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A Pilot Study of Increased Gene Expression of Growth Differentiation Factor 15 and Telomerase Reverse Transcriptase in the Middle-Aged with Acute Coronary Artery Disease

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Abstract

Background: Growth Differentiation Factor 15 (GDF15) and Telomerase Reverse Transcriptase (TERT) may be involved in cardiovascular disease. We hypothesised altered expression of the genes for these molecules in acute coronary artery disease (CAD).
Methods: Venous blood was obtained from 53 patients (27 with diabetes) presenting with an acute coronary syndrome and subsequently shown to have CAD, and from 46 age and sex matched controls free of cardiovascular disease and its risk factors. Relative expression of leukocyte transcriptome *GAPDH, GDF15* and *TERT* were determined by RT-PCR and quantified by quantitation-comparative Ct (ΔCt).
Results: Compared to controls, mean (95% CI) expression of *GDF15* in the patients was 1.38 (1.13-1.49) ($p<0.001$), and of *TERT* was 1.12 (1.04-1.20) $p=0.003$), with *GDF15* being greater than that of *TERT* ($p<0.001$). There was no difference in *GDF15* expression in 26 patients free of diabetes (1.6 [1.42-1.78]) versus 27 with diabetes (1.6 [1.29-1.91]) ($p=0.996$), and no difference in the *TERT* expression in patients free of diabetes (1.19 [1.06-1.33]) compared to those with diabetes (1.25 [0.98-1.50]) ($p=0.739$).
Conclusions: *GDF15* and *TERT* expressions are both increased in CAD and in CAD+diabetes, with no difference between the patient groups. These genes may have roles in the pathogenesis of acute CAD.

1. Introduction

The pathogenesis of coronary artery disease has long been linked to the four major risk factors of diabetes, hypertension, hypercholesterolemia and smoking, but numerous genetic influences are emerging (1,2). Growth differentiation factor 15 (GDF-15), also known as macrophage inhibitory cytokine-1, is a member of the transforming growth factor-β super family and has functions likely to have a role in numerous processes such as angiogenesis and inflammation (3). Coded for by *GDF15* at 19p13.11, increased serum levels have been reported in many cardiovascular diseases such as atrial fibrillation and heart failure and are linked to increased all-cause death and cardiovascular death in coronary artery disease and with death following an ischaemic stroke (4,5). Several studies have indicated that levels of circulating GDF-15rise with age and are directly, perhaps causally, linked to the etiology of these cardiovascular diseases (6,7). Data from the Framingham study points to effects of risk factors as well as genetics on plasma concentrations of GDF-15 (8), and although variants of *GDF15* are linked to ischaemic stroke in a Chinese population (9), this has been countered in a meta-analysis (10).

Telomere length has long been associated with a variety of diseases, including atherogenesis and CAD (11-13). Telomerase is a ribonucleoprotein enzyme with two catalytically essential subunits, the telomerase RNA, and telomerase reverse transcriptase protein (TERT) that regulates telomere length (14,15).

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As this enzyme effectively reduces telomere length, several commentators have suggested it may be a new therapeutic target (16,17). Over-expression of human TERT (h-TERT), coded for by *TERT* at 5p15.33, may be important in cancer (18), whilst variants of *TERT* may be linked to outcome in stable CAD (19) and risk of ischaemic stroke (20).

Thus, the literature points to potential roles for both *GDF15* and *TERT* and their protein products in coronary artery disease. However, the great majority of clinical data focuses on late-middle aged and elderly patients, with little data on the young middle-aged. Our literature search failed to find any report of both genes or molecules analysed together. In order to fill this gap in the knowledge, we therefore hypothesised altered expression of both *GDF15* and *TERT* in relatively young patients presenting to hospital with an acute coronary syndrome subsequently shown to be a myocardial infarction.

