, 172].

1.12.1 C-peptide and Pro-insulin:

Under normal physiological conditions the concentration of pro-insulin is quite low. However the pro-insulin concentration is raised in T2DM as it is not properly broken

down (176). Therefore pro-insulin can be used as a marker of insulin resistance (177). Similarly fasting insulin and C-peptide can be measured to assess β-cell production.

However as C-peptide has a much longer half-life (20-30 minutes) compared to insulin (5 minutes) it is considered a much more accurate marker of insulin production (178, 179). Intact pro-insulin is also considered an excellent marker of β-cell function correlating with insulin resistance and identifying the progression to T2DM in clinical practice (180, 181).

Some studies have also looked at using insulin derived markers to predict long term cardiovascular outcomes. One such study examined the role of C-peptide in this regard. The study showed C-peptide was superior to other insulin derived measures of insulin resistance in predicting cardiovascular and overall death in non-diabetic adults. C-peptide predicted cardiovascular death even in subjects with normal glucose tolerance and without metabolic syndrome. C-peptide levels were found to be a better predictor of cardiovascular and overall mortality compared with serum insulin levels as well as other well-known markers of insulin resistance such as HOMA-IR, QUICKI, and metabolic syndrome (182).

1.12.2 Homeostasis model assessment models:

In addition to the above markers i.e. pro-insulin and C-peptide different homeostasis model assessment (HOMA) models are also clinically used to assess β-cell function/dysfunction (HOMA-β), insulin resistance (HOMA-IR) and insulin sensitivity (HOMA-IS) (183). Other markers of insulin resistance such as HOMA-IR (using a product of serum insulin and plasma glucose levels) have also been shown to be a negative indicator of prognosis for cardiovascular and overall mortality (184).

Previously Wallander et al demonstrated that patients who had impaired glucose tolerance (NGT) at baseline but progressed to develop T2DM had higher triglycerides but lower insulin glycaemic index (IGI). The group who had T2DM at baseline and remained diabetic had higher triglycerides and HOMA-IR but lower IGI (81).

I decided to utilize some of these in our project and measured intact pro-insulin, C-peptide, glucagon as well as homeostasis model assessments HOMA- β, HOMA-IR and HOMA-IS in our participants.

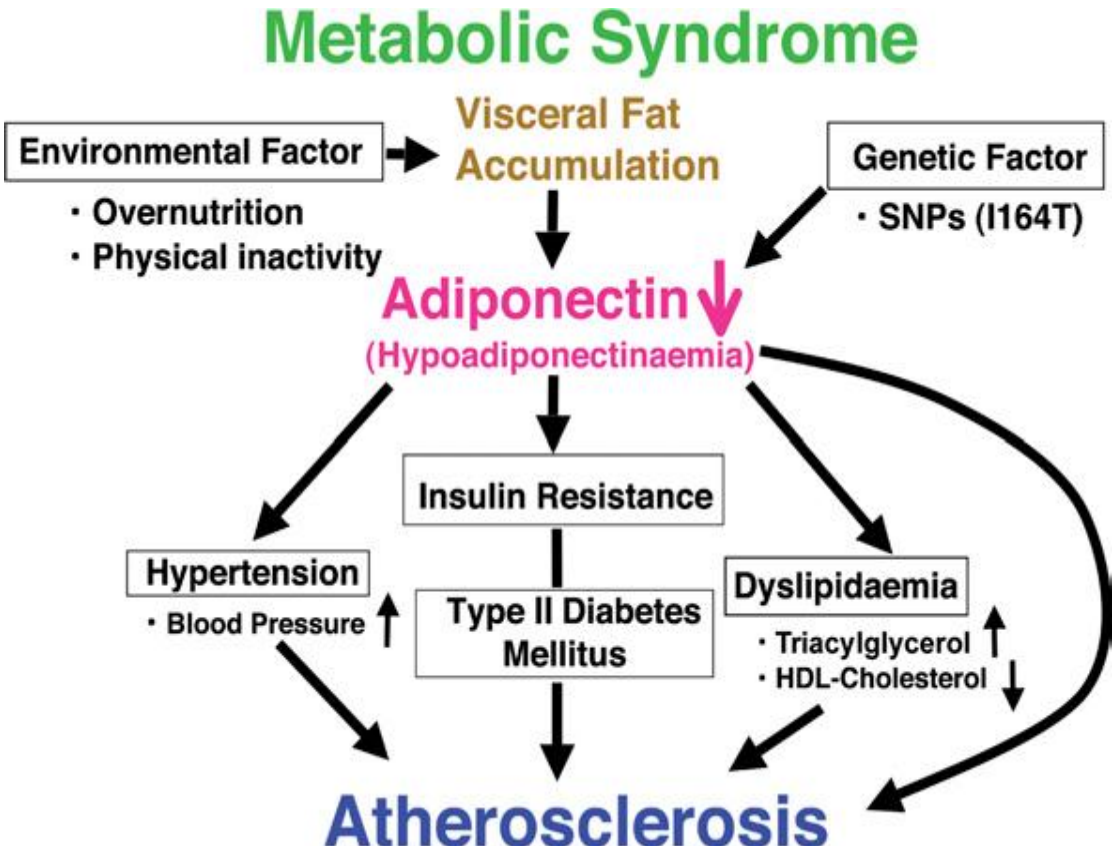
1.12.3 Adipokines:

There is evidence from the literature that obesity and more specifically excess visceral body fat plays a role in the development of T2DM and metabolic syndrome

as well as risk factors for CVD like dyslipidaemia and insulin resistance (185, 186). Adipose tissue produces some markers (adipokines) leptin and adiponectin which have been shown as having an association with the risk factors of CVD. Leptin is an adipokine which is mainly produced by adipose tissue and is known to play a role in regulation of food intake as well as insulin glucose and triglycerides (187). Its levels rise in individuals at high risk of CVD. Its levels positively correlate with body fat (185).

Adiponectin is a protein which is only synthesized in adipose tissue. It inhibits the production and release of glucose from the liver as well as reducing the levels of free fatty acids and oxidation. In addition it has antiatherogenic properties. Its concentration in the plasma is inversely proportional to the body weight (185). I decided to examine these biomarkers in our participants to examine potential benefit in developing a diagnostic model for high risk participants with CVD

A study of patients with kidney failure demonstrated that patients who suffered from new cardiovascular events had lower plasma adiponectin levels than controls (188). Following is a graphic illustration of some of the consequences of deficiency of adiponectin.



1-11 **Adiponectin in metabolic syndrome (Adapted from 189)**

1.12.4 Interleukin-1RA

As illustrated above a number of markers can be used to help to predict incident diabetes. Interleukin-1β (IL-1β) is considered one of the most crucial cytokines in the development of T2DM as it triggers a cascade of events which in turn result in β cell death (190, 191). The harmful effects of this cytokine can be prevented by its antagonist IL-1 receptor antagonist (IL-1Ra) which is produced once again by adipose tissue and occurs naturally (192).

This compound had also been used therapeutically and shown to improve glycaemic control and β cell function (193). A case control study has also demonstrated elevated levels of IL-1Ra being associated with T2DM (194). This could be a sign of developing immune and metabolic derangements which happen before development of T2DM (195).

I decided to look at levels of IL-1Ra in our cohort to examine if it could predict the onset of developing T2DM in our cohort.

To summarize this section I looked at measuring a number of novel biomarkers which included C-peptide, glucagon, intact pro-insulin, homeostatic assessment models, leptin, adiponectin, leptin adiponectin ratio and IL-1Ra in our cohorts. My aim was to examine if they aided in developing a mathematical model for T2DM and also to see which markers may play a more important role in predicting the onset of developing T2DM.

Rationale of Project:

T2DM is one of the major risk factors for CVD. Patients admitted to hospital with ACS have high levels of abnormal glucose homeostasis which has short and long term consequences. A number of centres across the UK are now recognising the need to assess glucose tolerance in all patients admitted to a coronary care unit (CCU) with ACS. Evidence suggests that only one third of patients have normal glucose tolerance and that one third have IGT and further third overt T2DM. Although we are starting to appreciate the magnitude of the problem less is known regarding the outcome of patients in these different groups, particularly those with IGT who go undiagnosed.

The overall aim of this study is to characterise glucose tolerance in all patients admitted to the Heartlands and Queen Elizabeth Hospitals (QEH) CCU with ACS on admission and 3 months after discharge and to evaluates the ability of international

criteria and our T2DM screening algorithm to accurately classify the glycaemic status. I also evaluated a panel of biomarkers in order to determine whether they enhanced the power of our screening tests.

Hypothesis:

In patients with acute coronary syndrome, long term glycaemic status can be determined on hospital admission using reproducible and easily obtainable measures other than the oral glucose tolerance test.

2. METHODS

2.1 Primary Aims:

1. To determine the prevalence of undiagnosed diabetes and impaired glycaemic state (IGS) in patients admitted with ACS.
2. To compare the WHO 1998 and IEC criteria for diagnosis of T2DM in patients admitted to hospital with ACS.
3. To investigate the role of a screening algorithm that includes fasting plasma glucose (<7.0 mmol/l) and HbA_{1c} (>6.0%) to accurately define glucose tolerance in patients admitted with an acute coronary syndrome (ACS).

2.2 Secondary Aims:

1. To determine whether screening for abnormalities or glucose tolerance are similar at the time of admission with ACS and at 3 months post−discharge.
2. To determine whether glycaemic abnormalities at admission to hospital in patients with ACS are secondary to stress hyperglycaemia.

2.3 Tertiary Aims:

1. To determine whether markers of oxidative stress, pancreatic function and inflammation are predictors of glycaemic status in patients with ACS.
2. To determine long term cardiovascular mortality in patients classified as normal glucose tolerance, IGS and undiagnosed type 2 diabetes.

2.4 Study Design and Settings:

We conducted a prospective study of patients admitted to hospital with ACS. The study was carried out at Queen Elizabeth and Heartlands Hospital in Birmingham. Patients were identified from ACS and coronary care units (CCU).

The potential participants were identified by visiting CCU and the ACS unit on a daily basis. They were approached on the ward and provided with a leaflet about the study. Details were verbally explained and the participant offered a chance to ask questions. If they agreed to participate in the study they were provided with a consent form to sign and recruited in the study. It was made clear that they can

withdraw consent at any stage. All patients admitted with ACS were reviewed by me or a research nurse (Mrs Susan Maiden) on a daily basis. Patients were given adequate time to make their mind and contacted the next day.

The study was approved by the Solihull East and North REC and also by the local R&D departments at Queen Elizabeth and Heartlands Hospitals. The study was supported by an initial educational grant by the Sanofi-Aventis Excellence in Diabetes Programme and further supported by Queen Elizabeth Hospital Charities. The funding bodies had no role to play in the design of the study protocol or interpretation of the results.

2.5 Study Protocol:

2.5.1 Subjects:

The subjects were patients admitted to Queen Elizabeth and Heartlands Hospital in Birmingham with ACS. Patients not known to have diabetes, based upon past medical history underwent fasting plasma glucose (FPG), oral glucose tolerance test (OGTT) and HbA_{1c}. In addition I also determined serum nitrotyrosine as well as vitamin D, rennin and other biomarkers of acute coronary syndrome and insulin resistance.

To detect glucocorticoid response to stress we measured spot urinary cortisol creatinine ratio. We performed these measurements within 7 days of hospital admission on midstream urine early morning sample.

All samples were annonymised and only the study investigators had a code in order to be able to link them to individual patients. All participants were asked to return to the hospital after 3 months for repeat FPG, OGTT and HbA_{1c} as well as the other biomarkers. Urinary cortisol creatinine ratios were repeated at the second visit.

At the follow-up visits, all participants were given healthy dietary and lifestyle advice. The relevant General Practitioners were informed of the results of the screening tests after second visit and glycaemic status were defined on basis of results of the second OGTT according to WHO 1998 criteria. Patients with venous FPG>7.0 mmol/l were classified with diabetes mellitus, if 6.1 to 6.9 mmol/l with impaired fasting glycaemia (IFG) and <6.0 mmol/l with normal fasting glucose. Patients with a 2 hour plasma glucose (2hPG) >7.8 and <11.1 mmol/l obtained from venous blood will be classified as having impaired glucose tolerance (IGT) and those with 2 hour plasma glucose >11.1 mmol/l with T2DM.

Any participants who were diagnosed as having diabetes mellitus were treated according to the local guidelines and followed up in the diabetes clinic. They were also offered full support of other staff like dieticians and diabetes specialist nurses.

2.5.2 Inclusion Criteria:

1. Patients admitted to Queen Elizabeth and Heartlands Hospital coronary care with ACS over a 3 year period. The diagnosis of ACS requires the presence of chest pain with elevated troponin or ECG changes suggestive of ischemic heart disease.
2. Age 18 to 90 years.

2.5.3 Exclusion Criteria:

1. Patients already known to have T2DM.
2. Those unable to give informed consent.
3. Participants under 18 or over 90 years of age.
4. Participants with normal troponin results and no previous history of ischemic heart disease.

2.6 Detailed methodology:

2.6.1 Glucose:

Venous blood was collected next morning after fasting the previous night and fluoride Vacutainers transported to the central laboratory for measurement of plasma glucose. Glucose was measured using a hexokinase kit (Cat. No. 11876899216) and C.f.a.s. calibrator for automated systems (Cat. No. 10759350190) on a Roche Modular platform (Roche Diagnostics, E Sussex, UK) with CVs across the range of <2%.

2.6.2 OGTT:

OGTTs were performed at admission and 3 months. Patients were requested to fast the previous evening for 10 hours and bloods collected the next morning. Plasma glucose and HbA_{1c} were measured on venous plasma. Glucose was measured using the same reagents. For the 75 g OGTT, the patient was asked to drink 113 ml glucose polymer drink, Polycal, (Nutricia Clinical Care, Wiltshire, UK) over a period of 5 min. A further venous blood sample was taken after 2 hours for plasma glucose measurement.

2.6.3 HbA_{1c}:

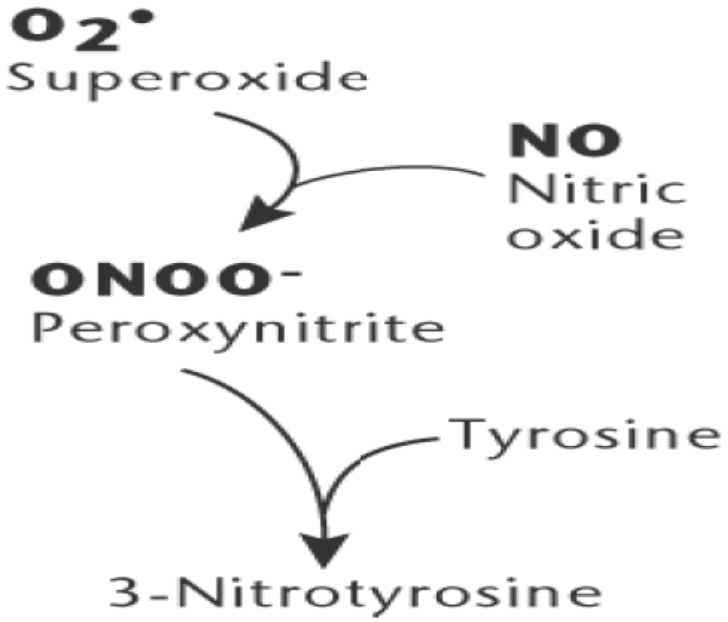
'DCCT aligned' HbA_{1c} was reported from an ion exchange, high performance, liquid chromatography analyser, TOSOH G7 A1c Variant Mode (Tosoh Bioscience Ltd, Worcs, UK) that detects haemoglobin variants. A reference interval of <6% HbA_{1c} was quoted by the manufacturer. HbA_{1c} was not reported in patients with variant haemoglobin.

2.6.4 Vitamin D:

Vitamin D status was assessed by measuring the concentration of 25 OH vitamin D in serum. LC-MS/MS method was used by the local laboratory which has been reported as having excellent sensitivity as well as being accurate, precise and quick. The principle is based on tandem mass spectrophotometry.

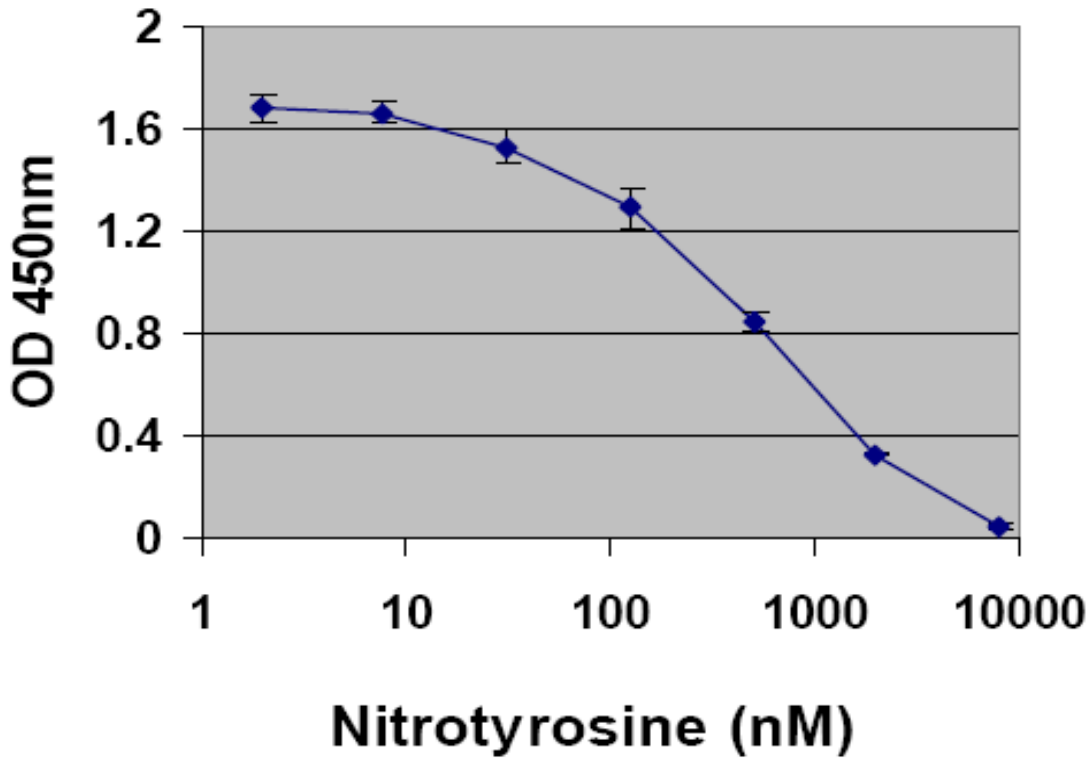
2.6.5 Nitrotyrosine:

Modification of tyrosine residues in proteins to 3-nitrotyrosine by peroxynitrite (Fig 2-1) or other agents has been detected in biological systems that are subject to oxidative stress. 3-Nitrotyrosine is formed after a hydrogen ion is removed from tyrosine to form tyrosyl. The active tyrosyl then interacts with peroxynitrite to form 3-nitrotyrosine (196). The efficiency of tyrosine nitration is also dependent on biological conditions like local production of reactive species, availability of antioxidants and scavengers and accumulation of inflammatory and presence of pro-inflammatory cytokines (196). It has been implicated in a number of medical conditions including T2DM and cardiovascular disease.



