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CHEMICAL PATHOLOGY

Interpretative comments specifically suggesting specialist referral increase the detection of familial hypercholesterolaemia

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Summary

Familial hypercholesterolaemia (FH) is an underdiagnosed inherited condition characterised by elevated low density lipoprotein (LDL)-cholesterol and premature coronary artery disease. The requesting general practitioner of individuals with extremely elevated LDL-cholesterol measured by St John of God Pathology receives an interpretative comment on the lipid results highlighting possible FH. We sought to determine whether specifically recommending referral to the regional Lipid Disorders Clinic (LDC) increased referral and FH detection rates. A prospective case-control study of individuals with LDL-cholesterol ≥6.5 mmol/L was conducted. All individuals received an interpretative comment highlighting the possibility of FH. The cases comment also suggested LDC referral, and a subset of cases received the LDC's fax number (fax-cases) in addition. There were 231 individuals with an LDL-cholesterol ≥6.5 mmol/L; 96 (42%) controls and 135 (58%) cases, of which 99 were fax-cases. Twenty-four (18%) cases were referred to clinic compared with eight (8%) controls ($p = 0.035$). After specialist review and genetic testing, four probable and four definite FH individuals were detected amongst controls, compared with seven possible, eight probable and nine definite FH amongst cases. Genetic testing was performed in 31 (94%) individuals, 13 (42%) had a causative mutation identified. Interpretative commenting specifically recommending specialist review augments the detection of FH in the community.

Key words: Familial hypercholesterolaemia; screening; laboratory; interpretative commenting.

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INTRODUCTION

Familial hypercholesterolaemia (FH) is an autosomal codominant condition characterised by elevated plasma levels of low density lipoprotein (LDL)-cholesterol and premature coronary artery disease (CAD). FH is generally caused by a mutation in one of three genes; the LDL receptor (*LDLR*), apolipoprotein B (*APOB*), or proprotein convertase subtilisin kexin type 9 (PCSK9).[1] Heterozygous FH occurs in 1 in 250–300 people.[2,3] However, the majority (~90%) of individuals with FH are undiagnosed.[4] FH can lead to a 13-fold increased risk of CAD.[4] Untreated, men with FH have a 50% risk of CAD by the age of 50 years, whereas women have a 30% risk by 60.[5] There are estimated to be over 45,000 cases of FH in Australia, although less than 10% are known to secondary care lipid clinics.[6]

The Dutch Lipid Clinic Network criteria (DLCNC) are preferred for the diagnosis of FH index cases in Australia.[7] Systematic testing of family members (cascade testing) is recommended from these index cases, which is the most cost effective method for detecting FH cases.[8] Australasian guidelines for the detection and management of FH recommend that those deemed at risk of FH be assessed at a specialist clinic.[7]

Community laboratories have the potential to detect FH and are well placed to opportunistically highlight individuals who are at very high risk of having FH.[9] Individuals with an LDL-cholesterol ≥6.5 mmol/L have previously been demonstrated to be at very high risk of FH.[10–12] Interpretative commenting provides additional clinical information on a pathology report to aid the requesting practitioner's interpretation of the results and subsequent management.[13] This function is typically performed by pathologists, but also senior scientists with appropriate professional qualifications.[14]

In Australia over 81% of the population visits a general practitioner (GP) at least once each year.[15] GPs order 92% of cholesterol tests from a community laboratory,[9] and prefer interpretative commenting to alert them when a patient is at risk of FH.[16] We have previously demonstrated that interpretative comments highlighting FH were associated with significant additional reductions in LDL-cholesterol.[17] A subgroup analysis of this study found that specifically suggesting specialist referral was associated with increased referrals. However, the applicability of this finding was limited by the subgroup analysis and the use of a historical control group.

In this study, we sought to determine the specialist referral rate and diagnostic yield of appending a specific

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interpretative comment to lipid profiles of individuals at high risk of FH in a prospective case-control study. In addition, we sought to determine if including the fax number for the regional Lipid Disorders Clinic (LDC) was associated with an additional increase in referral rates.

MATERIALS AND METHODS

This prospective case-control study was performed on individuals referred by a GP who were found to have an LDL-cholesterol ≥6.5 mmol/L measured by St John of God Pathology (SJGP), Western Australia, between 1 December 2012 and 1 December 2013. Individuals were excluded if there was an identifiable potential secondary cause for the hypercholesterolaemia, such as hypothyroidism (TSH >4.0 mU/L), mixed hyperlipidaemia (triglyceride >4.0 mmol/L), nephrotic syndrome (proteinuria >3 g/L and serum albumin <30 g/L), and cholestasis [alkaline phosphatase (ALP) >135 U/L and γ glutamyl-transferase (GGT) >55 U/L in males or >38 U/L in females] within ±30 days of the LDL-cholesterol result, or if they were included in a previous study investigating the impact of a telephone call from the chemical pathologist on FH detection.[12]

Interpretative comments were added to the lipid results with the assistance of an expert system, Ripple Down (Pacific Knowledge Systems, Australia),[18] with all comments reviewed by one of two chemical pathologists before being issued. All subjects received an interpretative comment that raised FH as a consideration, and stated that FH was an autosomal dominant condition associated with elevated LDL-cholesterol and premature atherosclerotic CAD. The cases received an additional recommendation for referral to a LDC, for example: 'Familial hypercholesterolaemia (an autosomal co-dominant disorder characterised by increased LDL-cholesterol, xanthomata and premature coronary heart disease) is an important consideration when LDL >6.4 mmol/L. Suggest review for clinical stigmata of FH, family history and consider specialist referral to the lipid disorders clinic at Royal Perth Hospital.'

