Sex determination using sex determining region Y primers by single conventional polymerase chain reaction

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The development of the polymerase chain reaction (PCR) as a means of amplifying known sequences of DNA allows determination of gender by amplification of sequences unique to the Y chromosome. Detection of Y-specific sequences has clinical importance as it would be useful in fetal gender identification in mothers who are carriers for X-linked disorders and in forensic medicine.[1] There were reports dealing with sex determination to detect Y-specific sequences by PCR. Established PCR-based on sex determination methods using the X–Y homologous region of the amelogenin gene,[2] the Y chromosome specific sequences include the Y-specific repeat sequences, DYS14,[3] and the sex determining region Y (SRY).[4] One study used a nested PCR using SRY forward/reverse as external primers and SRY-5'netsed/3'-nested as internal primers[5] for detection of Y-specific sequences. The difficulties with the nested PCR prompted us to develop a single conventional PCR by cross-using the SRY forward/SRY 3'-nested primers.

We prepared genomic DNA from the peripheral blood samples collected from adult males and females in EDTA-containing tubes. Cell-free DNA from plasma to plasma by using QIAamp DNA blood Mini Kits (Qiagen, UK) according to the blood and body fluid protocol with minor modifications. Primers for amplification of 173 bp sequence are as follows:

SRY forward: 5'GTG TCC TCT CGT TTT GTG AC 3,
SRY 3'-nested: 5'CTA GTA CCC TGA CAA TGT ATT C 3'.

We performed PCR amplification in a total volume of 25 µl containing extracted DNA, 200 µM dNTPs, 20 pmol of each primer, 1 x Taq polymerase buffer (containing 1.5 mM $MgCl_2$), and 1 U of Taq polymerase (Roche Biochemicals). The thermal cycling began with denaturation at 94°C for 7 minutes, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for one minute, with final incubation at 72°C for 12 minutes. The PCR amplification products were separated by 8% acrylamide gel electrophoresis and visualized by exposure to ultraviolet light after ethidium bromide staining. A female staff member performed all procedures including blood specimen collection and preparation, DNA extraction and PCR amplification. To determine the specificity of male genomic DNA in a single PCR using SRY forward / SRY 3'-nested primers; we examined 15 male genomic DNA and 15 female genomic DNA samples. All the male samples were positive and produced a 173 bp fragment while the 173 bp fragment was not detectable in the female samples (**Figure 1**). We amplified all female samples giving negative results for the Factor VIII gene, as a positive control (data not shown). To evaluate the sensitivity of the developed assay, we applied the single PCR, using SRY forward/SRY 3'-nested primers, to serial dilutions of 220 ng of male genomic DNA in female genomic DNA. Positive signals were detected up to the 1/10000 dilution, which is almost at the 3 cells level (data not shown).

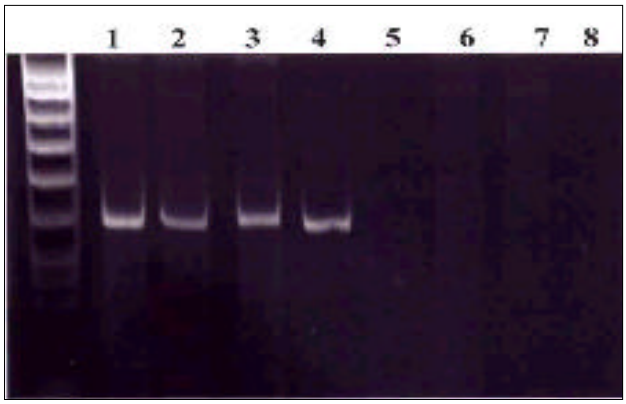

Figure 1 - Photograph of electrophoresed polymerase chain reaction products of 4 males and 3 females genomic DNA. Lane 1 represent molecular weight marker VIII (pUCBM21 DNA cleaved by Hpa II, Dra I and Hind III). Lane 2, 3, 4 and 5 represent male DNA. Lane 6, 7 and 8 represent female DNA.