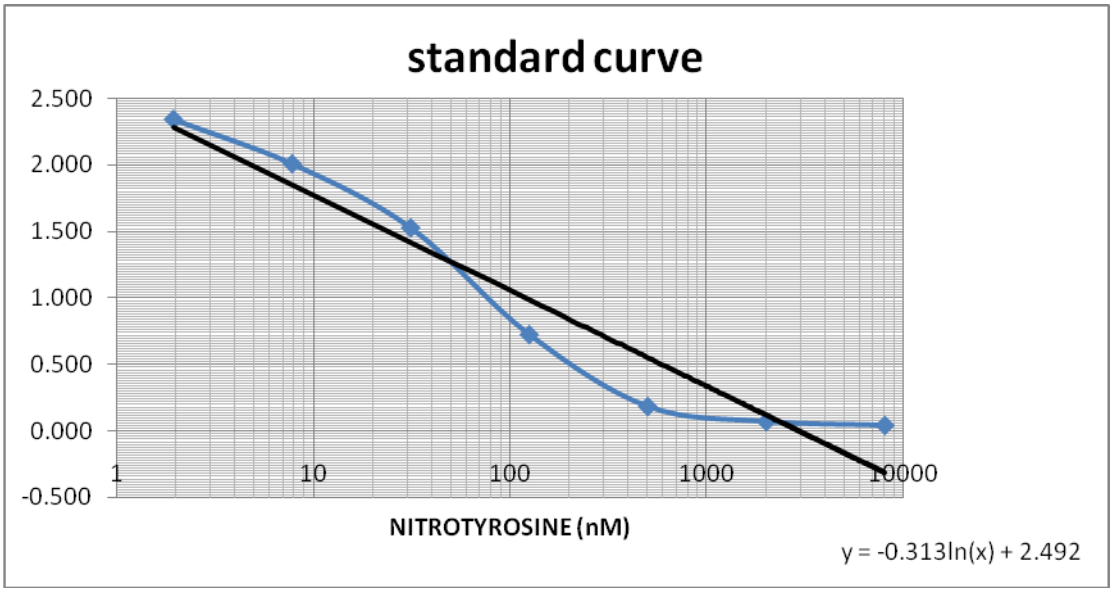
2-1: Nitrotyrosine formation

The OxiSelect Nitrotyrosine ELISA Kit (catalogue number STA-305) was used for detection and quantification of 3-nitrotyrosine in protein sample. The principle was based on competitive ELISA. Quantity of 3-nitrotyrosine is determined by comparing its absorbance with a known nitrated BSA standard curve. The sensitivity range of our assay was 20 nM to 8.0 µM. I followed the instructions issued by the manufacturers. The unknown sample or nitrated bovine serum albumin (BSA) standards were first added to preabsorbed E1A plate. After brief incubation, anti-nitrotyrosine antibody was added, followed by horseradish peroxidase (HRP) conjugated secondary antibody. The protein nitrotyrosine content was determined by comparing with a standard curve prepared from predetermined BSA standards. An example of manufacturers curve is shown below:



2-2: Nitrotyrosine ELISA curve provided by the manufacturer

An example of curve obtained by me is shown below:



2-3: Nitrotyrosine ELISA standard curve from my plates

2.6.6 Matrix metalloproteinase 9 (MMP-9):

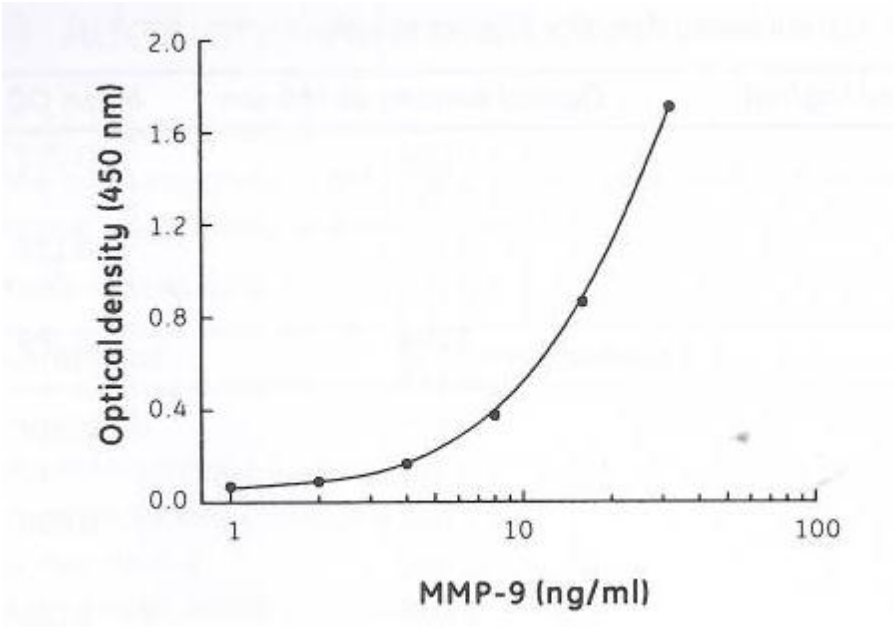
MMP-9 belongs to a series of enzymes called matrix metalloproteinase's which have the ability to breakdown components of the extracellular matrix. MMP-9 has a broad range of substrate specificity for a wide variety of native collagens including various collagen types as well as gelatin, elastin and proteoglycans (197-199). As explained in section 1.9 the activity of MMP-9 is inhibited by tissue inhibitor of matrix metalloproteinase's (TIMP) in a 1:1 molar ratio (197).

MMP 9 has been implicated in pathogenesis of a number of clinical conditions. While it has been implicated in CVD and acute and chronic hyperglycaemia its role has not been studied as a positive predictor of glycaemic status in patients with CVD.

The MMP 9 was measured by using the Biotrak MMP-9 ELISA from GE Healthcare. The assays principle is based on a two way ELISA sandwich format using two antibodies. During first step of the ELISA, MMP-9 present in samples was bound to micro plate precoated with the antibody. During the second step, detection antibody conjugated with HPO was added forming the immobilized complex.

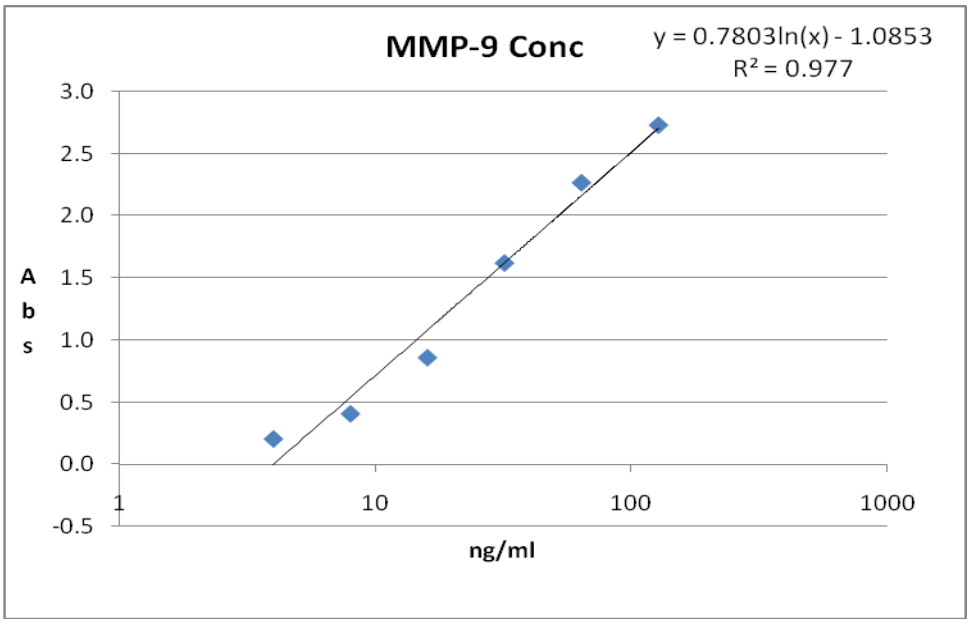
Amount of peroxidase bound to each well was determined by the addition of tetramethylbenzidine (TMB) 'ready to use' substrate. The reaction was stopped by addition of acid solution and the resulting colour change measured at 450 nm in a

micro plate spectrophotometer. The concentration of MMP-9 was measured by interpolation from a standard curve. The following is an example of a standard MMP-9 curve obtained by plotting mean optical density (y-axis) against ng/ml standard (x-axis). An example of a typical standard curve for the assay is shown.



2-4: Manufacturers standard curve for MMP-9 assay

An example of a curve obtained by me during analysis is shown below:



2-5: MMP-9 ELISA curves from my plates

2.6.7 Urinary cortisol:

Measurements of cortisol in urine were carried out by the Roche Modular E170 cortisol assay which uses Electrochemiluminescence immunoassay (ECLIA).

2.6.8 Novel Biomarkers:

C-peptide and glucagon were measured using commercial MSD assays and following manufacturer's instructions. Intact pro-insulin was measured using an Invitron assay. The assays for adiponectin, TIMP1 and TIMP2 used R&D duo-sets and were optimised for use on human plasma. The leptin and IL-1Ra assay used mouse monoclonal fabs and were again optimised for use on human plasma. Following are more details about the assays used:

The C-peptide kit MSD Cat no. was N45CA-1 was optimised for plasma by the manufacturer. Assays were carried out according to manufacturer's instructions.

The leptin and IL-1Ra assays were in-house assays from Mologic and had been optimised for use with plasma by performing dilution of linearity and spiked sample tests. These test the linear range of the assay within plasma and find if there are any issues with the matrix effect with regard to the concentration of plasma used.

Human Glucagon kit MSD Cat no. K151HCC-2
is validated kits for use with serum/ EDTA plasma. Full details available from MSD website. Assays carried out according to manufacturers' instructions

Intact Proinsulin Luminescence Assay Kit Invitron Cat no. IV2-002
http://www.invitron.co.uk/intact-proinsulin-luminescence-assay.html
is validated kits for use with Heparin and EDTA plasma
Assays carried out according to manufacturers' instructions.

Human TIMP-1 Duo Set 15 Plates, R&D Systems, Cat no. DY970
http://www.rndsystems.com/Products/DY970
Validated in-house using dilutional linearity and spike/recovery assays
Samples diluted 1:200 in PBS+10% Foetal calf serum assay diluent for testing

Human TIMP-2 DuoSet, 15 Plate, R&D Systems , Cat no. DY971
http://www.rndsystems.com/Products/DY971
Validated in-house using dilutional linearity and spike/recovery assays
Samples diluted 1:200 in 2x R&D assay diluent for testing.

Human Adiponectin/Acrp30 DuoSet, 15 Plate, R&D Systems , Cat no. DY1065
http://www.rndsystems.com/Products/DY1065
Validated in-house using dilutional linearity and spike/recovery assays
Samples diluted 1:5000 in 1x R&D assay diluent for testing.

2.7 Data collection:

2.7.1General:

The following data was collected**:**

Age, gender, ethnicity, medications, past medical history, family history, smoking history, history of hypertension and dyslipidaemia.

2.7.2 Metabolic:

The following data was collected:
Height, weight, Body Mass index (BMI), HbA1c, OGTT, Lipids, Blood Pressure (B.P), and Vitamin D. These were all measured in the respective laboratories. BP was measured by an automated device while the patient in sitting position and the left arm resting on a table. The 2 measurements were at least 10 minutes apart and the first measurement was after about 30 minutes after the start of the consultation. The average of the two readings was used in the database.

2.7.3 Cardiac history:

The following data was collected:
The nature of the cardiac event, treatment offered and outcome of the underlying cardiac condition.

2.7.4 Blood samples:

These were collected from consenting patients and were used to measure levels of nitrotyrosine (as a marker of nitrosative stress) and other biomarkers. Plasma and serum samples were collected and stored in a -80 degree Celsius freezer following centrifuge. These samples were collected following fasting.

2.8 Statistical analysis:

Data analyses were performed using SPSS 15.0 software (SPSS Inc, Chicago, IL). Data are presented as mean ± SD or SE or median (interquartile range) or frequencies. Independent continuous variables were compared using the Student *t* test or the Mann-Whitney test. Categorical variables were compared using the Chi-square test. Correlations between continuous variables were performed using the Pearson or Spearman tests. Differences between independent groups were assessed by analysis of variance (ANOVA). If the homogeneity of variance assumption of ANOVA was violated, the Welch statistics were used to calculate the P values. Post-hoc analyses were performed using Games-Howell or Gabriel tests depending on whether or not the equal of variance assumption was violated.

To assess the relationship between continuous and/or categorical variables and dichotomous outcomes multiple logistic regressions (forced entry method) was used. Variables included in the regression models were based on known outcome-related risk factors. We assessed multicolinearity in both multiple linear and logistic regression models using simple correlations between variables plus the tolerance values, and the condition indices. No tolerance values were < 0.1 and no variables had strong correlations (r > 0.8). In multiple linear regression models, the residuals were examined. In all the models presented, residuals followed a normal distribution with uniform variance and there was no relationship between the residual and the predictor of interest. Data distribution was assessed using histograms and the Shapiro-Wilk test. A *P* value < 0.05 was considered significant unless stated otherwise.

Sample Size: 110 allowing for dropout of 10%

Justification of Sample Size:

Based on previous publications (3) we expected a 33% prevalence of T2DM in our cohort. Using our screening algorithm (7) in order to achieve sensitivity of 97% (95% confidence interval 84-100%) and specificity of 94% (95% confidence interval 85-98%) we needed a sample size of 100 participants.

3. PREVALENCE OF DIABETES MELLITUS AND IMPAIRED GLYCAEMIC STATUS

3.1 Clinical Characteristics:

118 participants (age range 31-90, mean 61.3 years) were recruited over a period of 3 years with a higher number of male participants. While we had representation from all ethnicities, we had a majority of white Caucasians recruited in our study. The distribution was male and female participants were in keeping with the number of admissions to CCU.

3-1: Sex and ethnicity based distributions of participants

	Number	**(%)**
Male	96	81
Female	22	19
White Caucasian	94	80
South Asian	19	16
Afrocaribbean	5	4

We aimed to recruit patients admitted to coronary care units with both ST segment elevation and non ST segment elevation myocardial infarction. This information was available for 113 participants.

3-2: Nature of cardiac events

	Number	**(%)**
NSTEMI	64	57
STEMI	49	43

The following is an illustration of the distribution of some of the baseline parameters in our cohort of participants. Mean age was 61.3 (range 31-90) years while mean body mass index (BMI) was 28.3 (range 17-47) kg/m^2. Admission plasma glucose was only available for 47 participants (Mean 6.7 mmol/l Range 4.7-12.9 mmol/l) thus highlighting deficiencies in comprehensive diabetes screening on admission to hospital.

3-3: Clinical and metabolic parameters of study participants

Variables	Mean	Minimum	Maximum	±S.D
Age (Years)	61	31	90	11.9
BMI (kg/m²)	28	17	47	5.1
Baseline FPG (mmol/l)	5.7	4.3	13.1	1.2
Baseline 2hr PG(mmol/l)	8.5	2.4	23.5	3.7
Baseline HbA1c (%)* mmol/mol	6.1 43	4.8 29	10.6 92	0.84
Systolic BP (mmHg)	126	91	192	20.5
Diastolic BP (mmHg)	74	47	115	12
Admission glucose (mmol/l)	6.7	4.7	12.9	2
Total cholesterol (mmol/l)	4.4	1.3	8	1.5
Triglycerides	2.0	0.3	6.7	1.4
Fructosamine (µmol/l)	213	169	401	28.7
Vitamin D (nmol/l)	37.6	2.1	119	24.2

* HbA1c was not reported in one participant due to the presence of variant Haemoglobin

Medication history was available for approximately 100 participants.

3-4: Drug history at admission

DRUGS	NUMBER OF PARTICIPANTS (%)
Antiplatelet agents (Aspirin and clopidogrel)	93
Lipid lowering therapy (Statins and ezetimibe)	88
Beta Blockers	81
ACE inhibitors/ Angiotensin receptor antagonists	87
Nitrates	10
Diuretics (Thiazides and K sparing)	19
Calcium channel blockers	10
Antiarrhythmic drugs	4

Note: Other miscellaneous agents included agents such as doxazosin, ivadrabine, rivoraxaban, finasteride and inhalers. One patient was also on long term prednisolone and alendronic acid and was not included in the analysis for urinary cortisol creatinine ratio. Most of these drugs were commenced at hospital admission apart from antihypertensive and lipid lowering therapy. Data concerning past medical history were available on 92 participants. At least a third of the participants had no known risk factors for CVD. Smoking appeared to be the most significant risk factor in our cohort with at least two thirds of participants giving a history of smoking.

3-5: Past Medical History

PAST MEDICAL HISTORY	YES	NO
Hypertension	32	60
Smoking (Current and Ex-smokers)	62	30
Dyslipidaemia	29	63
Ischaemic heart disease	22	70

3.2 Classification of Diabetes Mellitus according to W.H.O 1998 and IEC diagnostic criteria:

On the basis of W.H.O 1998 diagnostic criteria, at baseline 48.3% of participants had normal glucose tolerance (NGT) 19.5% T2DM and 32% impaired glycaemic status (IGS) which includes patients with IFG 2.5%, IGT 25.4% as well as a combination (IFG+IGT) 4.2%.

8 out of these 23 participants diagnosed with diabetes mellitus were started on diabetes treatments based on the original OGTT results due to the severity of their glycaemic abnormalities. The remaining 15 underwent a second OGTT and the glycaemic status was re-classified.

3-6: Baseline glycaemic classification

	Number of participants	**(%)**
NGT	57	48.3
IFG	3	2.5
IGT	30	25.4
IFG+IGT	5	4.2
T2DM	23	19.5

Due to the limited number of subjects with IFG alone, for the purpose of some of the analyses of the data, the three groups classed as IFG, IGT and IFG+IGT were grouped together and called impaired glycaemic status (IGS). This group would be considered as high risk for developing T2DM in future.

3-7: Baseline glycaemic classification for analysis

	Number of participants	**(%)**
Normal	57	48.3
IGS	38	32.1
T2DM	23	19.5

Following is an illustration of ethnicity based glycaemic classification at baseline. Although the frequency of patients with T2DM was higher among South Asians compared to white Caucasians the trend did not reach statistical significance.

3-8: Baseline glycaemic classification according to ethnicity

Ethnicity	NGT	IGS	T2DM
White Caucasian	45(48%)	31(33%)	18(19%)
South Asian	9 (47.3%)	5(26.3%)	5(26.3%)
Afrocaribbean	3(60%)	2(40%)	0

The glycaemic status was reclassified again at 3 months following the initial event. 3 month data were available for 101 participants (14 drop outs, 3 died). As described above 8 participants with DM did not undergo a second OGTT, therefore follow-up results for FPG and 2 hour PG were only available for 93 participants. 54% participants had NGT, 21% T2DM and 25% IGS with 9% having IFG, 11% IGT and 5% having a combination of IFG+IGT. The number of participants diagnosed with T2DM at 3 months included a combination of those from an initial OGTT who did not undergo a second one as well as those whose diagnosis was based on 2nd OGTT results. The majority of participants lost to follow-up had NGT at initial OGTT (10 out of 14). It was noted that the prevalence of IFG nearly tripled at 3 months and at least 4 participants who had NGT at hospital discharge had IFG at 3 months.