2. Methods

We tested our hypothesis in consecutive patients with existing cardiovascular disease (myocardial infarction, stroke, arterial surgery) aged up to 55 years old presenting to the Cardiac Catheter Unit, Cardiology Department, Assuit University Hospital in an acute coronary syndrome subsequently shown by angiography to have CAD precipitating a myocardial infarction. Data from patients was controlled by samples from healthy hospital and laboratory staff free of cardiovascular disease or its risk factors. Exclusion criteria were age >55 years, psychological disorders, cancer, autoimmune diseases, acute or chronic inflammatory diseases, and diabetic patients treated with an inhibitor of dipeptidyl peptidase. The purpose and method of the study were explained to each participant and informed consent was obtained. A predesigned case record form was used to record age and clinical data of the consenting subjects. This study was approved by the ethical committee of the Faculty of Medicine, Assiut University (IRB no. 17200057).

Venous blood was collected from each patient on presentation, and from each control. Serum urea, creatinine, creatine kinase, creatine kinase MB, Troponin-T (patients only), HbA1c, total cholesterol, triglycerides, and high-density lipoprotein were performed by the routine hospital clinical chemistry service (which subscribes to the national quality control service) by established methods. Low-density lipoprotein was calculated according to the Freidwald formula.

Total RNA was extracted from EDTA-anticoagulated whole blood using Direct-zol™ RNA Miniprep Plus (Zymo Research, Irvine, CA, USA), followed by a SensiFAST™ SYBR® Hi-ROX One-Step Kit for cDNA by RT-PCR (Meridian Bioscience, Memphis, TN, USA). The reactions were performed in a thermal cycler (MJ Research, Inc., Waltham, MA, USA) and the reaction products stored at -20°C until the subsequent step 2. The thermal profile consisted of 20 minutes at 46°C for reverse transcription (RT) then at 95°C for 1 minute for RT inactivation. Gene expression was standardized to that of *GAPDH* as a control and is registered as fold change. We applied 1 μM of each forward and reverse primer specific for each target gene: *TERT* ,5' GGAGCAAGTTGCAAAGCATTG3'; 5' TCCCACGACGTAGTCCATGTT-3'.*GDF15* ,5 'TCAAGGTCGTGGGACGTGACA 3'; 5' GCCGTGCGGACGAAGATTCT 3'and *GAPDH*, 5'-AGCCACATCGCTCAGACAC-3'and5'-GCCCAATACGACCAAATCC-3'. The products were hybridized to the SYBR green dye. The mean expression levels of*GDF15* and *TERT* mRNA relative to those of *GAPDH* were analysed utilizing the quantitation-comparative Ct (ΔΔCt) method on a fluorescence quantitative PCR analyser (ABI 7500, Applied Biosystems, Foster City, CA, USA).

We hypothesised a difference in relative expression of *GDF15* or *TERT* of at least 12.5% compared to expression in the controls. To achieve this at alpha <0.05 and 1-beta >80% calls for 40 per group, and for additional assurance we recruited in excess of this requirement. A sample size of 80 provides the power to defend a Pearson correlation coefficient of >0.31. In post-hoc analyses we noted the large number of diabetics and considered their analysis in relation to non-diabetics. A sample size of 25 per group provides the power to robustly defend a difference of 16% in a test statistic compared to a control group, and a sample size of 50 provides the power to defend a Pearson correlation coefficient of >0.39. Categorical data were analysed by the chi-squared test, continuously variable data by student's t test or the Mann-Whitney U test and correlated by Pearson's method. All analyses were performed on Minitab release 21.

3. Results

3.1: The larger case-control analysis

Clinical, demographic, and laboratory data of the controls and patients are shown in table 1.

Table 1: Clinical, demographic and laboratory data on Cases and Controls

	Controls (n=46)	Cases (n=53)	P value
Age (years)	40.3 (11.1)	42.9 (9.6)	0.205
Sex (m/f)(n)	31/15	41/12	0.267
Urea (mg/dL)	4.2 (1.8)	4.2 (1.4)	0.835
Creatinine (mg/dL)	0.88 (0.27)	1.15 (0.46)	<0.001
CK (IU/L)	39 (13)	138 (46)	<0.001
CK-MB (ng/mL)	0.8 (0.3)	7.5 (4.0)	<0.001
Cholesterol (mg/dL)	135 (12)	211 (59)	<0.001
Triglycerides (mg/dL)	93 (17)	111 (33)	0.001
HDL (mg/dL)	37 (7)	42 (10)	0.003
LDL (mg/dL)	79 (16)	147 (58)	<0.001
HbA1c (%)	4.7 (0.4)	8.1 (3.6)	<0.001
GADPH expression (units)	17.7 (5.5)	18.6 (5.7)	0.403
GDF15 expression (units)	19.9 (5.7)	26.8 (3.6)	<0.001
TERT expression (units)	17.7 (5.4)	21.3 (6.3)	0.003

Data are mean (standard deviation) or number of subjects. CK = creatine kinase, CK-MB = creatine kinase isotype MB. HDL = high density lipoprotein, LDL = low density lipoprotein.