We developed a new single PCR of a nested PCR for sex determination, which is able to identify Y-specific signals with no false-positives and false-negatives. Honda et al[5] developed a single PCR by use of the internal pair of primers used in the nested PCR devised by Lo et al.[3] This study is an attempt to cross-use one primer from the external pair of primers and another from the internal pair of primers of a nested PCR to develop a single PCR. Although this method is incomparable to real time quantitative PCR, it has overcome the difficulties of nested PCR and provided a feasible methodology with an acceptable specificity and sensitivity. However, the sensitive nature of this assay, which can detect copies at the 3 cells level, implies that great care must be taken to avoid contamination.

Due to the sensitivity of this method it could potentially be used for prenatal sex identification using free fetal DNA in maternal plasma or serum in addition to chorionic villous samples. Therefore, considering the sensitivity of the assay, it could be used in parallel with other Y-specific assays to increase the certainty of the results.

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Serum calcium and phosphorus levels in patients with ischemic heart disease

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Ischemic heart disease (IHD) is estimated to be the leading cause of lost life years until at least 2020.[1] There is a long list of well known risk factors for cardiovascular diseases but limited work has been carried out on the relation of serum calcium and phosphorus levels with IHD, and their results are controversial. Calcium is a vital electrolyte, in normal adults plasma level ranges from 2.2-2.6 mmol/l (8.8-10.4 mg/dl). Plasma concentration of phosphorus is normally 2.5-4 mg/dl. In a case control study of men in Finland with 10 years follow up, there were no significant differences in concentrations of serum calcium, and serum magnesium between cases who died from cardiovascular diseases and controls.[2]

Jorde et al[3] shows that total serum calcium is a predictor of cardiovascular disease in men. We performed this cross-sectional study in order to compare the status of total serum calcium and phosphorus levels in patients with coronary artery disease (CAD) with normal controls. We used a questionnaire for demographic data and we excluded patients with malignancy, renal failure (creatinine >1.5 mg/dl), liver diseases, digestive disorders, hyperthyroidism, and those undergoing treatment with thiazide diuretics. We collected blood samples for measurement of serum total calcium, phosphorus, urea, creatinine, triglyceride, uric acid, cholesterol and fasting blood sugar after overnight fasting, and we determined biochemical parameters with ordinary kits using RA 1000 apparatus.

The patients underwent routine coronary angiography using optimus-M 200 Philips angiography system. The number of the vessels with significant stenosis (>50%) was detected by a cardiologist who was not aware of the investigation. Stenosis less than 50% was known as non-significant lesions and were excluded. We analyzed all data through EPI info 2000 and p values <0.05 were considered as statistically significant, after gathering the samples and data recording in the questionnaire. We studied a total of 230 consecutive patients (149 males and 81 females) admitted for coronary angiography in Shafa Hospital. Their age ranged from 23-78 years with a mean age of 52.8 $\pm$ 11.2 years, 142 (61.7%) patients had CAD (group I) and 88 (38.3%) subjects had normal coronary angiogram, as control group (group II). Single vessel disease was detected in 58 (40.8%), 2 vessel disease 33 (23.3%), and 3 vessel disease 51 (35.9%) of patients with CAD. Mean age was (55.1 $\pm$ 10.2 years in group I, 47.9 $\pm$ 11.6 years in group II). The mean age was significantly higher in group I (p<0.0001). Patients with CAD had diabetes mellitus, high blood pressure, hyperlipidemia, and smoking habitus more than control group, (all p-values <0.05). The mean calcium and phosphorus concentrations in the 2 groups are given in **Table 1**. The concentration of calcium and phosphorus did not significantly differ between patients with CAD and normal controls. Serum calcium and phosphorus levels had no significant association with the number of vessels diseased. We indicated calcium has an important role in the pathogenesis of atherosclerosis, an association between serum calcium and the metabolic syndrome.[4] If such an association exists, one would expect serum calcium to be related to CAD. We suggested high serum total calcium concentration to be an independent risk factor for myocardial infarction in middle aged men.[5] In our study, the mean levels of total serum calcium and phosphorus were not significantly different between the patients with and without