The 2 hour plasma glucose was significantly different between baseline and follow-up with mean value being higher at admission suggesting possible stress hyperglycaemia (8.5 vs. 6.8 mmol/l). The possibility was studied in more detail and explained in the section on stress hyperglycaemia.

3-9: Follow-up glycaemic classification

	Number of participants	(%)
Normal	55	54
IFG	9	9
IGT	11	11
IFG+IGT	5	5
T2DM	21	21

For the purpose of some of the analyses of the data, the three groups classed as IFG, IGT and IFG+IGT were grouped together as IGS. This group would be considered as high risk for developing T2DM in future.

3-10: Follow-up glycaemic classification for analysis

	Number of participants	**(%)**
Normal	55	54
IGS	25	25
T2DM	21	21

I analysed the glycaemic classification according to ethnicity at 3 months and the frequency of T2DM was lower among South Asians compared to White Caucasian and also when compared with baseline. However the trend once again did not reach statistical significance.

3-11: Follow-up glycaemic classification according to ethnicity

Ethnicity	**NGT**	**IGS**	**T2DM**
White Caucasian	46(58%)	17(21%)	17(21%)
South Asian	7 (41%)	7(41%)	3(18%)
Afrocaribbean	2(50%)	1(25%)	1(25%)

The following is an illustration of the incidence of diabetes in our cohort when using the IEC criteria as a diagnostic modality. Sections 3.4 and 3.5 offer more detailed comparison of the two diagnostic criteria at baseline and follow-up.

3-12: Classification of glycaemic status at baseline based on IEC diagnostic criteria

	Number of participants	**(%)**
Normal (HbA1c<6.5%)	98	84
Diabetes (HbA1c ≥6.5%)	19	16

3.3 Relationship of other parameters with glycaemic status:

My next aim was to explore possible associations of any of the background parameters with the glycaemic classification. The main parameters which appeared to be positively associated with the glycaemic status at baseline were age, body mass index (BMI) and serum fructosamine levels.

3-13: Associations of means of basic parameters with background glycaemic status

	NGT	IGS	T2DM	Trend for significance
Age (Years)	57± 11	64± 10	67± 12	0.001
BMI (kg/m²)	27±4	29± 4	31±7	0.02
Fructosamine	207±17	208± 22	236±47	0.001

The mean age of NGT participants was 57 years. However mean age was higher in the IGS and T2DM groups at 64 and 67 years, respectively. This was statistically significant with a p value of 0.01 and 0.002 respectively. Mean BMI was 27 kg/m² in the normal, 29 kg/m² in the IGS and 31 kg/m² in the diabetic group. Mean BMI was higher in the DM group when compared to normal (p=0.02).

Mean fructosamine was higher in the diabetic group (236) as compared to normal and IGS groups (207 and 208 respectively). This was associated with p values 0.004 and 0.001 respectively.

The next step was to look at a possible association of any of the clinical parameters with the glycaemic classification according to W.H.O 1998 diagnostic criteria at 3 months. BMI, serum fructosamine were the parameters significantly related to the glycaemic classification as illustrated. The association with vitamin D and age was of borderline statistical significance only.

3-14: Association of means of basic parameters with follow-up glycaemic status

	NGT	IGS	T2DM	Trend for significance
Age	59± 10	61± 10	66± 12	0.08
Vitamin D	43±26	27±16	34±20	0.05
BMI (kg/m²)	28±5	28±5	32±5	0.008
Fructosamine	207±18	213±19	232±49	0.01

The mean BMIs were 28, 28 and 32 kg/m² in the normal, IGS and DM groups. Mean fructosamine values were 207, 213 and 232 respectively in the normal IGS and DM groups. There was a statistically significant difference between normal and DM groups (p=0.01).

Mean vitamin D levels were 43, 27 and 34 International Units in the normal, IGS and DM groups. It was interesting to note that there was a difference between normal and IGS groups with the mean vitamin D levels being higher in the normal group when compared with the IGS cohort (p=0.05).I also looked at possible association of the clinical parameters with glycaemic stratification based on IEC diagnostic criteria. The parameters significantly related were age, BMI and serum fructosamine levels only. All the three parameters were higher in the diabetic cohort compared to the normal group as illustrated in the table below.

3-15: Association of basic parameters with IEC based glycaemic classification

	Normal	DM	Trend
Mean Age	60±11	67±14	0.05
Mean BMI	28±5	31±6	0.04
Mean Fructosamine	209±19	242±50	0.03

In addition I did not see any differences based on sex or ethnicity for baseline or follow-up glycaemic classification.

3.4 Comparison of W.H.O 1998 and IEC diagnostic criteria:

I decided to compare the W.H.O 1998 diagnostic criteria with the IEC diagnostic criteria (HbA1c >=6.5%) at baseline in our study. Currently Diabetes U.K does not recommend routine use of HbA1c to diagnose diabetes mellitus in acute hospital admissions.

The two diagnostic criteria were positively related to each other. Figure 3.1 illustrates our findings. All the participants classified as normal on W.H.O 1998 diagnostic criteria were all also normal on the IEC diagnostic criteria. However by implementing the IEC criteria we would have missed 10 (43%) participants classified as T2DM on WHO 1998 diagnostic criteria. As expected the group classified as IGS were represented in both groups with 6(16%) of them being classified as having T2DM according to the IEC criteria. This does raise the possibility of identifying different cohort of patients from the two different diagnostic criteria.

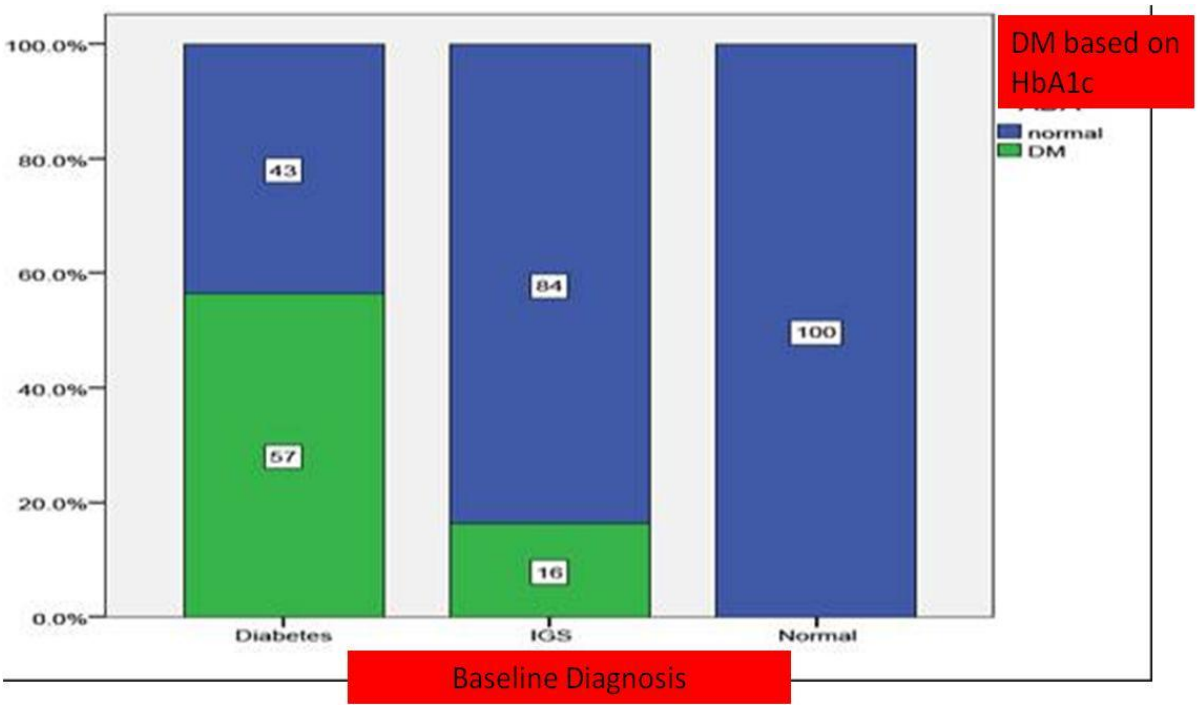


Figure 3-1: Comparison of outcome of WHO 1998 (x-axis) and IEC diagnostic criteria (y-axis) at baseline.

Another way of illustrating the comparison between the two is shown in the graph below. Among participants classified as normal on the IEC diagnostic criteria, 10(10%) had T2DM on the W.H.O 1998 diagnostic criteria while 31(32%) had IGS and

57(58%) NGT. On the contrary among those classified as T2DM on IEC diagnostic criteria 6(31.58%) had IGS and 13(68.32%) T2DM on W.H.O 1998 diagnostic criteria.

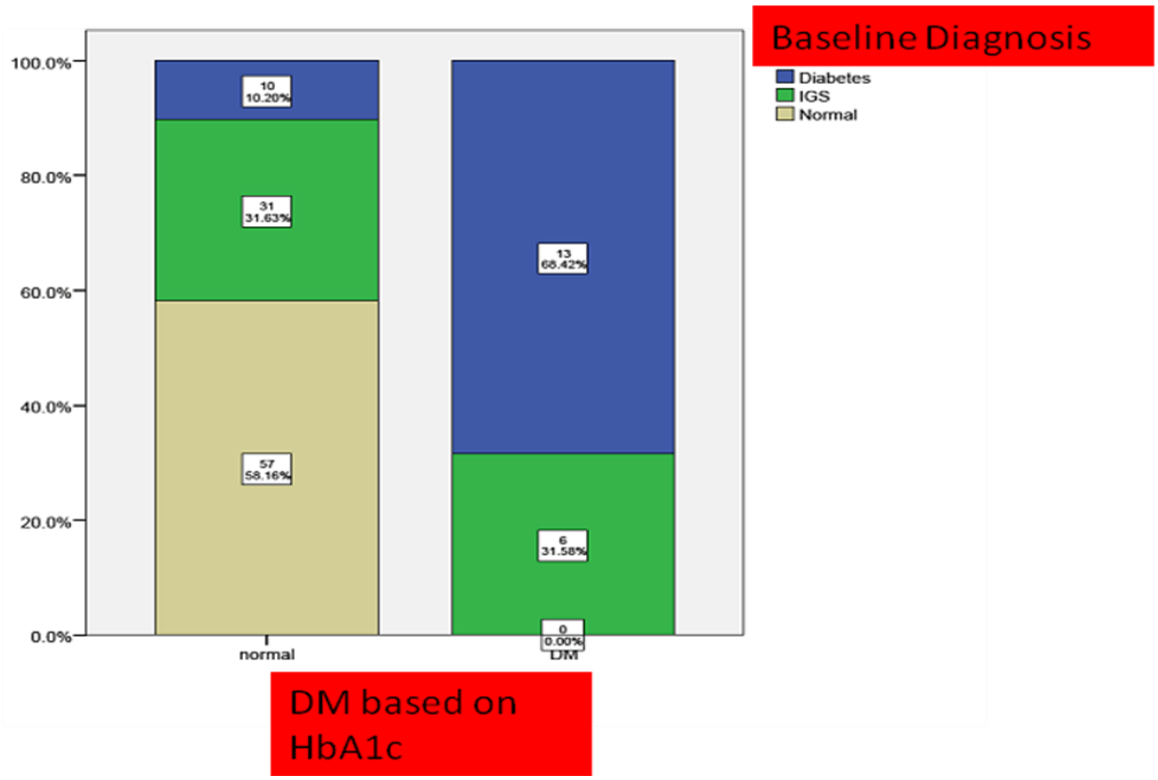


Figure 3-2: Comparison of outcome of WHO 1998 (y-axis) and IEC diagnostic criteria (x-axis) at baseline

HbA1c diagnostic criteria and WHO 1998 diagnostic criteria were also positively related to each other at 3 months. Closer examination of the graph below however shows that the HbA1c criteria would have missed at least 7 (33%) participants who had been classified as T2DM on the W.H.O 1998 criteria. However only one participant classified as normal on the W.H.O 1998 criteria had T2DM based on IEC criteria at 3 months.

Participants classified as having IGS were mostly normal 22(88%) on the A1C criteria with only 3(12%) having T2DM.

The ADA guidelines do recommend using an HbA1c range of 5.7 to 6.4% to define pre-diabetes state. The following table illustrates comparison of individual normal, impaired and diabetic groups in the two diagnostic criteria. In all three categories we appear to be identifying different cohorts. However the difference appears to be most marked in the pre-diabetes group as only a third (35%) of participants

classified in this group according to ADA criteria were in the same category according to WHO criteria. Indeed over half (54%) of the participants were normal according to WHO criteria.

3-16: Comparison of outcome of ADA and WHO criteria at baseline

ADA criteria	W.H.O Criteria NGT (%)	W.H.O Criteria IGT (%)	W.H.O Criteria T2DM (%)
Normal 18	14 (78)	3 (17)	1 (5)
IGS 80	43 (54)	28 (35)	9 (11)
T2DM 19	0	6 (32)	13 (68)

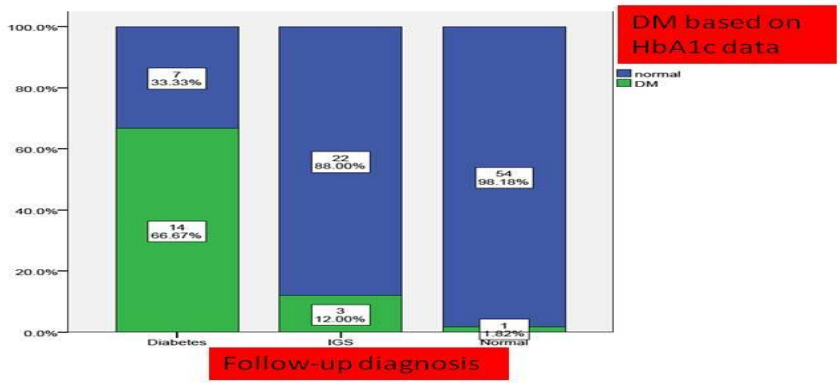


Figure 3-3: Comparison of outcome of WHO 1998(x-axis) and IEC diagnostic criteria (y-axis) at 3 months

Looking at the data in another way at least 7(8.4%) participants classified as normal on IEC criteria had T2DM on the OGTT while 22(26.5%) had IGS and 54(65.1%) NGT. On the contrary only 1(5%) participant classified as having T2DM on the A1C criteria had normal OGTT results. The majority 14(78%) had T2DM on the W.H.O criteria, however 3(17%) had IGS. The results for IGS were variable and they were represented in both groups (26.5% of normal and 16.67% of T2DM groups on IEC criteria).

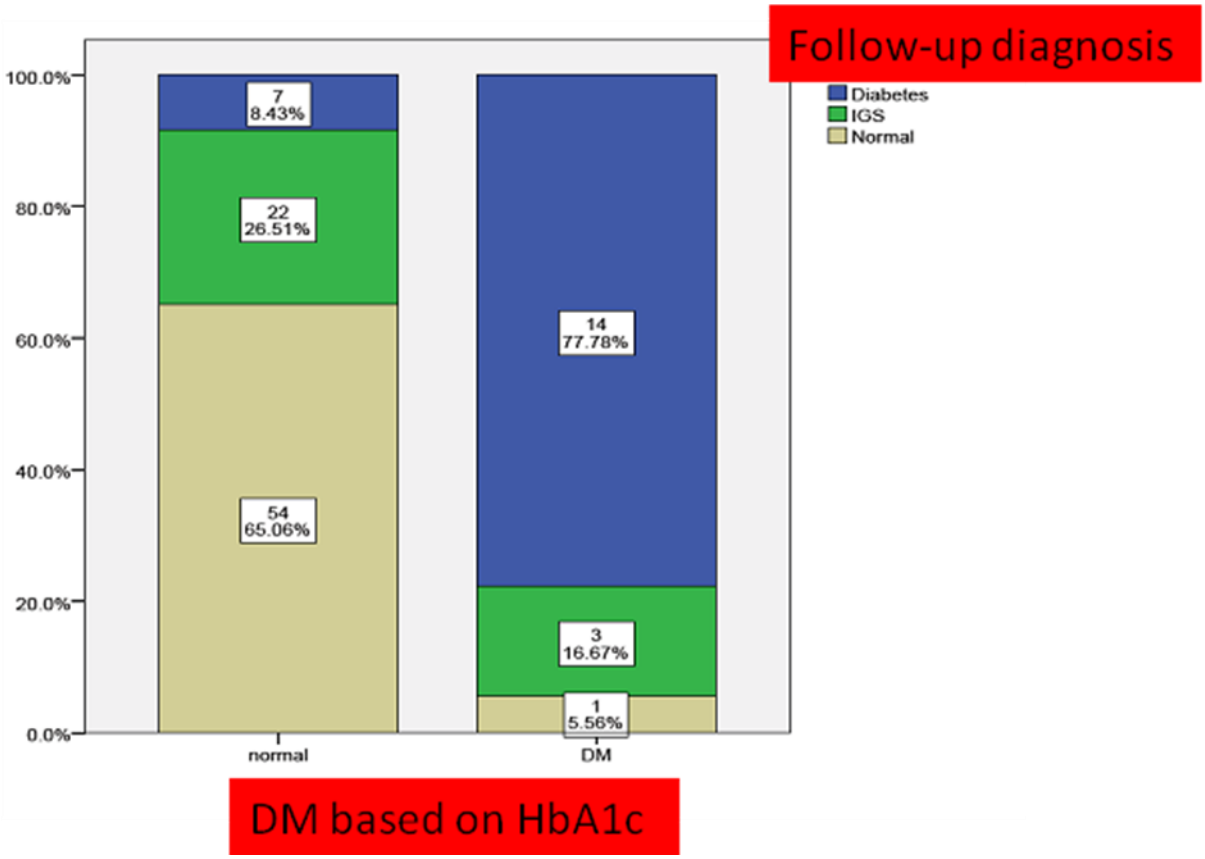


Figure 3-4: Comparison of outcome of WHO 1998 (y-axis) and A1C based (x-axis) diagnostic criteria at 3 months.

The following table illustrates comparison of individual normal, impaired and diabetic groups in the two diagnostic criteria at 3 months. In all three categories it is obvious that we appear to be identifying different cohorts. However the difference appears to be most marked in the pre-diabetes group as less than a third (27%) of participants classified in this group according to ADA criteria were in the same category according to WHO criteria. Indeed over half (63%) of the participants were normal according to WHO criteria.

3-17: Comparison of outcome of ADA and WHO criteria at 3 months

ADA criteria	W.H.O Criteria NGT (%)	W.H.O Criteria IGT (%)	W.H.O Criteria T2DM (%)
Normal 16	12 (75)	4 (25)	0
IGS 67	42 (63)	18 (27)	7 (10)
T2DM 18	1(5)	3 (17)	14 (78)

3.5 Comparison of Screening Algorithm and W.H.O 1998 diagnostic criteria:

I analysed the utility of our screening algorithm in this acute setting. We should specify that this algorithm was originally designed to minimize the number of OGTTs in patients referred to hospital biochemistry department with IFG.