The two groups were matched for age and sex. There was no difference in levels of serum urea between the groups, but (as partially expected) creatinine, HbA1c, all cardiology metrics, and all lipid metrics were increased in the patients. There was no difference in the expression of *GAPDH* between the groups, but the expression of *GDF15* and *TERT* were both increased. Compared to expression in controls, mean (95% confidence interval) relative expression of *GDF15* in the patients was 1.38 (1.13-1.49) ($p<0.001$), and of *TERT* was 1.12 (1.04-1.20) ($p=0.003$), with relative expression of *GDF15* being greater than that of *TERT* ($p<0.001$).

Expression of *GDF15* and *TERT* failed to correlate together significantly in the controls with $r=0.22$, $p=0.131$, but in the patients the correlation was significant at $p=0.55$, $p<0.01$. Expression of *GDF15* and *TERT* failed to correlate significantly with age in the controls with $r=0.15$, $p=0.327$ and $r=0.23$, $p=0.131$ respectively, or the patients with $r=0.08$ $p=0.576$ and $r= -0.02$ $p=0.879$ respectively.

3.2: Analysis according to diabetes

Clinical, demographic, pharmacotherapy, and laboratory data of the two patient groups are shown in table 2. The two groups were matched for smoking, age, and sex. Serum creatinine and HbA1c were higher in the diabetic group, but there was no difference in levels of serum urea, or in cardiology or lipid metrics. There was no difference in the relative expression of *GDF15* in the patients free of diabetes (1.6 [1.42-1.78]) compared to those with diabetes (1.6 [1.29-1.91]) ($p=0.996$). Similarly, there was no difference in the expression of *TERT* in patients free of diabetes (1.19 [1.06-1.33]) compared to those with diabetes (1.25 [0.98-1.50]) ($p=0.739$). *GDF15* and *TERT* correlated significantly in the patients with CAD ($r=0.48$, $p=0.013$) and also in patients with CAD and diabetes ($r=0.57$, $p=0.002$).

Table 2: Clinical, demographic, pharmacotherapy, and laboratory data on patient subgroups at presentation

	CAD (n=26)	CAD and diabetes (n=27)	P value
Age (years)	41.3 (11.6)	44.6 (7.2)	0.223
Sex (m/f)	23/3	18/7	0.138
Smoking (y/n)	16/10	12/15	0.213
Urea (mg/dL)	4.5 (1.2)	4.0 (1.5)	0.157
Creatinine (mg/dL)	1.0 (0.3)	1.3 (0.5)	0.032
CK (IU/L)	126 (45)	149 (45)	0.067
CK-MB (ng/mL)	8.4 (3.9-10.9)	6.0 (3.5-8.30	0.118
Troponin T (ng/mL)*	0.12 (0.06-1.25)	0.08 (0.05-1.00)	0.676
Cholesterol (mg/dL)	203 (48)	219 (68)	0.306
Triglycerides (mg/dL)	115 (39)	107 (28)	0.394
HDL (mg/dL)	40 (8)	45 (11)	0.073
LDL (mg/dL)	140 (44)	153 (70)	0.404
HbA1c (%)	4.6 (0.4)	11.4 (1.6)	<0.001
GAPDH expression (units)	18.6 (5.2)	18.7 (6.2)	0.901
GDF15 expression (units)	27.7 (2.4)	26.0 (4.4)	0.104
TERT expression (units)	21.7 (6.0)	21.0 (6.6)	0.699
Aspirin	26	27	-
Beta-blocker	7	7	-
Insulin	0	12	-
Metformin	0	10	-
Sulphonylurea	0	5	-
Statin	21	8	-
Ezetimibe	6	8	-
ACEI	7	11	-