A subset of the cases (fax-cases) also had the LDC's fax number included. The selection of cases and controls varied according to the day of the week the subject had their lipid results authorised, and thus was not completely randomised; cases were authorised on Mondays, Thursdays and the weekend, and controls Tuesdays, Wednesdays and Fridays. The fax-cases were selected based on the pathologist reviewing the lipid report; with one always including the fax number and the other never including it.

The LDL-cholesterol results from the preceding 365 days were reviewed for these individuals to determine the particular interpretative comment assigned to any previous LDL-cholesterol report, as part of routine care. A number of GPs who had utilised SJGP may have been previously telephoned by a consultant chemical pathologist to discuss FH as a consideration and suggesting referral to the LDC as part of a previous trial.[12] In order to explore whether a referral bias existed because of these previous interventions, the GPs who had been called were identified amongst the cases and controls in this study.

Total cholesterol, triglyceride, and high density lipoprotein (HDL)-cholesterol analyses were performed with enzymatic, colorimetric assays using Siemens reagents on a Siemens Vista or Dimension EXL chemistry analyser (Siemens Healthcare Diagnostics, USA). LDL-cholesterol was calculated according to the Friedewald equation.[19]

The community laboratory database was searched with CrystalReports software version 11.0.0.1282 (SAP AG, Business Objects, Germany) and Microsoft Access 2007 (Microsoft, USA). This information was transferred to a Microsoft Excel 2010 spread sheet for analysis. After allowing 365 days for the referral and specialist review to have occurred, the individuals in this database were then cross referenced to the database at the regional LDC to identify those who were referred, and the database of the regional Cardiovascular Genetics Laboratory database to determine if genetic testing had been requested.

Lipid specialists at the LDC at Royal Perth Hospital assessed and managed individuals according to the Australian FH Model of Care.[7] Genetic testing was performed after obtaining informed consent. Genetic testing was performed as previously described.[20] In brief, all 18 exons of the *LDLR*, part of exons 26 and 29 of *APOB*, and exon 7 of *PCSK9* were Sanger sequenced, and multiplex ligation-dependent probe amplification (MLPA) of the *LDLR* was performed to detect large deletions or duplications.

Statistical analysis was performed using Microsoft Excel 2010. Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as absolute values and percentages of total. Statistical significance between groups was determined by Pearson chi-squared test and analysis of variance.

This study was approved by the Royal Perth Hospital Quality Improvement and Human Research Committees.

RESULTS

There were 231 individuals found to have an LDL-cholesterol ≥6.5 mmol/L requested by GPs who were highlighted as being at risk of FH in this period. There were 96 controls (42%) and 135 cases (58%). Of the cases, 99 (73%) received a fax number (fax-cases). There were no significant differences in demographics or lipid concentrations between the cases and controls (Table 1). There was a female predominance (~65%) in both cases and controls.

Eight individuals (8%) were referred to the lipid clinic from the control group compared with 24 (18%) from the cases ($p = 0.035$). Each of the individuals from the control group was referred by a different GP. Among the cases, 18 were referred in the fax-case group (18%), compared with six (20%) in the non-fax group ($p = 1.0$). Four unrelated cases were referred by the same GP. This GP had not been previously telephoned in relation to referral of individuals to the lipid disorders clinic. The remaining 20 cases all had different GPs.

After specialist review and genetic testing, there were four probable and four definite FH individuals identified from the control group, compared with seven possible, eight probable and nine definite FH individuals among the referred cases. No individuals were deemed to have definite FH (DLCNC score >8) based on clinical information alone. Thirty-one of the 33 (94%) individuals referred were genetically tested. A mutation was detected in 13 (42%) of these individuals.

There were 108 different GPs caring for the 135 cases, and 72 received an additional fax number. There were 92 different GPs for the 96 controls. Eighteen GPs were providing care to different patients where one was assigned as a case and the other a control. There was no significant difference in the referral rates from these GPs who received both the cases and control comments when compared to GPs receiving just a control ($p = 0.18$) or case ($p = 0.16$) comment.

In the 365 days prior to the selection period, 51 (53%) of the 96 controls had a previous LDL-cholesterol measurement. FH was raised as a consideration in 24 (25%), while interpretative comments did not mention FH for the remaining 75% as the LDL-cholesterol was <5.0 mmol/L. Thirty-nine (28%) of the 135 cases had LDL-cholesterol measured in the previous year. Twenty-five (25%) individuals in the fax-case group had a previous measurement, eight of which were >5.0 mmol/L and included a comment that raised FH as a consideration. Fourteen (37%) individuals from the non-fax group had previous measurements, of which ten (25%) were >5.0 mmol/L and included a comment that raised FH as a consideration. The remaining LDL-cholesterol measurements were all below 5.0 mmol/L.

Six (20%) of the individuals referred to the LDC had GPs who had previously been telephoned by a Chemical