The following figure (3-5) demonstrates a comparison of WHO 1998 diagnostic criteria with our screening algorithm. The left hand column represents the group of participants with T2DM. The algorithm had a sensitivity of 87% in detecting them and missed only 3 (13%) participants with T2DM. The second column represents the group of participants with IGS and by using our screening algorithm we would have referred 24 (65%) for an OGTT while missing the remaining 13(35%). The third column represents the NGT group and we would have only referred 15(26%) while correctly identifying the remaining 42(74%). This has cost saving implications. Currently in our two hospitals the cost of an OGTT including laboratory analysis and glucose drink etc. comes out as around £62.00. On the other hand the cost of testing for HbA1c is only around £9.00 suggesting clear cost savings.

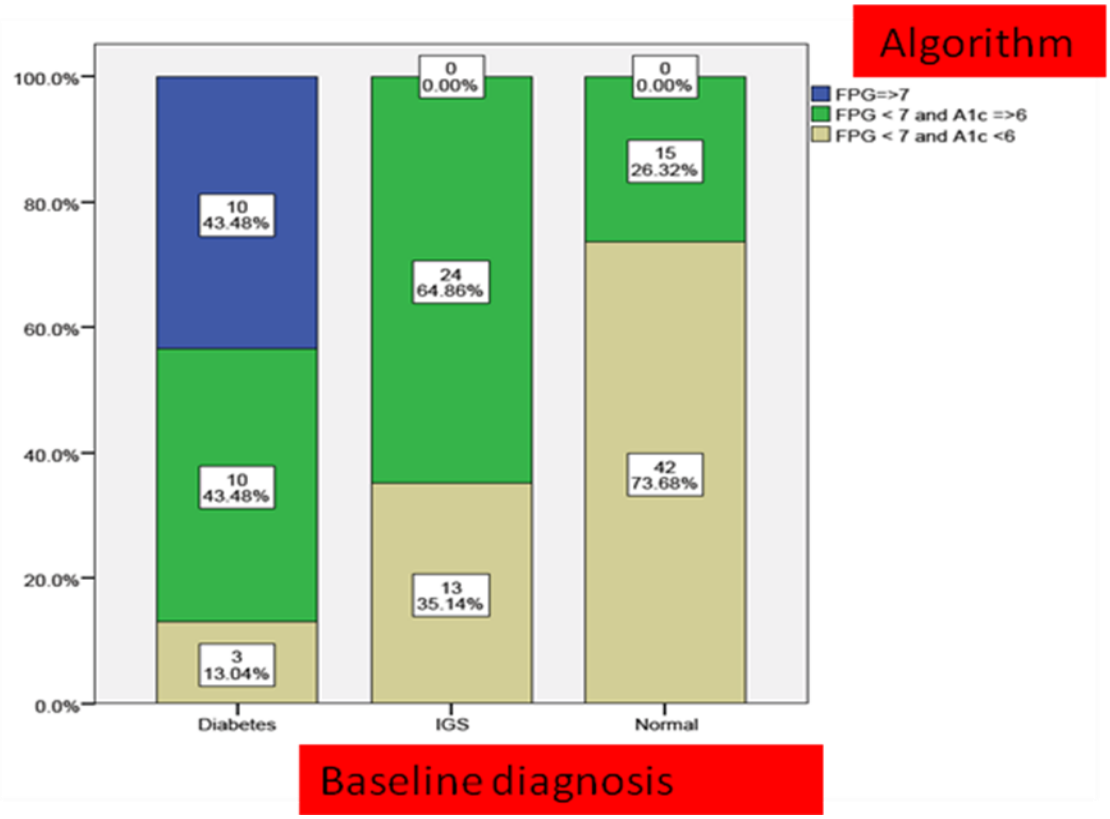


Figure 3-5: Comparison of outcome of WHO 1998 (x-axis) diagnostic criteria with screening algorithm (y-axis) at baseline

Following (Fig 3-6) is another graphical illustration of the same findings. The first column represents group of participants who would have been diagnosed as having DM based on elevated FPG. We would have referred the second group for an OGTT and it has representation of all the three categories i.e. 15(30.6%) NGT, 24(49%) IGS and 10 (20.4%) T2DM. The third column represents patients who would not have been referred for OGTT due to our algorithm and we would have missed 3 participants with DM. The majority of participants in this category belonged to NGT 42(72%) with 13(22%) having IGS. In total we would have reduced the number of OGTTs from 118 to 49 only (58% reduction).

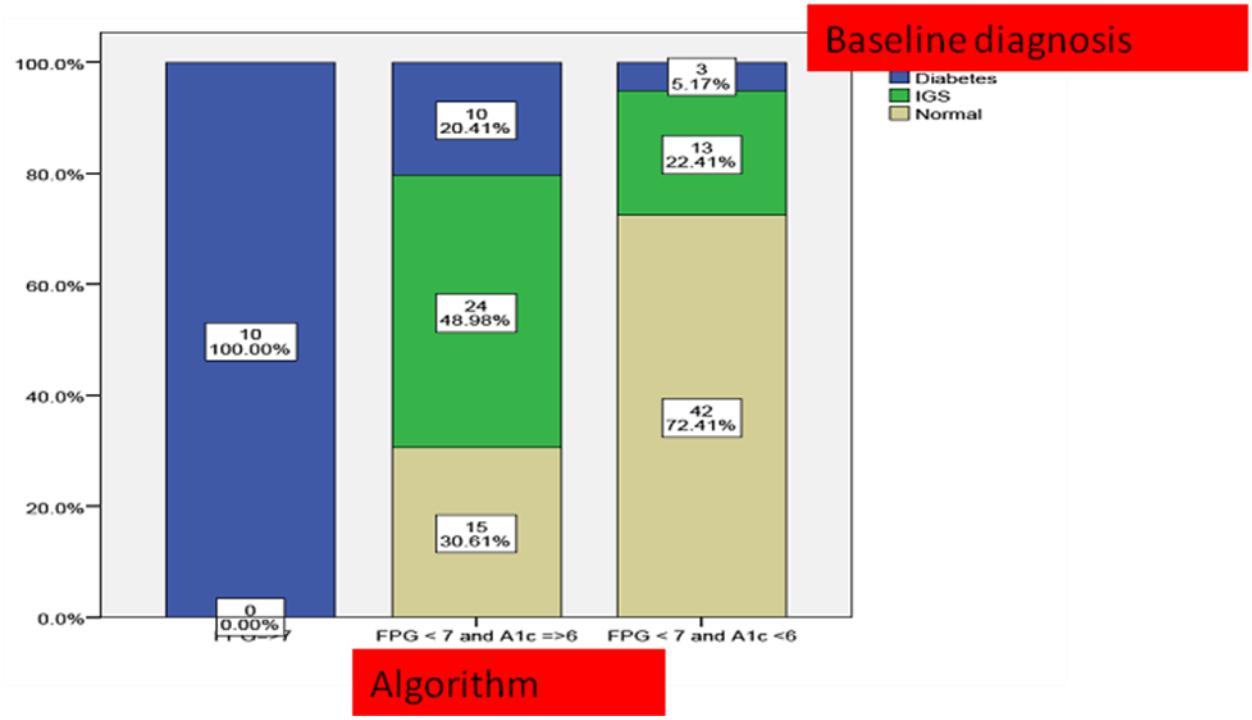
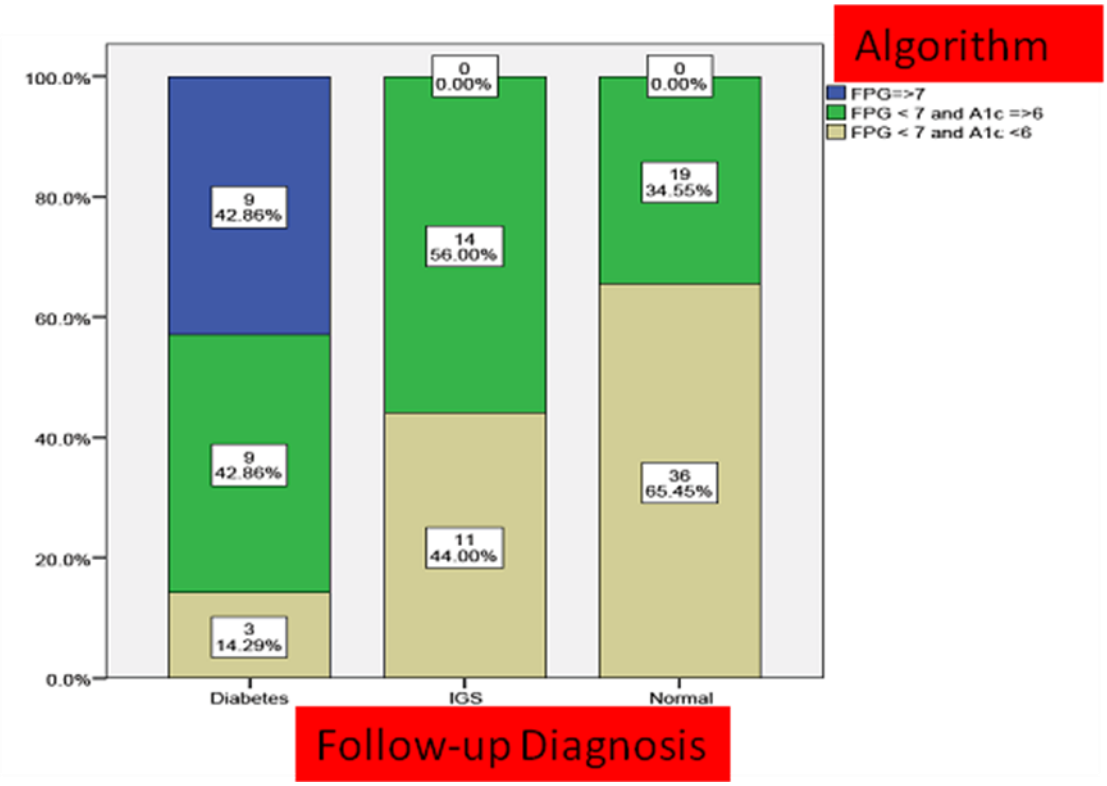


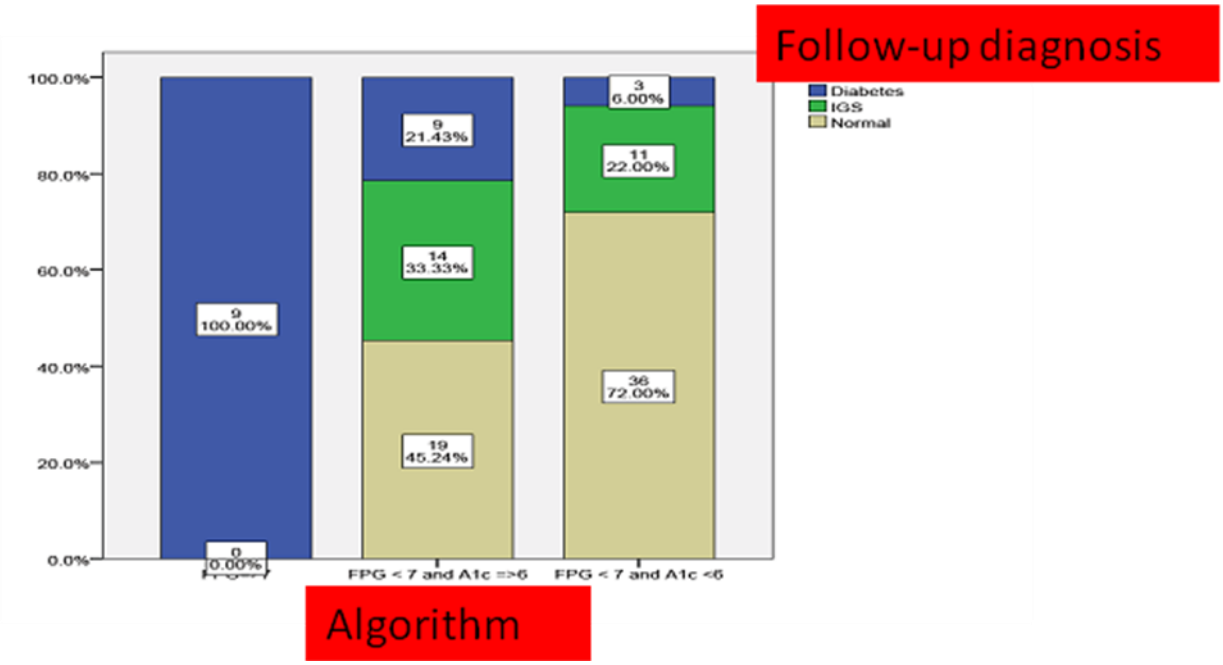
Figure 3-6: Comparison of outcome of WHO 1998 (y-axis) diagnostic criteria and screening algorithm (x-axis) at baseline

One of the primary aims of our project was to look at how our algorithm performed with respect to long term glycaemic classification. Our algorithm would have again led to a reduction in the number of OGTTs. The left hand column represents the group with T2DM and we would have only missed 3 participants (14.3%) with T2DM giving a sensitivity of 85.7%. The second column is the group with IGS and we would have detected 14(56%) of this category while missing the remaining 11(44%). The third column is NGT and we would have correctly identified 36(65.45) of them while referring the remaining 19(34.55%) for an OGTT. If we had used our screening algorithm we would have referred 18 out of 21 patients with DM for an OGTT giving us a sensitivity of 85.7%. On the contrary we would not have referred 36 out of 55 patients with normal OGTT results (65.5%).



3-7: Comparison of outcome of WHO 1998 (x-axis) diagnostic criteria with screening algorithm (y-axis) at 3 months

Following is another graphical illustration of the same findings. The first column represents group of participants who would have been diagnosed as having DM based on elevated FPG. We would have referred the second group for an OGTT and it has representation of all the three categories i.e. 19 (45.2%) NGT, 14(33.3%) IGS and 9(21.4%) T2DM. The third column represents patients who would not have been referred for OGTT due to our algorithm and we would have missed 3 participants with DM. The majority of participants in this category were in the NGT category 36(72%) with 11(22%) having IGS. In total we would have reduced the number of OGTTs from 101 to 42 only (58% reduction).

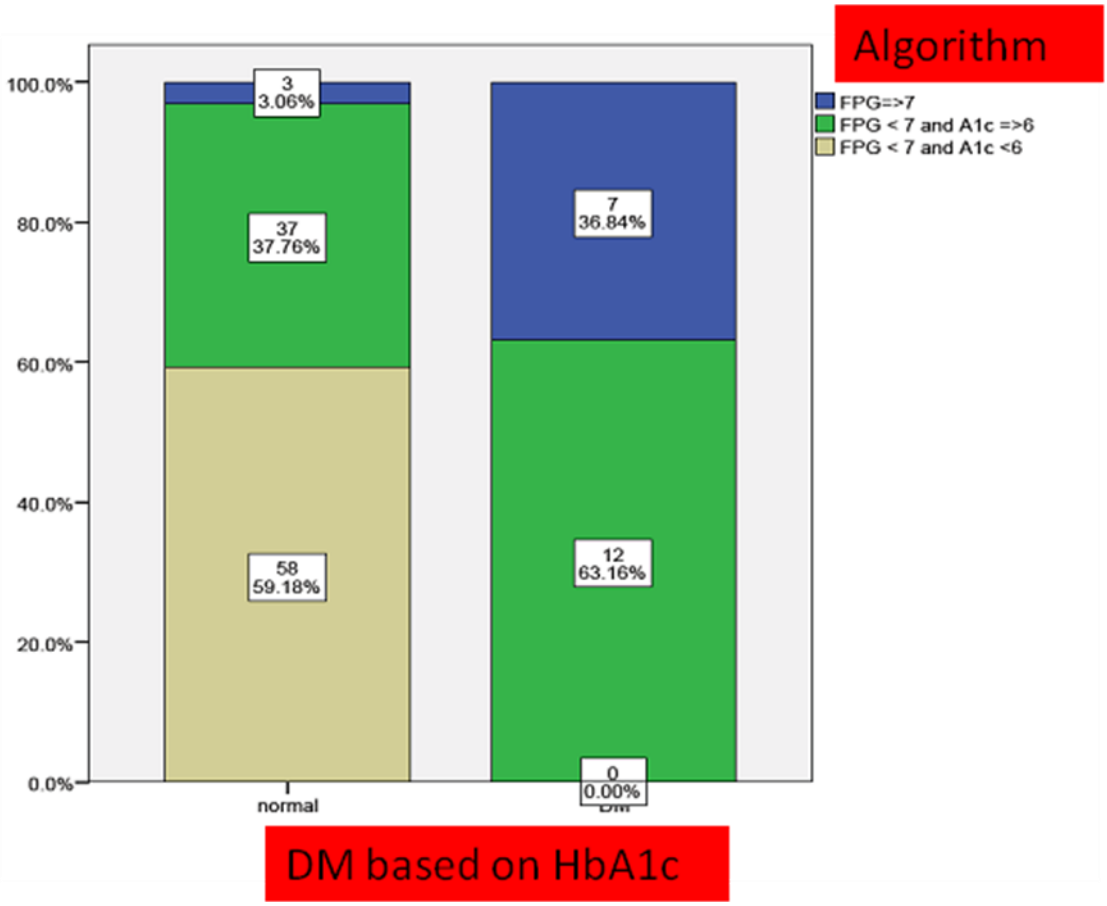


3-8: Comparison of outcome of WHO 1998 (y-axis) diagnostic criteria and screening algorithm (x-axis) at 3 months

3.6 Comparison of Screening Algorithm and IEC diagnostic criteria:

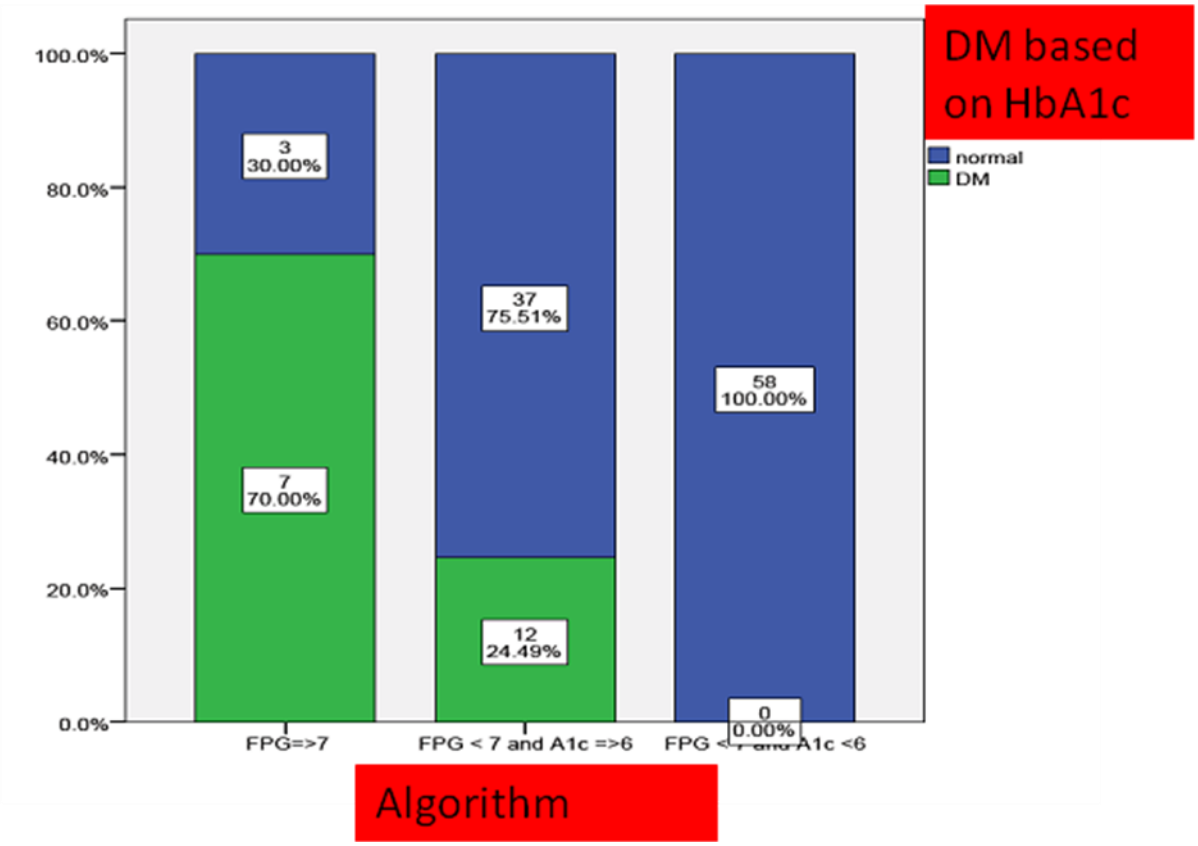
We also decided to look at how our algorithm would perform compared to the newer IEC diagnostic criteria. The two perform quite well paired together and all the participants classified as not requiring OGTT based on our algorithm were normal on the IEC diagnostic criteria giving a specificity of 100%. On the other hand among participants classified as diabetic and not requiring an OGTT according to our screening algorithm, 3 (13.6%) were normal according to ADA criteria.