Data are mean (standard deviation), median (interquartile range) or number of subjects. CK = creatine kinase, CK-MB = creatine kinase isotype MB. HDL = high density lipoprotein, LDL = low density lipoprotein. CAD = coronary artery disease. ACEI = angiotensin converting enzyme inhibitor. *reference value <0.01 ng/mL

4. Discussion

Although the four major risk factors for coronary artery disease can be addressed and possibly treated, myocardial infarction and stroke still remain the leading global cause of death (21). In Egypt, ischaemic heart disease is the leading cause of death in each 5-year age band from 20-24 years to 85-plus years (22).

Molecular genetics are providing other potential causes of cardiovascular disease (1,2,23), among which are the genes *GDF15* and *TERT* that code for molecules with markedly different physiological functions (3,14). Under certain circumstances, increased circulating levels of their protein products are present in the blood, and as such may be markers and/or targets of particular diseases that include cardiovascular disease and cancer (4,17,24-27). These increased levels may be related to features that include age and/or genetic control (6,28).

The principal results of our study contribute to the literature in that we report (a) increased expression of both *GDF15* and *TERT* in relatively young patients with CAD, with the relative expression of *GDF15* being greater than that of *TERT*, (b) that this expression does not vary with the presence of diabetes, (c) that the co-expressions of these two genes is unrelated in healthy controls but correlate significantly in the patients regardless of their diabetes status, and (d) expression of either gene failed to correlate with age in patients or in controls. This last point is in contrast to other data on this risk factor (14,29), although our population has a maximum age of 55 years, and so excludes the elderly who are more likely to carry a greater burden of disease. As both genes and their products are altered in diabetics without cardiovascular disease (30-32), we note that this risk factor does not have a modulating effect on gene expression on a platform of acute coronary artery disease, although some have reported increases in those with more extensive disease (32-34). However, the precise clinical science of our data is clouded by the use of metformin by over a third of our patients with diabetes, as this drug has a positive influence on circulating GDF-15 (35). Furthermore, serum GDF-15 correlates with blood glucose and HbA1c (36), although it is unknown if these, or the effect of metformin, are due to the direct promotion of the transcription of *GDF15*, post-transcription changes, or other mechanisms. Although various SNPs in *TERT* have been reported in diabetes, there is no comparable data on the quantitative expression of this gene (37-39).

Our cross-sectional study is unable to answer the question of whether the increased expression of these genes were present before the patients were admitted to hospital, or they are the product of the acute coronary syndrome. We accept that it is entirely possible that either of these two processes could be present. However, whatever the mechanism, it is notably that the relative expression of the two genes correlated strongly in the patients but not in the controls for reasons that demand an explanation that at present we cannot supply. In this pilot study we accept the limitations of a small sample size, and so can criticised for being at risk of false positive or false negative, although the case/control and CAD with/without diabetes differences we found exceeded those of our hypothesis, so we believe our findings are robust. This limited sample size also precludes more complex multivariate analyses. We also acknowledge possible influences of other pathology we have not addressed, such as ACS-derived inflammation, and that we lack a diabetes control group. Nevertheless, we submit that our data has potential implications for the aetiology and/or pathogenesis of coronary artery disease.

5. Conclusions

Our pilot data provides evidence for alterations in the relative expression of *GDF-15* and *TERT* in middle aged patients suffering an acute myocardial infarction, with a firm correlation between the two indices. There was no difference in the expression of these genes according to the presence of diabetes. We believe our data justifies a larger study to determine the mechanisms and implications of these findings, which, we speculate, may contribute further to our knowledge of the pathogenesis and management of cardiovascular disease.

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Authors' contributions

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Idriss NK: Genetic analysis, acquisition and interpretation of data, drafting manuscript.

Blann AD: Performed final statistical analysis, interpretation of data, wrote final draft of the manuscript, corresponding Author.

Mosa AA: Genetic analysis and drafting the manuscript

Fouad DA: Study design and writing the protocol, consideration of the manuscript.

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Nasreldin E: Study design and writing the protocol, consideration of the manuscript.

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Meki AA: Study design and writing the protocol, consideration of the manuscript.

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