Following is an illustration of similar data in the form of two graphs. In the first graph the left hand column represents those who would be classified as normal based on IEC criteria. As illustrated 3 out of those 98 (3%) would have been classed as T2DM based on our screening algorithm (FPG=>7 mmol/l). The right hand column represents 19 participants who were classified as T2DM based on IEC criteria and none of them was missed by our algorithm. 7(37%) would have been detected by an elevated FPG and remaining 12(63%) on OGTT results.



3-9: Comparison of outcome of HbA1c based diagnostic criteria (x-axis) and screening algorithm (y-axis)

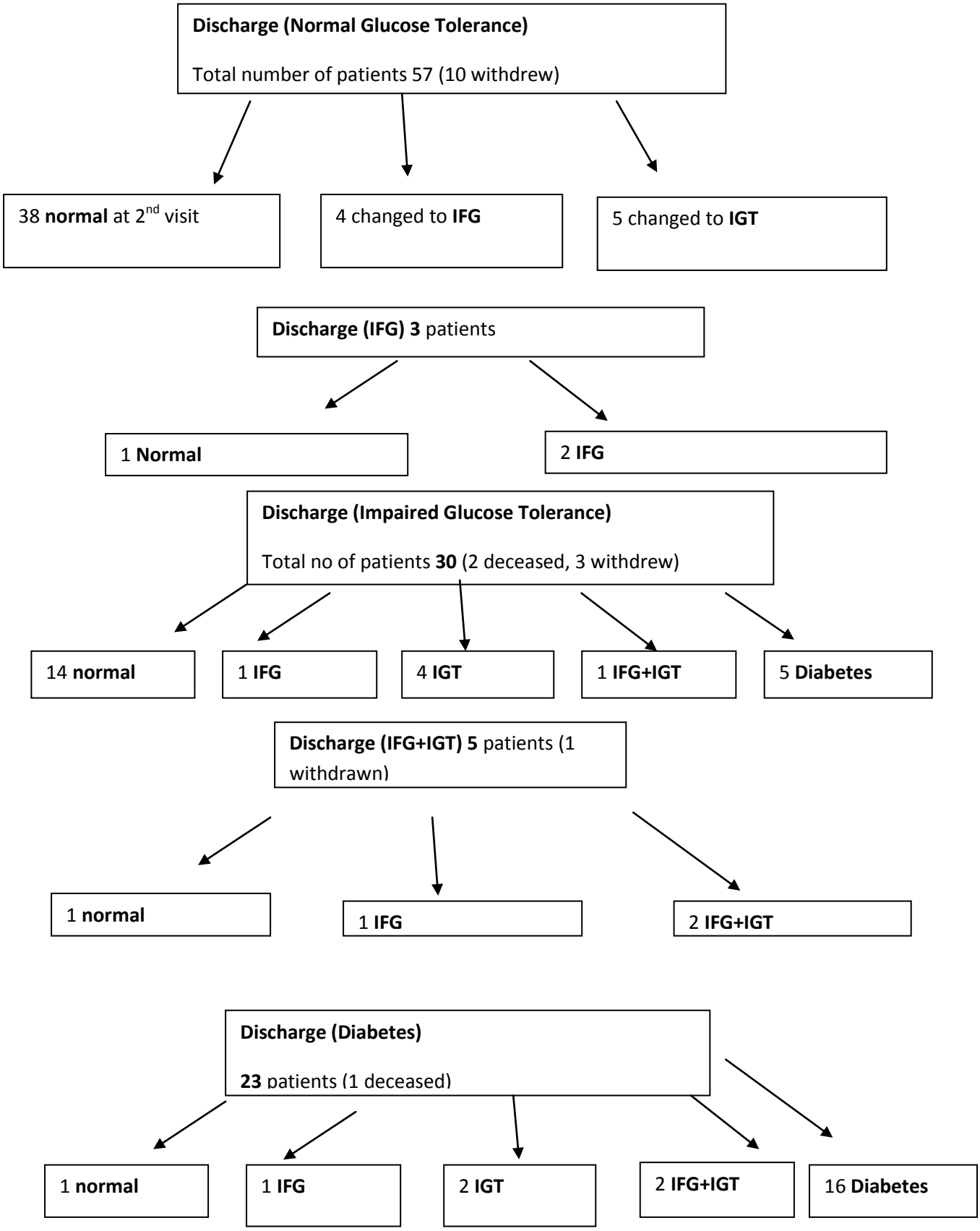
Following is the other graphical illustration of the same findings. The first column represents group of participants who would have been diagnosed as having T2DM based on elevated FPG. 3 out of the 10 (30%) participants in this category would have been missed on IEC criteria. We would have referred the second group for an OGTT and it has representation of both categories i.e. 37(75.5%) NGT and 12 (24.5%) T2DM. The third column represents patients who would not have been referred for OGTT due to our algorithm and we would have missed no participants with T2DM. In total as described earlier we would have reduced the number of OGTTs from 118 to 49 only (58% reduction).



3-10: Comparison of outcome of HbA1c based diagnostic criteria (y-axis) and screening algorithm (x-axis)

3.7 Glycaemic changes from baseline to follow-up:

One of the major concerns regarding OGTT has also been its poor reproducibility because of the high co-efficient of variation of the 2 hour value. It has also been suggested that the timing of the GTT following acute coronary event may be important due to possible effects of stress hyperglycaemia. However more recent publications have suggested concordance between the GTT results at admission and at 3 and 12 months (81). We also decided to look at the possible movements between the various groups in our cohort.



3-11: Glycaemic changes from baseline to 3 months in the individual groups based on WHO 1998 diagnostic criteria.

Analysing the data it was clear that 62 (61%) remained similar at 3 months. However in 39 participants (39%) the diagnosis was altered at 3 months with the vast majority of participants having IGT at baseline changing at 3 months. This could be possibly explained by stress hyperglycaemia. In the NGT category 38 (81%) remained similar while the remaining 9(19%) changed to IGS at 3 months. In the group with T2DM 16(73%) remained in the same category while 5(23%) changed to IGT and only 1(4%) to NGT. In the third category with IGS only 11(34%) remained similar while 16(50%) changed to NGT and 5(16%) changed to T2DM.

3.8 Urinary cortisol creatinine ratios:

In order to detect glucocorticoid response to stress I decided to look at estimating spot urinary cortisol creatinine ratio at baseline and then repeated it at 3 months. I postulated that that it will be positively related to the underlying diagnosis at baseline but not follow-up. Following is a basic illustration of the means and standard errors (S.E) at baseline and follow-up.

3-18: Baseline ratio and baseline diagnosis

Baseline Diagnosis	Number	Mean ratio	S.E
Diabetes	21	14.22	2.2
IGS	35	8.29	0.78
Normal	51	9.03	0.75
Total	107	9.8	0.64

P =0.016

3-19: Follow-up ratio and baseline diagnosis

Baseline Diagnosis	Number	Mean ratio	S.E
Diabetes	14	11.49	2.49
IGS	28	8.77	1.52
Normal	37	11.41	1.37
Total	79	10.49	0.95

P=0.41

Post-hoc analysis suggested the urinary cortisol creatinine ratio at baseline was associated with the glycaemic status at baseline. Following is a tabulated illustration of the individual relationships showing the baseline ratio being higher in the participants with diabetes compared with both IGS and normal cohorts. (p=0.003 and 0.005 respectively).

3-20: Post hoc analysis for baseline ratio and baseline diagnosis

Dependent Variable	Mean Difference of ratio	Trend
Diabetes vs. IGS	5.93	0.003
Diabetes vs. Normal	5.19	0.005
IGS vs. Normal	-0.74	0.933

3-21: Post hoc analysis for follow-up ratio and baseline diagnosis

Dependent Variable	Mean Difference of ratio	Trend
Diabetes vs. IGS	2.71	0.68
Diabetes vs. Normal	0.075	1
IGS vs. Normal	-2.64	0.51

I then went on to look at a possible link between spot urinary cortisol creatinine ratios and follow-up glycaemic classification. Following is a basic illustration of the means and standard errors (S.E) for urinary cortisol creatinine ratios at baseline and follow-up between the individual groups.

3-22: Baseline ratio and follow-up diagnosis

Follow-up Diagnosis	Number	Mean ratio	S.E
Diabetes	18	12.21	2.11
IGS	24	10.68	1.69
Normal	51	8.54	0.73
Total	93	9.80	0.73

P=0.12

3-23: Follow-up ratio and follow-up diagnosis

Follow-up Diagnosis	Number	Mean ratio	S.E
Diabetes	12	8.8	1.79
IGS	20	10.54	1.86
Normal	46	10.99	1.34
Total	78	10.54	0.96

P=0.74

Post-hoc analysis did not show a possible association between the ratios and follow-up glycaemic stratification. Following is a tabulated illustration of the relationships between the individual groups suggesting no relationship between the ratios and the follow-up diagnosis.

3-24: Post hoc analysis for baseline ratio and follow-up diagnosis

Dependent Variable	Mean Difference of ratio	Trend
Diabetes vs. IGS	1.53	0.86
Diabetes vs. Normal	3.67	0.14
IGS vs. Normal	2.14	0.50

3-25: Post hoc analysis for follow-up ratio and follow-up diagnosis

Dependent Variable	Mean Difference of ratio	Trend
Diabetes vs. IGS	-1.69	.93
Diabetes vs. Normal	-2.13	.80
IGS vs. Normal	-.44	.1

To summarise I noted a clear association between spot urinary cortisol creatinine ratio at baseline and the baseline diagnosis.

Discussion:

I aimed to recruit participants with a wide range of age, sex, ethnic and socioeconomic status. However I had a clear majority of white Caucasian males in our study. My work is not unique in this regard. Indeed other studies have also demonstrated a clear bias towards male recruitment. Study from Holland by de Mulder et al had 81% men with 98% being white Caucasian (93). Possible reasons for this obvious bias could be the well documented increased risk of cardiovascular disease in men compared to women.

One of the major reasons for the lack of recruitment of the ethnic minority groups was that a large proportion of them admitted with ACS were already known to have T2DM and therefore automatically excluded. In addition other studies have also documented that some of these groups can be difficult to reach for research studies. I also noted this despite the ability of members of the research team to speak a variety of locally relevant languages. The major risk factor in our study for cardiovascular disease appeared to be smoking with at least two thirds of the participants being current or ex-smokers. Other studies (93) have also suggested smoking as the commonest risk factor although the frequency was 38% compared to our study (67%).

I also noted that in comparison with previous studies (81, 93) the prevalence of diabetes was relatively lower in our cohort. Other studies have suggested the prevalence being around 33 %(81) or 35 %(93) in patients with ACS. This may be due to several factors. Firstly because of the nature of our population, a large number of participants being admitted to hospital with ACS already had T2DM and were therefore automatically excluded. Indeed a previous audit conducted a couple of months before starting our project had identified at least 50 patients with DM being admitted every month with an ACS. Secondly I also included patients who were admitted with non-ST segment elevation myocardial infarction while previous studies have mainly focused on patients with ST segment elevation myocardial infarction who are at higher risk of diabetes. If I compare my study with de Mulder et al they recruited two patient groups i.e. ST segment elevation myocardial infarction or troponin positive chest pain (93). In contrast in my study I also included participants with chest pain and dynamic ECG changes. This may be a lower risk group accounting for a lower prevalence of T2DM.

As expected, age, BMI and serum fructosamine appeared to have a close relationship with glycaemic status with all of them being higher in the groups with T2DM compared to NGT or IGS at baseline as well as at follow-up. Previous studies have also identified age as being a factor associated with dysglycaemia with de Mulder et

al reporting mean age of 71 in the T2DM group compared to 56 and 63 in the NGT and IGS groups (93). However they did not find any association with BMI and most studies have not reported fructosamine levels. I examined this relationship with age in more detail to construct my own diagnostic algorithm in comparison with the WHO 1998 diagnostic criteria and this is discussed in more detail in section 5. The only other parameter which appeared to be associated with glycaemic status was vitamin D status which was higher in the NGT compared to IGS levels. Whilst vitamin D levels can be affected by seasonal testing etc. which was not taken into account in our study this finding is of some interest since vitamin D has been postulated as having anti-oxidant effects as discussed in the introduction.

One of my main aims was to compare the two currently commonly used diagnostic criteria for glucose intolerance. The WHO 1998 and IEC diagnostic criteria gave a different prevalence of diabetes in our cohort (20% vs. 16% respectively at baseline or 21% vs.16% at 3 months). As illustrated the two diagnostic criteria appeared to identify different cohorts of people with T2DM. The obvious conclusions are that while there are some similarities in the patient groups mostly using the two criteria we are in fact identifying different cohort of participants. This does raise concerns with regards to long term screening in this very high risk group of patients with cardiovascular risk factors. In addition by implementing the IEC criteria we are potentially missing the group with IGS who are known to be at an increased risk of progression to T2DM as well as increased risk of cardiovascular disease and mortality. Other studies have also demonstrated similar findings with the prevalence of T2DM being only 10% according to IEC criteria in comparison with 35% according to WHO 1998 diagnostic criteria in one such study (93).

ADA also recommends pre-diabetes as being 5.7-6.4%. When I looked at a comparison between the two pre-diabetes groups from WHO and ADA criteria it was obvious that we would be identifying different cohorts with only a third of those according to ADA criteria being pre-diabetic according to WHO criteria.

Our screening algorithm had better sensitivity (87%) in comparison with IEC diagnostic criteria (57%) when using WHO criteria as gold standard at baseline. At 3 months the sensitivity of screening algorithm was 86% in comparison with IEC criteria (67%). Previous studies have illustrated similar disparities with one such study suggesting sensitivity of IEC criteria being only 29% in comparison with WHO diagnostic criteria (93). In comparison with IEC criteria the sensitivity of our screening algorithm was 100%. In addition the algorithm would also lead to around 60% reduction in the number of patients referred for an OGTT. In the current financial climate this has potentially significant cost saving implications. In our own Trust the cost of a single OGTT was £62 for analysis only while the cost of the drink itself was £7.50. More importantly we are still detecting the majority of participants

with T2DM. The main reason for improved performance of the screening algorithm was due to the inclusion of FPG in the design of the algorithm.

Other studies have suggested a better specificity of IEC criteria at 100% compared to our study (94%) at baseline (93). However previous studies conducted in this area have their own limitations. For example one study only conducted OGTTs at hospital discharge and did not follow-up participants therefore possibly having bias due to the effects of stress hyperglycaemia (93). A different study which conducted OGTTs at baseline and then repeated them at 3 and 12 months, however did not compare the two diagnostic criteria (81).My work on the other hand takes these factors into account. I have compared the two criteria and also looked at tackling the issue of stress hyperglycaemia by repeating an OGTT at 3 months.

Earlier I mentioned that glycaemic stratification based on WHO 1998 diagnostic criteria changed from baseline to 3 months. Closer examination reveals most of the patients in the NGT category remained unchanged (81%). While some of the participants in the T2DM category also changed (27%) most remained unchanged (73%) suggesting in these two categories we could rely on glycaemic classification at baseline as a predictor of long term glycaemic status. In comparison most of the participants in the IGS category did change (66%) with some of them becoming NGT (50%) and some also progressing to T2DM (16%). Previous studies (81) had suggested we could rely on glycaemic diagnosis on baseline; however my data suggest that at least in the IGS category a second OGTT is required at around 3 months to predict long term glycaemic diagnosis. This also demonstrates why we cannot rely on findings from other studies like de Mulder et al which only rely on one OGTT at hospital discharge to detect dysglycaemia not accounting for stress hyperglycaemia.

I measured urinary cortisol creatinine ratio as a possible marker of glucocorticoid response to stress. I found a clear association of urinary cortisol creatinine ratio being higher in participants with T2DM at baseline compared to IGS and NGT as well as those with T2DM at follow-up. This raises the possibility of elevated glucocorticoids being part of the reason behind the changes in glycaemic status from baseline to follow-up at 3 months. In the previous section, I demonstrated that 30% of the participants (7 out of 23) diagnosed with diabetes at baseline did not remain diabetic at repeat testing at 3 months. However I did not see a similar association in the group of participants with IGS which was the group in whom the majority of patients had a change in their glycaemic status. One of the possible limitations of my study was the timing of spot urinary collections. For urinary cortisol measurements ideally the collections should be early morning, however in a small minority of cases I struggled to get the samples at the absolutely correct times. In our study we examined the effects of stress by repeating second OGTT at 3 months. It is not

entirely clear how long we should wait before repeating the investigations. Some studies have looked at 3 and 12 months however other people have also recommended 6 weeks. It is also not entirely clear what impact this may have on the long term prognosis of these patients. In our study we had 3 patients who passed away and all of them had either DM or IGS at diagnosis with at least one of them returning to normal at 3 months suggesting the initial changes could have been related to a stress related response. This may also be related to how sick some of the patients were at their original presentation. Briefly we have suggested a novel association between urinary cortisol and glycaemic status in patients admitted to hospital with ACS which has not been established before.

4. RELATIONSHIP OF NOVEL BIOMARKERS WITH GLYCAEMIC MEASURES

4.1 Association of novel biomarkers with individual glycaemic measurements:

In addition to the other biomarkers already described, I also looked for an association between novel biomarkers and some of the glycaemic measures used in our study.

These markers included c-peptide, glucagon, interleukin-1RA, adiponectin, leptin, pro-insulin, leptin adiponectin ratio, tissue inhibitors of matrix metalloproteinases 1 and 2 (TIMP 1 and TIMP2) and homeostatic assessment models for insulin sensitivity, insulin resistance and beta cell function. I also looked at 3-nitrotyrosine as a measure of oxidative stress.

The following table illustrates the relationship between these biomarkers and some of the glycaemic measures at baseline

4-1: Association of biomarkers with baseline glycaemic measurements

Biomarkers and associations		Mean Baseline FPG	Mean Baseline 2-hr PG	Mean Baseline HbA1c
C-peptide	Pearson Correlation	.41	.41	.41
	Sig	<0.0001	<0.0001	<0.0001
	Number	81	81	81
Glucagon	Pearson Correlation	.13	.25	.12
	Sig	.2	.02	.3
	Number	80	80	80
3 NT	Pearson Correlation	-.013	-.014	.1
	Sig	.9	.7	.3
	Number	99	99	98
Adiponectin	Pearson Correlation	-.19	-.03	-.05
	Sig	.09	.8	.7
	Number	81	81	81
Leptin	Pearson Correlation	0.14	0.24	0.12
	Sig	.2	.03	.3
	Number	80	80	80
IL 1 RA	Pearson Correlation	0.17	0.29	0.33
	Sig	0.19	.02	.009
	Number	60	60	60
TIMP 1	Pearson Correlation	-.06	0.22	0.03
	Sig	.6	.05	.8
	Number	81	81	81
TIMP 2	Pearson Correlation	-.02	.09	0.12
	Sig	0.8	0.4	0.3
	Number	81	81	81
Pro-insulin	Pearson Correlation	0.39	0.49	0.40
	Sig	<0.0001	<0.0001	<0.0001
	Number	78	78	78
L/A ratio	Pearson Correlation	0.12	0.25	0.11
	Sig	0.29	0.03	0.31
	Number	79	79	79
HOMA I.R	Pearson Correlation	0.44	0.37	0.27
	Sig	<0.0001	0.001	0.017
	Number	80	80	80
HOMA β	Pearson Correlation	-0.398	-0.18	-0.298
	Sig	<0.0001	0.12	0.007
	Number	80	80	80
HOMA I.S	Pearson Correlation	-0.376	-0.321	-0.21
	Sig	0.001	0.002	0.06
	Number	80	80	80

I noted interesting correlations for a number of these biomarkers. Mean c-peptide was noted to be positively associated with mean baseline fasting and 2 hour PG, as well as HbA1c (p=<0.0001).

Mean glucagon, leptin and leptin adiponectin ratio were noted to be positively associated with mean baseline 2 hour PG levels (p=0.02, 0.03 and 0.03 respectively).

Mean Interleukin-1RA was positively associated with mean baseline 2 hour PG as well as HbA1c (p=0.023 and 0.009 respectively).

Mean TIMP 1 was positively related to mean baseline 2 hour PG (p=0.049). I did not see any relationship between TIMP 2, 3 NT or adiponectin and any of the mean glycaemic markers.

Mean intact pro-insulin levels were positively related to mean baseline FPG, 2 hour PG, as well as HbA1c (p<0.0001).

Insulin resistance was noted to be positively associated with all the mean baseline variables FPG, 2 hr PG and HbA1c (p<0.0001, 0.001 and 0.017 respectively).

Insulin sensitivity was negatively associated to the glycaemic markers including mean baseline FPG and 2 hour PG (p=0.001 and 0.002 respectively) On the other hand beta cell function was negatively associated with mean baseline FPG and HbA1c only (p<0.0001 and 0.007 respectively).

I than went on to look at the relationship between these biomarkers and the follow-up glycaemic measures including mean follow-up FPG and 2 hour PG. Following table illustrates the relationship between them in detail.

4-2: Association of biomarkers with follow-up glycaemic measurements

Biomarkers and associations		Mean Follow-up FPG	Mean Follow-up 2-hr PG
C-peptide	Pearson Correlation	0.26	0.25
	Sig	0.04	0.05
	Number	62	61
Glucagon	Pearson Correlation	.13	.33
	Sig	0.3	0.009
	Number	61	60
3 NT	Pearson Correlation	-.01	-.09
	Sig	0.9	0.4
	Number	79	78
Adiponectin	Pearson Correlation	-0.2	-0.2
	Sig	0.1	0.1
	Number	62	61
Leptin	Pearson Correlation	0.17	0.31
	Sig	0.2	0.02
	Number	61	60
IL 1 RA	Pearson Correlation	0.32	0.17
	Sig	0.03	0.23
	Number	44	43
TIMP 1	Pearson Correlation	0.16	0.13
	Sig	0.2	0.3
	Number	62	61
TIMP 2	Pearson Correlation	0.11	0.19
	Sig	0.38	0.14
	Number	81	81
Pro-insulin	Pearson Correlation	0.14	0.35
	Sig	0.3	0.006
	Number	61	60
L/A ratio	Pearson Correlation	0.15	0.38
	Sig	0.25	0.003
	Number	60	59
HOMA I.R	Pearson Correlation	0.30	0.28
	Sig	0.01	0.03
	Number	60	59
HOMA β	Pearson Correlation	-0.1	-0.05
	Sig	0.4	0.7
	Number	60	59
HOMA I.S	Pearson Correlation	-0.35	-0.27
	Sig	0.006	0.03
	Number	62	61

Mean c-peptide and HOMA I.R were positively associated with mean follow-up FPG and 2 hour PG (p=0.04 and 0.05 for C peptide and 0.01 and 0.03 for I.R respectively). Similarly HOMA I.S was negatively associated with both variables (p=0.006 and 0.03 for FPG and 2 hour PG).

Mean glucagon, leptin, pro-insulin and leptin adiponectin ratio were positively associated with mean 2 hour PG at follow-up (p=0.009, 0.02, 0.006 and 0.003

respectively). IL 1RA was positively associated with the follow-up FPG only (p=0.03).

I did not observe any association between 3 NT, adiponectin, TIMP 1 and TIMP 2 and any of the glycaemic measures at follow-up. I wanted to look at the baseline associations after adjusting for some of the background variables. As a first step I adjusted for age, body mass index, sex and ethnicity. The following table illustrates these associations after correcting for the above 4 parameters.

4-3: Association of biomarkers with baseline glycaemic measurements after adjusting for age, BMI, sex and ethnicity

Biomarkers and associations	Mean baseline FPG	Mean baseline 2-hr PG	Mean baseline HbA1c
C-peptide Correlation	.32	.34	.34
Sig	0.007	0.005	0.005
Glucagon Correlation	.05	0.19	0.05
Sig	.69	0.12	0.69
3 NT Correlation	-0.04	-0.07	0.09
Sig	0.72	0.49	0.41
Adiponectin Correlation	-0.15	-.04	-.002
Sig	0.23	0.76	0.99
Leptin Correlation	-0.03	0.04	-0.06
Sig	0.82	.76	0.63
IL 1 RA Correlation	0.08	0.17	0.27
Sig	0.56	0.23	0.05
TIMP 1 Correlation	-0.076	0.19	0.006
Sig	0.54	0.11	.96
TIMP 2 Correlation	-0.11	-0.08	0.04
Sig	0.38	0.50	0.73
Pro-insulin Correlation	0.32	0.47	0.35
Sig	0.009	<0.0001	0.005
L/A ratio Correlation	-0.05	0.095	-0.05
Sig	0.65	0.45	0.7
HOMA I.R Correlation	0.38	0.33	0.18
Sig	0.002	0.007	0.14
HOMA β Correlation	-0.51	-0.28	-0.39
Sig	<0.0001	0.02	0.001
HOMA I.S Correlation	-0.304	-0.26	-0.12
Sig	0.01	0.04	0.33

Mean c-peptide, intact pro-insulin levels and beta cell function still remained associated with all the baseline glycaemic measurements i.e. mean baseline FPG, 2 hour PG and HbA1c (p=0.007, 0.005 and 0.005 for C-peptide, p=0.009, < 0.0001 and 0.005 for pro-insulin and <0.0001, 0.02 and 0.001 for beta cell function respectively). Beta cell function had a negative correlation while others were positive.

Insulin resistance and beta cell sensitivity were still associated with the baseline FPG and 2 hour PG (p=0.002 and 0.007 for I.R and p=0.01 and 0.04 for HOMA I.S respectively). Insulin sensitivity had a negative while HOMA I.R had a positive association with the glycaemic measurements. IL 1RA also still remained positively associated with the baseline HbA1c (p=0.05).

The associations for other biomarkers such as glucagon, leptin, leptin adiponectin ratio and TIMP 1 were lost after adjusting for age, BMI sex and ethnicity.

I decided to look at these confounders individually and it appeared that BMI was the main confounding variable for most of these biomarkers. Following table illustrates this in detail as it shows the associations after adjusting for age, sex and ethnicity only.

As illustrated in the table, after adjusting for age, sex and ethnicity the associations for glucagon, leptin and leptin adiponectin ratio still remained suggesting BMI as being the main confounder as expected for leptin and adiponectin.

4-4: Association of biomarkers with baseline glycaemic measures after adjusting for age, sex and ethnicity.

Biomarkers and associations		Baseline FPG	Baseline 2-hr PG	Baseline HbA1c
C-peptide	Correlation	.40	.44	.41
	Sig	<0.0001	<0.0001	<0.0001
Glucagon	Correlation	0.1	0.25	0.1
	Sig	.38	0.03	0.38
3 NT	Correlation	-0.04	-0.07	0.08
	Sig	0.72	0.49	0.44
Adiponectin	Correlation	-0.24	-0.17	-0.10
	Sig	0.04	0.14	0.38
Leptin	Correlation	0.16	0.26	0.13
	Sig	0.18	0.02	0.28
IL 1 RA	Correlation	0.19	0.29	0.35
	Sig	0.16	0.03	0.008
TIMP 1	Correlation	-0.07	0.18	0.007
	Sig	0.54	0.11	.95
TIMP 2	Correlation	-0.09	-0.07	0.05
	Sig	0.41	0.56	0.67
Pro-insulin	Correlation	0.38	0.52	0.39
	Sig	0.001	<0.0001	0.001
L/A ratio	Correlation	0.13	0.29	0.13
	Sig	0.28	0.01	0.28
HOMA I.R	Correlation	0.44	0.42	0.27
	Sig	<0.0001	<0.0001	0.02
HOMA β	Correlation	-0.43	-0.19	-0.32
	Sig	<0.0001	0.09	0.005
HOMA I.S	Correlation	-0.37	-0.36	-0.21
	Sig	0.001	0.002	0.07

4.2 Relationship of novel biomarkers with glycaemic classification based on WHO 1998 diagnostic criteria:

I next compared these biomarkers with baseline glycaemic stratification based on WHO 1998 diagnostic criteria as illustrated below.

4-5: Mean levels of biomarkers in the individual groups based on WHO 1998 criteria at baseline

Variables		Number	Mean	S.E
C-peptide	DM	18	3654.2	602
	IGS	20	2870.4	209
	Normal	43	2501.5	206
Glucagon	DM	18	82.9	14.8
	IGS	20	75.4	5.9
	Normal	42	59.6	3.6
3 NT	DM	18	28.9	4.5
	IGS	34	28.7	2.3
	Normal	47	27.7	2.2
Adiponectin	DM	18	3.9	0.6
	IGS	20	4.1	0.8
	Normal	43	3.6	0.26
Leptin	DM	18	18.7	3.4
	IGS	19	19.5	4.4
	Normal	43	11.1	1.2
IL-1RA	DM	12	0.35	.06
	IGS	17	0.36	.05
	Normal	31	0.26	.03
TIMP-1	DM	18	112.7	12
	IGS	20	94.5	6
	Normal	43	92.8	5
TIMP-2	DM	18	76.6	3.3
	IGS	20	75.8	1.7
	Normal	43	71.7	1.9
Pro-insulin	DM	18	15.7	3.7
	IGS	19	8.3	1.6
	Normal	41	6.6	.8
Lep/Ad ratio	DM	18	6.2	1.2
	IGS	19	8	2.0
	Normal	42	3.6	.4
I.R	DM	17	2.5	.27
	IGS	20	2.2	.16
	Normal	43	1.8	.15
B-fun	DM	17	104.6	8.4
	IGS	20	130.8	8.6
	Normal	43	130.0	7
I.S	DM	17	50.5	6.9
	IGS	20	51.9	4.5
	Normal	43	69.9	6

4-6: Strength of association between biomarkers and baseline diagnosis

BIOMARKERS	SIGNIFICANCE OF TREND (p values)
C-peptide	0.04
Glucagon	0.05
3 NT	0.94
Adiponectin	0.77
Leptin	0.04
IL 1 RA	0.13
TIMP 1	0.32
TIMP 2	0.35
Pro-insulin	0.02
Leptin adiponectin ratio	0.03
HOMA IR	0.04
HOMA β	0.09
HOMA IS	0.05

I noted that mean c-peptide, glucagon, intact pro-insulin and HOMA IR were higher in the diabetic group compared to normal and IGS groups. (p=0.04, 0.05, 0.02 and 0.04 respectively)I also noted a trend for leptin and leptin adiponectin ratio to be higher in both diabetic and IGS groups compared to normal groups (p=0.04 and 0.03 respectively).The only other biomarker which showed a clear association was HOMA IS which was noted to be lower in the diabetic and IGS groups compared to normal cohort (p=0.05).

I than went on to compare these biomarkers with follow-up glycaemic stratification based on W.H.O 1998 diagnostic criteria. Following 2 tables illustrate the mean values along with standard error and the significance of the association.

4-7: Mean levels of biomarkers in the individual groups based on WHO 1998 diagnostic criteria at 3 months

Variables		Number	Mean	S.E
C-peptide	DM	16	2548.8	637.2
	IGS	14	1204.3	321.8
	Normal	38	1402.3	227.5
Glucagon	DM	16	73.9	8.1
	IGS	14	87.9	18.3
	Normal	37	58.7	3.7
3 NT	DM	15	29.5	4.7
	IGS	22	26.1	3.0
	Normal	47	29.1	2.4
Adiponectin	DM	16	3.3	0.6
	IGS	14	3.4	0.4
	Normal	38	3.9	0.37
Leptin	DM	16	18.9	3.5
	IGS	14	15.2	3.0
	Normal	37	13.5	1.9
IL-1RA	DM	12	0.41	.06
	IGS	9	0.27	.05
	Normal	28	0.27	.03
TIMP-1	DM	16	105.5	8.2
	IGS	14	98.4	10.6
	Normal	38	94.8	6.1
TIMP-2	DM	16	70.8	2.9
	IGS	14	73.8	1.8
	Normal	38	70.7	1.7
Pro-insulin	DM	16	15.5	3.6
	IGS	14	8.3	1.9
	Normal	36	7.0	1.1
Lep/Ad ratio	DM	16	6.5	1.2
	IGS	14	5.5	1.1
	Normal	36	4.5	.8
I.R	DM	15	2.6	.22
	IGS	14	1.96	.25
	Normal	38	1.86	.17
HOMA β	DM	15	108.9	10.5
	IGS	14	118.6	8.9
	Normal	38	131.5	7.9
HOMA I.S	DM	15	46.1	5.7
	IGS	14	58.9	5.7
	Normal	38	69.4	5.9

4-8: Strength of association between biomarkers and baseline diagnosis

BIOMARKERS	SIGNIFICANCE OF ASSOCIATION(p values)
C-peptide	0.05
Glucagon	0.11
3 NT	0.75
Adiponectin	0.54
Leptin	0.33
IL 1 RA	0.05
TIMP 1	0.63
TIMP 2	0.01
Pro-insulin	0.02
Leptin adiponectin ratio	0.37
HOMA IR	0.07
HOMA β	0.23
HOMA IS	0.05

I noted that mean c-peptide, IL 1 RA, TIMP 2 and intact pro-insulin were higher in the diabetic group compared to normal and IGS groups. (p=0.05, 0.05, 0.01 and 0.02 respectively). The only other trend noticed was for HOMA IS to be lower among diabetics (p=0.05)

4.3 Comparison of individual NGT, IGS and T2DM Groups:

I decided to compare the three individual groups with respect to the movements within these groups. The first group we compared was the DM group. We noted higher adiponectin, HOMA I.S and TIMP 1 levels among NGT and IGS compared to DM group in this cohort at 3 months.

4-9: Mean levels of biomarkers in the DM group based on WHO 1998 diagnostic criteria at 3 months

Variables		Number	Mean	S.E
C-peptide	DM	12	3920.7	836.1
	IGS	4	3184.2	881.1
	Normal	1	1139.9	
Glucagon	DM	12	72.1	8.8
	IGS	4	127	62.1
	Normal	1	48.5	
3 NT	DM	12	31.2	5.8
	IGS	4	23.9	11.5
	Normal	1	23.3	
Adiponectin	DM	12	3.7	0.5
	IGS	4	2.5	0.4
	Normal	1	12.7	
Leptin	DM	12	20.3	4.5
	IGS	4	20.1	6.5
	Normal	1	2.2	
TIMP-1	DM	12	105.7	10.6
	IGS	4	111.5	27.4
	Normal	1	244.6	
TIMP-2	DM	12	79.9	3.8
	IGS	4	67.2	4.6
	Normal	1	93.8	
Pro-insulin	DM	12	16.9	4.7
	IGS	4	15.4	6.7
	Normal	1	3.2	
Lep/Ad ratio	DM	12	6.1	1.5
	IGS	4	8.5	2.5
	Normal	1	0.17	
I.R	DM	11	2.6	0.3
	IGS	4	2.3	0.7
	Normal	1	0.8	
HOMA β	DM	11	95.8	9.4
	IGS	4	122.2	22.2
	Normal	1	113.2	
HOMA I.S	DM	11	45.5	6.9
	IGS	4	51.7	9.9
	Normal	1	126	
Age	DM	16	67.7	3.2
	IGS	5	61.8	5.7
	Normal	1	76	
BMI	DM	15	31.6	1.5
	IGS	5	30.4	4.4
	Normal	1	17	
FPG	DM	16	7.5	0.55
	IGS	5	5.8	0.2
	Normal	1	4.3	
2hr PG	DM	16	15.3	1.1
	IGS	5	12.7	0.3
	Normal	1	12	
HbA1c	DM	16	7.5	0.4
	IGS	5	6.1	0.2
	Normal	1	6.2	
Fructosamine	DM	11	239.7	17.1
	IGS	4	218.2	8.6
	Normal	1	246	
Systolic B.P	DM	15	129.1	4.9
	IGS	5	133.6	11.5
	Normal	1	103	
Diastolic B.P	DM	15	77.5	2.6
	IGS	5	72.6	6.9
	Normal	1	78	

4-10: Strength of association between biomarkers and baseline diagnosis

BIOMARKERS	SIGNIFICANCE (p values)
C-peptide	0.59
Glucagon	0.31
3 NT	0.80
Adiponectin	<0.001
Leptin	0.51
IL 1 RA	0.13
TIMP 1	0.02
TIMP 2	0.13
Pro-insulin	0.75
Leptin adiponectin ratio	0.37
HOMA IR	0.27
HOMA β	0.46
HOMA IS	0.01
Age	0.52
BMI	0.15
FPG	0.12
2 hr PG	0.34
HbA1c	0.14
Fructosamine	0.75
Systolic B.P	0.42
Diastolic B.P	0.7

I than went on to compare the IGS group and looked at the three groups within this group at 3 months. TIMP 2 levels were lower in the NGT group compared to IGS and DM groups. There was also a trend towards higher FPG and systolic B.P in the IGS group compared to NGT and DM groups. Following is a tabulated illustration of this cohort

4-11: Mean levels of biomarkers in the IGS group based on WHO 1998 diagnostic criteria at 3 months

Variables			Number		Mean		S.E	
C-peptide	DM	IGS	4	5	3190.3	2702.3	544.5	583.5
	Normal		8		2769.1		296.1	
Glucagon	DM	IGS	4	5	79.5	78.4	21	10.4
	Normal		8		71.9		9.5	
3 NT	DM	IGS	3	10	23	27	0.99	4.4
	Normal		15		30.9		4	
Adiponectin	DM	IGS	4	5	1.97	4.9	0.29	1.5
	Normal		8		3.2		0.7	
Leptin	DM	IGS	4	5	15	14.6	4.1	5.3
	Normal		7		22.2		7.7	
TIMP-1	DM	IGS	4	5	104.9		10.4	15.3
	Normal		8		89.9	91.1	11	
TIMP-2	DM	IGS	4	5	83.6	82.5	2	4.8
	Normal		8		61.6		2.1	
Pro-insulin	DM	IGS	4	5	11.3	6	3.3	1.8
	Normal		7		8.9		3.9	
Lep/Ad ratio	DM	IGS	4	5	7.4	4	1.4	1.36
	Normal		7		9.3		3.2	
I.R	DM	IGS	4	5	2.4	2.1	0.42	
	Normal		8		2.1		0.47	0.24
HOMA β	DM	IGS	4	5	145	107	22.6	11.7
	Normal		8		137		14.2	
HOMA I.S	DM	IGS	4	5	47.6	59.8	11.7	14.4
	Normal		8		51.7		4.9	
Age	DM	IGS	5	11	59.6		4.5	2.5
	Normal		16		64.3	60.7	2.1	
BMI	DM	IGS	5	10	32	28.8	2	0.9
	Normal		15		29		1.2	
FPG	DM	IGS	5	11	5.36	5.85	0.18	0.18
	Normal		16		5.34		0.14	
2hr PG	DM	IGS	5	11	9.6	8.3	0.5	0.3
	Normal		16		8.8		0.3	
HbA1c	DM	IGS	5	11	6.4	6.0	0.3	0.1
	Normal		16		6.0		0.06	
Fructosamine	DM	IGS	5	8	213.8	214.1	10.6	7.9
	Normal		9		200.3		5.1	
Systolic B.P	DM	IGS	5	11	114	135	6.9	6.8
	Normal		16		119		4.3	
Diastolic B.P	DM	IGS	5	11	74.6		4	4.8
	Normal		16		75.3	74.2	3.5	

4-12: Strength of associations

BIOMARKERS	SIGNIFICANCE (p values)
C-peptide	0.75
Glucagon	0.89
3 NT	0.62
Adiponectin	0.18
Leptin	0.66
IL 1 RA	0.53
TIMP 1	0.71
TIMP 2	<0.001
Pro-insulin	0.63
Leptin adiponectin ratio	0.36
HOMA IR	0.78
HOMA β	0.29
HOMA IS	0.71
Age	0.48
BMI	0.34
FPG	0.06
2 hr PG	0.12
HbA1c	0.15
Fructosamine	0.32
Systolic B.P	0.06
Diastolic B.P	0.98

I than went on to look at the NGT group and compared the cohorts which remained normal with those that developed IGS. The only trend was diastolic B.P being higher in the normal compared to IGS groups. Following is a tabulated illustration of the findings:

4-13: Mean levels of biomarkers in the NGT group based on WHO 1998 diagnostic criteria at 3 months

Variables		Number	Mean	S.E
C-peptide	IGS	5	2149.1	126.9
	Normal	29	2481.7	283.8
Glucagon	IGS	5	65.9	13.1
	Normal	28	55.2	3.9
3 NT	IGS	8	26.1	4
	Normal	31	28.4	3.1
Adiponectin	IGS	5	2.7	0.44
	Normal	29	3.8	0.32
Leptin	IGS	5	11.8	4.6
	Normal	29	11.8	1.4
IL-1RA	IGS	3	0.28	0.08
	Normal	20	0.27	0.03
TIMP-1	IGS	5	96.5	17
	Normal	29	90.7	5.3
TIMP-2	IGS	5	70.6	2.3
	Normal	29	72.4	1.9
Pro-insulin	IGS	5	4.9	1.2
	Normal	28	6.7	1.1
Lep/Ad ratio	IGS	5	4.6	1.6
	Normal	28	3.5	0.44
I.R	IGS	5	1.56	0.08
	Normal	29	1.8	0.2
HOMA β	IGS	5	127.3	13.6
	Normal	29	130.6	9.1
HOMA I.S	IGS	5	63.9	3.3
	Normal	29	72.3	7.3
Age	IGS	9	56.7	3.7
	Normal	38	58.7	1.8
BMI	IGS	9	27.2	1.3
	Normal	35	27.2	0.78
FPG	IGS	9	5.18	0.13
	Normal	38	5.19	0.06
2hr PG	IGS	9	6	0.29
	Normal	38	5.9	0.21
HbA1c	IGS	9	5.8	0.13
	Normal	38	5.76	0.04
Fructosamine	IGS	6	208	6.2
	Normal	30	207.2	3.1
Systolic B.P	IGS	9	122	5.2
	Normal	37	127	3.3
Diastolic B.P	IGS	9	68	2.6
	Normal	37	75.5	1.9

4-14: Strength of association between biomarkers and baseline diagnosis

BIOMARKERS	**SIGNIFICANCE (p values)**
C-peptide	0.63
Glucagon	0.32
3 NT	0.73
Adiponectin	0.17
Leptin	0.98
IL 1 RA	0.95
TIMP 1	0.69
TIMP 2	0.69
Pro-insulin	0.51
Leptin adiponectin ratio	0.37
HOMA IR	0.59
HOMA **β**	0.89
HOMA IS	0.64
Age	0.63
BMI	0.98
FPG	0.97
2 hr PG	0.89
HbA1c	0.73
Fructosamine	0.92
Systolic B.P	0.53
Diastolic B.P	0.07

Discussion:

I examined the relationship between individual glycaemic measures and novel bio-markers. At baseline I found a clear positive association between fasting plasma glucose and c-peptide, pro-insulin, HOMA I.R and HOMA β. In contrast HOMA I.S was negatively associated with baseline FPG. The post-load glucose concentrations were positively associated with the vast majority of variables including C-peptide, pro-insulin, glucagon, leptin, leptin adiponectin ratio, HOMA I.R, TIMP-1 and IL-Ira. HOMA I.S was negatively associated with post-load glucose. The HbA1c on the other hand was positively associated with C-peptide, pro-insulin, HOMA I.R, HOMA β and IL-Ira. These findings suggest the role some of these variables can play in predicting glycaemic abnormalities in patients with ACS.

The 2 hour plasma glucose was the most consistently associated variable with the pancreatic and insulin related biomarkers. Part of this could be related to stress hyperglycaemia. However apart from an association of glucagon levels with urinary cortisol creatinine concentrations at baseline I was unable to demonstrate any other associations between these markers. Earlier in the first chapter I have mentioned studies have suggested a clear association between elevated post-load glucose concentrations and cardiovascular mortality.

The HOMA IR was used as a marker of insulin resistance as other measures such as euglycaemic hyperinsulinaemic clamp study are too expensive and technically demanding to conduct (182). Some studies have also demonstrated association of c-peptide and HOMA-IR with all cause and cardiovascular mortality. These studies have looked at them independent of other factors such as BMI and increased waist hip ratio and still found that all-cause mortality is independently predicted (181, 183).

One of the novel aspects of my work is that I also studied these relationships at 3 months after the initial cardiovascular event in contrast with other studies which only looked at measuring glycaemic status at baseline (93). At 3 months I noted the FPG was positively associated with c-peptide and IL-IRA and negatively associated with HOMA I.S. On the other hand 2 hour plasma glucose was associated with C-peptide, pro-insulin, HOMA I.R, leptin, leptin adiponectin ratio and glucagon positively and with HOMA I.S negatively. The association of glucagon, leptin and leptin adiponectin ratio appears to be driven by the BMI as demonstrated by logistic regression analysis.

I also examined the relationship between the pancreatic biomarkers and glycaemic stratification according to WHO diagnostic criteria. C-peptide, pro-insulin and HOMA I.S were the three biomarkers related to both baseline and follow-up diagnosis with c-peptide and pro-insulin being higher and HOMA I.S lower among those with T2DM. This suggests insulin resistance and sensitivity being the main parameters associated with baseline and follow-up glycaemic status. By contrast leptin, glucagon, leptin adiponectin ratio and HOMA I.R were associated with baseline diagnosis only. IL-1RA and TIMP-2 were associated with follow-up diagnosis only.

These findings suggest we can in future potentially look at utilizing some of the markers of insulin production and sensitivity as helpful in predicting future glycaemic status. I will explore this in more detail in the next section where we tried to utilize some of these biomarkers in designing our novel formula i.e. Diabetes Predictor Score.

In the previous section I demonstrated that there were movements between individual groups according to WHO 1998 diagnostic criteria from baseline to follow-up at 3 months. Although the importance of treating elevated plasma glucose during admissions with cardiovascular disease is accepted, it is also important to determine long term glycaemic status. I analysed the three groups individually to check if any measurements could help predict long term glycaemic status.

Previous studies of assessing dysglycaemia in ACS demonstrated that those with T2DM who remain diabetic long term have higher HbA1c, triglycerides and HOMA I.R and lower IGI (81). In my study I noted that in this group patients who remained diabetic at 3 months had lower adiponectin and TIMP 1 and lower HOMA I.S. This would suggest that performing a complete metabolic profile assessment can provide useful information in predicting long term glycaemic status.

In subjects with IGS, I noted TIMP 2 levels were lower in those who became NGT at 3 months. There was also a trend towards higher FPG and systolic B.P in the IGS group compared to NGT and DM groups. In the NGT group there were no statistically significant differences between the NGT and IGS groups.

We have to accept limitations of our study in this regard as the number of participants in each group were very few. Ideally we would have liked to recruit more participants in each category so that our subsequent sub-analysis would have more strength. We were surprised to find no significant associations of parameters such as HbA1c and fructosamine as well as urinary cortisol creatinine concentrations. However this is likely to be due to the lack of statistical power.

5. DIABETES PREDICTOR SCORE

5.1 Diabetes Predictor Score:

My aim was to determine whether a score could be designed to diagnose diabetes with improved sensitivity and specificity compared to IEC criteria and the screening algorithm using the W.H.O 1998 diagnostic criteria as gold standard. I looked at various parameters including age, BMI, sex, ethnicity, FPG, HbA1c, novel biomarkers, nature of the cardiac event as well as serum fructosamine by using logistic regression. However I was unable to utilize some of the parameters like fructosamine and the pancreatic and insulin related biomarkers as they were not available for all the participants. The following table illustrates the relationship of glycaemic measures and background parameters like age, sex, BMI and nature of the cardiac event.

5-1: Initial Logistic regression table for computing Diabetes Predictor Score

Variable	Regression co-efficient	Standard Error	Significance (p value)	Odds ratio	Confidence interval
Age	0.11	0.04	0.008	1.1	1.03-1.30
BMI	0.05	0.08	0.49	1.0	0.90-1.24
Sex	0.84	1.0	0.40	2.3	0.32-16.7
FPG	1.7	0.57	0.003	5.5	1.8-16.9
HbA1c	1.7	0.75	0.025	5.4	1.2-23.7
Cardiac event	-0.5	0.76	0.46	0.57	0.13-2.52

The best predictors appeared to be age, FPG and HbA1c. We did initially include BMI as we thought it would have a clear impact, however as shown in Table 2, BMI did not appear to have a positive correlation. The equation we designed was as follows:

Diabetes predictor score= (0.1 * Age) + (1.7 * FPG) + (1.6 * HbA1c).

5-2: Final Logistic regression table for computing Diabetes Predictor Score

Variable	Regression co-efficient	Standard Error	Significance (p value)	Odds ratio	Confidence interval
Age	0.1	0.04	0.007	1.1	1.03-1.19
BMI	0.05	0.08	0.49	1.0	0.91-1.23
FPG	1.7	0.54	0.002	5.4	1.89-15.8
HbA1c	1.6	0.7	0.03	4.8	1.19-19.1

ROC curve for comparison with the baseline diagnosis showed excellent correlation with area under curve (AUC) of 0.90.

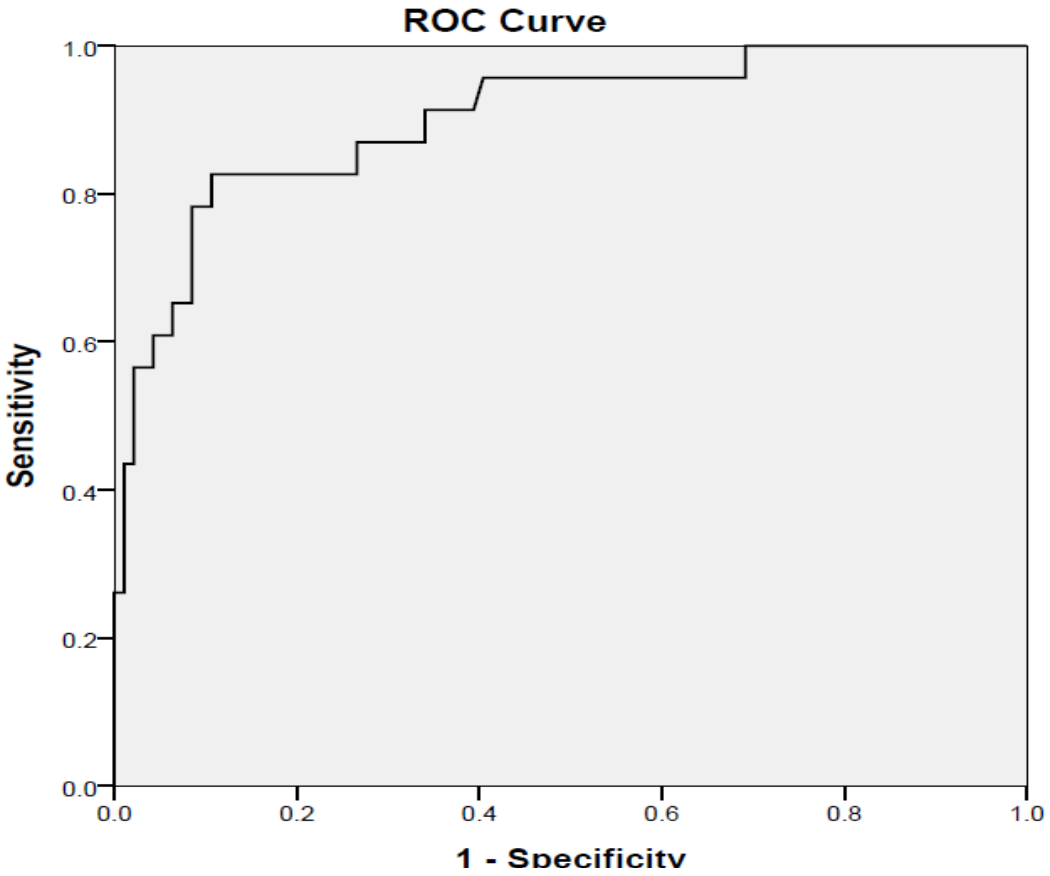


Figure 5-1: ROC curve for Diabetes Predictor Score against WHO 1998 diagnostic criteria at baseline

A score of 25.80 was associated with sensitivity and specificity of 83 %. Positive and negative predictive values were 54 and 95% respectively. Using a higher cut-off at 26.32 achieved similar sensitivity with better specificity at 87%. Positive and negative predictive values were 61 and 95% respectively. In comparison IEC criteria was associated with sensitivity of 57% and specificity of 94%. Positive and negative predictive values were 68 and 90% respectively.

ROC curve for comparison with follow-up diagnosis also had excellent correlation with an AUC of 0.89.

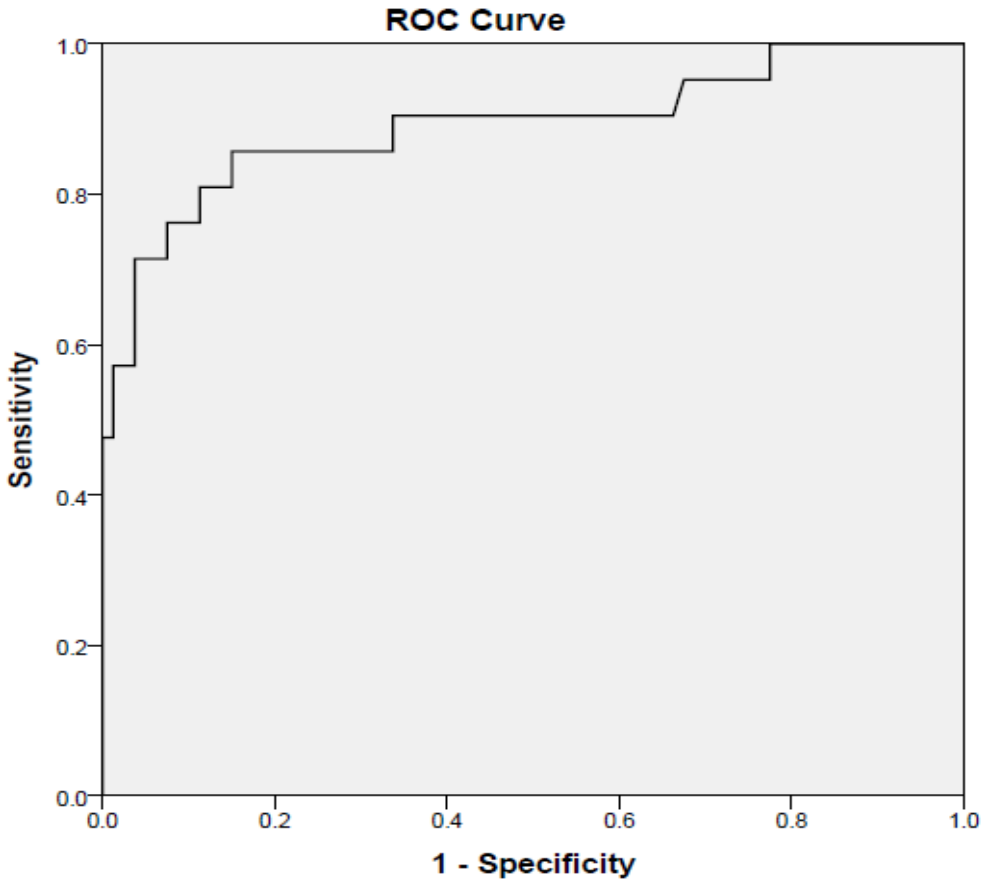


Figure 5-2: ROC curve for Diabetes Predictor Score against WHO 1998 diagnostic criteria at follow-up

A score of 25.80 was associated with a sensitivity of 86% and specificity of 85%. Positive and negative predictive values were 60 and 96% respectively. Using a higher cut-off at 26.32 achieved sensitivity of 81 and specificity of 89%. Positive and negative predictive values were 66 and 95% respectively. In comparison IEC criteria was associated with sensitivity of 67% and specificity of 95%. Positive and negative predictive values were 78 and 91% respectively.

Discussion:

Most guidelines recommend the OGTT as a gold standard investigation for assessing glycaemic status in patients with cardiovascular disease. However some recent studies have also compared a number of currently used diagnostic criteria for diabetes mellitus against W.H.O criteria ACS patients. One such study looking at comparing admission HbA1c, FPG and admission plasma glucose (APG) with OGTT results has suggested poor correlation. Indeed the AUCs measured in this study were 0.72, 0.75 and 0.61 respectively for the three parameters (93). While this study provided some useful information it did not offer any solutions. More importantly the investigators only looked at data at hospital admission while I have also clearly

demonstrated the change in glycaemic status from baseline to follow-up at 3 months in the dysglycaemic group.

On the other hand work done by Tahrani et al clearly demonstrated that using any of the parameters alone i.e. FPG or HbA1c is not enough. They initially performed univariate analysis with dichotomization of variables to obtain the optimum odds ratios (ORs) (200). For example, age was dichotomized to ≥65 years, BMI to 25 and waist circumference to 110cm as these gave improved significance over continuous variables and provided improved ORs over other clinically-meaningful cut-offs (200). Afterwards a stepwise logistic regression model was used showing only FPG ($P<0.001$, coefficient 1.23), HbA1c ($P = 0.002$, coefficient 1.59) and age≥ 65 years ($P = 0.049$, coefficient 0.87) were significant variables (200). The model was simplified by multiplying the coefficients by three and rounding to the nearest integers to generate the predictive model (PI) below:

(3 * Age≥65) + (4 * FPG) + (5 * HbA1c). (200)

Although the performance of this model was better than quoted in any of the criteria used by de Mulder et al (93) it was still worse than the Diabetes Predictor Score designed by us. The sensitivity, specificity and AUC of this model were 76%, 77% and 0.77 respectively. The model did have an excellent negative predictive value at 92.4%; however the positive predictive value was quite poor at 47% (200).By comparison our model gives us better sensitivity, specificity and AUC of 83%, 87% and 0.90 when compared to baseline diagnosis. Positive and negative predictive values were 68 and 90% respectively. When comparing it at 3 months the sensitivity, specificity and AUC were 81%, 89% and 0.89 respectively. Positive and negative predictive values were 66% and 95% respectively. Once again a major limiting factor of study done by Tahrani et al was the lack of complete glycaemic assessment at 3 months as they only relied on repeat FPG at 3 months (200).

To summarize employing a single glycaemic parameter alone does not seem to have a good correlation with WHO criteria. On the other hand as shown by our work as well as Tahrani et al a combination of age, fasting plasma glucose and HbA1c in a predictive model appeared to have the best correlation (200). The positive predictive values of these scores is relatively poor (although DPS does give≥65% at baseline as well as 3 months compared to PI value of only 47%). This would suggest that using these models to exclude diabetes may be the most logical use. These parameters should also be relatively easy to obtain at an acute admission making them more practical rather than an OGTT which is associated with poor reproducibility as well as logistic constraints.

6. Summary and Future Directions

6.1 Summary:

Despite the recognition of the importance of identification of undiagnosed pre-existent diabetes mellitus in patients hospitalized with acute cardiac events, diabetes can often be overlooked or inappropriately labelled as an acute stress response. In part, this may reflect a lack of consensus about the best screening modality to use in these patients or the complexity and costs associated with some methodology such as the OGTT (90, 97). Additionally limitations of the OGTT such as poor reproducibility, reflecting the high coefficient of variation of the 2 hour value, may limit its utility especially after acute medical stress. Alternative simple screening methods such as the FPG and HbA1c would appear to be attractive methods in comparison. However, HbA1c testing can be expensive, needs to be standardized and may not be universally available world-wide (97). HbA1c can be affected by racial origin and ethnicity (97, 99 and 100) and by anaemia and hemoglobinopathies. (97).

The overall aim of my project was to explore the role of alternative screening methods which are more reproducible, easier to perform, less expensive and suitable for large scale screening in predicting long term glycaemic status in patients admitted to hospital with ACS. I therefore evaluated a screening algorithm based on FPG and HbA1c (101) and the HbA1c based IEC criteria in a cohort of patients admitted to hospital with ACS. I was also able to design and evaluate a novel Diabetes Predictor Score which included basic parameters such as age, FPG and HbA1c.

My study was carried out in two large inner city hospitals in United Kingdom and had a representation from wide age, sex and multi-ethnic groups. The participants were all admitted to hospital with ACS and underwent an initial OGTT within 7 days of hospital admission which was followed up by glycaemic stratification at 3 months

Results demonstrated that on admission to hospital, 48% of participants had normal glucose tolerance (NGT) 32% impaired glycaemic status (IGS) and 20% met the criteria for T2DM based upon the 1998 WHO criteria. These data illustrate the importance of screening for diabetes mellitus in patients admitted to hospital with ACS. In comparison with previous studies (81) the prevalence of diabetes was lower in our cohort. This is likely to be related to our inclusion criteria and the nature of our population.

At 3 months, 54% of participants had NGT while 25% IGS and 21% T2DM. While the prevalence of diabetes mellitus remained similar the percentage of participants in

the NGT group increased and IGS group decreased. I also noted an association of baseline glycaemic stratification with baseline urinary cortisol creatinine ratio suggesting stress hyperglycaemia may play a role in some of these glycaemic abnormalities detected at baseline. However this relationship was lost at 3 months. There is also considerable debate about stress hyperglycaemia and whether it is cause or effect.

Some studies have suggested the use of admission plasma glucose (APG) as a diagnostic tool for glycaemic status in comparison with WHO criteria (93). However as illustrated earlier the correlation between APG and WHO criteria is very poor (93). In my study only a third of the total number of participants had bloods checked for venous APG suggesting poor inpatient diabetes care. The number of participants in the category where APG was checked was so few that we could not reliably look at its association with final glycaemic outcomes. The rise in APG demonstrated in other studies during the acute admission can be looked as both a response due to stress as well as independent of stress and an isolated marker of both short term and long term dysglycaemia and poor cardiovascular outcomes as described in previous sections. I would therefore recommend that a combination of fasting plasma glucose and HbA1c should be the bare minimum in terms of glycaemic investigations in these patients.

I would like to add here that the role of treating acute hyperglycaemia in the setting of acute myocardial infarction with therapies such as insulin has sparked some debate. In studies like DIGAMI for example it was suggested that treatment with insulin-glucose infusion followed by intensive subcutaneous insulin in diabetic patients with acute myocardial infarction improves long term survival. It was also demonstrated that the effect seen at one year continues for at least 3.5 years, with an absolute reduction in mortality of 11%. This means that one life was saved for nine treated patients. The effect was most apparent in patients who had not previously received insulin treatment and who were at a low cardiovascular risk (201). The investigators also looked at long term outcomes in these patients and suggested that mortality in these diabetic patients predicted by age, previous heart failure, and severity of the dysglycaemia at admission but not by conventional risk factors or sex. Intensive insulin treatment reduced long-term mortality despite high admission blood glucose and HbA1c (202). However results from DIGAMI 2 study were contrary to this. DIGAMI 2 suggested treatment with insulin may be associated with an increased risk of non-fatal cardiac events, but not mortality while metformin appeared protective against mortality (203).

There are two important messages from my work. Firstly it is important to detect and treat acute hyperglycaemia in both diabetic and non-diabetic patients admitted to hospital with acute myocardial infarction. I have confirmed in the previous

sections that even 1 mmol/l rise in blood glucose on admission in these patients can increase mortality by 4 times (116). This would suggest the importance of acute management of hyperglycaemia. DIGAMI and DIGAMI 2 studies also showed the importance of management of hyperglycaemia in the acute setting. Although in DIGAMI 2 insulin was associated with slight increase in non-fatal cardiovascular events while metformin was associated with improved cardiovascular and cancer outcomes (201-203).

Secondly on a long term basis studies like UKPDS and DCCT have demonstrated the importance of strict glycaemic control in improving micro vascular and macro vascular complications of diabetes. There is still considerable debate about the macro vascular outcomes, however it is clear that the micro vascular complications of diabetes can be delayed if not prevented by achieving strict glycaemic control specially early after the diagnosis. This also shows the importance of our findings of detecting these patients and intervening early to prevent long-term complications.

The application of either the WHO 1998 or the IEC diagnostic criteria gave a different prevalence of diabetes in our cohort (20% vs. 16% respectively at baseline or 21% vs.16% at 3 months). Fig 3-1 illustrates that at baseline we would miss almost half (43%) of the participants with DM diagnosed on W.H.O criteria by using IEC criteria. Similarly we would incorrectly identify 16% of participants in the IGS category as having DM on IEC criteria. This clearly suggests by using the two different criteria we are identifying different cohort of patients with diabetes. Fig 3-3 explores this relationship at 3 months. We would miss almost a third (33%) of the participants with DM diagnosed on W.H.O criteria by using IEC criteria. Similarly we would incorrectly identify 12% of participants with IGS and 2% of NGT as having DM based on IEC criteria. . Although the IEC criteria perform better at 3 months we are still identifying different cohort of participants by using the two diagnostic criteria. The reason for IEC criteria performing better at 3 months may be due to HbA1c reflecting longer term glycaemic status compared to OGTT.

The difference in population detected with T2DM by using the two different criteria is clinically relevant as we are basing screening in a high risk population on these criteria. Another important point is the importance of recognizing patients with IGS. The American Diabetes Association have recommended using an HbA1c cut-off of 5.7 to 6.4% to determine which patients may be at an increased risk of developing T2DM (118). Previous studies have suggested that in these groups of patients in the clinical setting of acute admissions with ACS 31% of them were diabetic according to WHO criteria (93). By contrast in my data a vast majority of participants 80(68%) had an HbA1c in this category and therefore this did not correlate very well with the group of participants with IGS according to WHO criteria. 9(11%) participants in this group had T2DM and 28(35%) had IGS with 43(54%) having NGT in comparison

with WHO criteria. Once again this poor correlation confirms our suspicion that the two criteria are identifying completely different cohorts. In addition we also need to look at the clinical and financial burden of managing the huge number of people in this category. As demonstrated in my study two thirds of the participants would have been classed as at risk of pre-diabetes if we had implemented ADA rather than WHO criteria

These findings are particularly relevant as it is well known that patients with IGS have increased cardiovascular morbidity and mortality (76-80, 101). Patients with IGS are also known to progress to T2DM (89) with studies suggesting an incidence of around 57.2 per 1000 patient years (204). Other studies have suggested similar findings with incidence rates ranging from 35 to 58 per 1000 patient-years depending on ethnicity (205). IGT has also been known to be associated with increased cardiovascular mortality. The Whitehall study suggested a 2 fold increase in cardiovascular mortality in patients with abnormal OGTT results (2 hour PG>5.3 mmol/l) vs. normal results (206). Studies have also examined the role of lifestyle or metformin (off label) therapy to reduce this progression (89). In this study 3 groups were compared i.e. placebo versus lifestyle intervention and metformin therapy. In the lifestyle intervention the incidence of diabetes was reduced by 58 percent and in the metformin group by 31 percent, as compared with placebo; suggesting life style intervention was significantly more effective than metformin. Putting these results in another way, in order to prevent a single case of diabetes during a time frame of three years, approximately 6.9 persons would have to be treated in the lifestyle-intervention program, and 13.9 would have to receive metformin (89) suggesting lifestyle intervention as being superior to metformin therapy. More recently a large meta-analysis was published looking at the effects of lifestyle intervention in reducing the incidence of T2DM and mortality in patients with IGT.

Diabetes incidence in this meta-analysis varied from 3% to 46% in the intervention arm and 9.3% to 67.7% in the control cohort. These studies were carried out in various countries like India, Japan; Sweden etc. making it difficult to discern the effects of ethnicity (207).Mortality and morbidity was only studied in one of the included studies and did not suggest any statistically significant changes (208). The Da Qing study did show that group-based lifestyle interventions over 6 years could prevent or delay diabetes for up to 14 years (208). However the study was not powered enough to look at mortality and morbidity outcomes (208).The investigators of the large meta-analysis concluded that lifestyle intervention can have a beneficial effect on the incidence of T2DM in patients with IGT (207). However, rather disappointingly several studies found the effect of lifestyle intervention decreased after intervention was terminated

In my study the 3 participants who died all have abnormal glycaemic status with two of them having IGS at baseline. IEC criteria on its own would miss all these participants as the glycaemic stratification is only based on HbA1c.

The screening algorithm developed locally appeared to have a good correlation with the WHO 1998 diagnostic criteria with sensitivity of over 85% at baseline as well as 3 months. In comparison with IEC criteria it performed very well with sensitivity of 100% as I did not miss any participants with diabetes mellitus. However I would have classified 3% incorrectly with diabetes mellitus. It would also lead to an over 60% reduction in OGTTs compared to WHO criteria. This has potentially significant cost saving implications for National Health Service (N.H.S). The screening algorithm does have an advantage over IEC criteria with better sensitivity. In addition it also ensures we identify at least half (50%) of the participants with IGS. The IEC criteria in comparison will detect less than 20% of IGS subjects and would actually label them all as having T2DM. Earlier I mentioned that glycaemic stratification based on WHO 1998 diagnostic criteria changed from baseline to 3 months. Closer examination reveals most of the patients in the NGT category remained unchanged (81%). While some of the participants changed in the T2DM category also changed (27%) most remained unchanged (73%) suggesting in these two categories we could rely on glycaemic classification at baseline as a predictor of long term glycaemic status. In comparison most of the participants in the IGS category did change (66%) with some of them becoming NGT (50%) and some also progressing to T2DM (16%). Previous studies had suggested we could rely on glycaemic diagnosis on baseline, however my data suggest in the IGS category a second OGTT is required at around 3 months to predict long term glycaemic diagnosis.

The screening algorithm was originally designed to reduce the number of OGTTs rather than accurately defining glucose tolerance. Therefore I wanted to look at a different algorithm in our cohort which performed better in comparison with WHO criteria. I utilised a logistic regression model and used basic parameters such as age, sex, ethnicity, serum fructosamine, BMI, FPG and baseline HbA1c. Surprisingly I did not notice any significant association with BMI or fructosamine; however the other parameters such as age, FPG and HbA1c were all significantly associated as illustrated in table 5-2.

Diabetes predictor score based on age, FPG and HbA1c appeared to perform better than IEC criteria if we use WHO diagnostic criteria as gold standard in this setting with both sensitivity and specificity of over 80%. ROC curves for DPS suggested excellent correlation with WHO diagnostic criteria at baseline and 3 months with AUC of 0.90 and 0.89 respectively. Sensitivity for DPS was much better than IEC criteria. It also appeared to show good concordance with the WHO criteria as it was

based on 3 parameters and not solely reliant on HbA1c. The DPS has excellent negative predictive values of over 90% at baseline as well as 3 months suggesting it can be used reliably as a rule out test. The positive predictive values were lower however suggesting we would identify some patients incorrectly with false positive results. We would postulate that DPS can be used in future as a test to exclude T2DM i.e. if DPS is negative T2DM can be excluded with a degree of certainty. However due to the poor positive predictive value, relying on it solely can lead to detection of a number of false positives. However based on our work as well as that of Tahrani (200) and de Mulder (93) it appears that our score has the best evidence as a test looking for concordance with WHO 1998 criteria.

One of the novel features of my project was the exploration of pancreatic biomarkers in the participants. I noticed some interesting associations between the individual biomarkers and individual glycaemic measures. I noted that at baseline mean c-peptide, glucagon, intact pro-insulin and HOMA IR were higher in the diabetic group compared to normal and IGS groups. I also noted a trend for the leptin and the leptin adiponectin ratio to be higher in both diabetic and IGS groups compared to normal groups. However this relationship may have been driven by BMI. The only other biomarker which showed a clear association was HOMA IS which was found to be lower in the diabetic and IGS groups compared to normal cohort. At 3 months I noted that mean c-peptide, IL 1 RA, TIMP 2 and intact pro-insulin were higher in the diabetic group compared to NGT and IGS groups. The only other trend noticed was for HOMA IS to be lower among diabetics. This relationship may also be clinically relevant as there is considerable interest in developing commercial assays for these biomarkers.

I was particularly interested in the concordance between the three glycaemic categories and the interchange between them at 3 months. I looked at them individually to discern any features which may help predict the group's which were more likely to retain dysglycaemia. In the DM group I noted higher adiponectin, HOMA I.S and TIMP 1 levels among NGT and IGS compared to DM group at 3 months. In the IGS group, TIMP 2 levels were lower in the NGT compared to IGS and DM groups at 3 months. There was also a trend towards higher FPG and systolic B.P in the IGS group compared to NGT and DM groups. In the NGT group, the only trend was diastolic B.P being higher in the normal compared to IGS groups.

My study does have some limitations. The prevalence of diabetes was lower compared to previous studies which could impact some of our subsequent analysis. This is particularly relevant in some of the analysis about the three sub-groups and the interchanges between the groups at 3 months. Some of the numbers in these subgroups were extremely small. It is difficult to apply all our results to ethnic minority groups as 80% of our participants were white Caucasian. Another major

limitation appears to be related to gender as there is clearly a vast majority of males recruited in our study. However as illustrated in the previous sections my work is not unique in this regard.

Despite these limitations my study has provided interesting data about the different diagnostic modalities and their impact on prevalence of diabetes. I have demonstrated that the two main diagnostic methods currently used i.e. WHO 1998 and IEC criteria appear to identify different populations with diabetes mellitus.

6.2 Future Directions:

I would have liked to follow-up these participants for a longer duration to look at their long term glycaemic status and its correlation with future cardiovascular events and cardiovascular mortality. I believe employing some of our algorithms and scores can help refine diagnosis of diabetes in this high risk category. My future aspiration would be to look at extending this work in other ethnic groups and also follow-up participants for a much longer duration. In addition this will also have potentially significant cost saving implications within N.H.S. In our own hospital the cost of an OGTT is around £64 suggesting clear reduction in acute hospital costs.

A novel aspect of my work was the use of novel biomarkers in predicting glycaemia in patients admitted with ACS. Indeed some of these biomarkers such as urinary c-peptide are already in clinical use to aid diagnosis of diabetes mellitus. They may also help us determine long term glycaemic outcomes. One of our future aspirations is to look at relationship of these biomarkers with cardiovascular outcomes i.e. morbidity and mortality independent of the glycaemic status

To summarize different diagnostic criteria appear to suggest differing prevalence of diabetes mellitus. The performance of screening algorithms and predictor scores appears to be much better compared to IEC criteria on its own when using W.H.O 1998 diagnostic criteria as gold standard. This is likely to be due to the inclusion of FPG in addition to HbA1c (and age in case of diabetes predictor score).I would also like to look at long term cardiovascular outcomes in participants with IGS and DM both with relevance to the novel biomarkers and on its own.

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