ABSTRACT

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency disease (PID) in which the defect lies at the neutrophil intracellular killing step of the phagocytosis process. It is caused by a dysfunctional respiratory burst enzyme Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-oxidase, a multi-protein complex making CGD a heterogeneous disease. X-linked CGD (X-CGD) is due to a mutation in the *CYBB* gene that codes for gp91-phox, while autosomal CGD is caused by mutations in genes that code for p22-phox, p47-phox, p67-phox, p40-phox and Rac2. The incidence of Klinefelter's Syndrome and CGD occurring together is extremely rare. This study describes a family where the youngest male child suffered from X-CGD while his older brother was both an X-CGD carrier and a Klinefelter. Flow cytometry was used to study the respiratory burst and gp91-phox protein expression in neutrophils, while genetic investigations was done by RT-PCR, PCR and X-chromosome short tandem repeat analysis. The Dihydrorhodamine (DHR) assay revealed that the patient's neutrophils failed to produce a respiratory burst while both the mother and the older brother showed a bimodal response. gp91-phox protein expression mirrored the DHR findings. The patient's cDNA showed a C>T change at nucleotide 676 of the *CYBB* gene and similar change was observed in patient's genomic DNA. The mother and the older brother were heterozygous, with C and T, in this position. The c.676C>T is a nonsense mutation that leads to premature termination of the gp91-phox protein. The brother was karyotyped as 47, XXY and X chromosome analysis revealed that he had inherited both of his mother's X chromosomes. This study is the first to report concurrence of X-CGD carrier and Klinefelter's Syndrome in an individual and therefore argues for family members to be included in PID investigations.

Keywords: Primary Immunodeficiency Disease, Chronic Granulomatous Disease, Klinefelter's Syndrome, NADPH-oxidase, gp91-phox protein

ABSTRAK

‘Chronic Granulomatus Disease’ (CGD) adalah suatu penyakit imunodefisiensi primer (PID) di mana kecacatan wujud pada langkah pembunuhan intrasel dalam proses fagositosis. Ia disebabkan oleh kompleks enzim multi-protein ‘respiratory burst’ yang tidak berfungsi iaitu ‘Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-oxidase’. CGD yang terwaris melalui kromosom X (X-CGD) adalah disebabkan oleh mutasi pada gen *CYBB* yang mengkodkan gp91-phox. Insiden bagi Sindrom Klinefelter dan CGD untuk berlaku serentak adalah amat jarang. Kajian ini adalah berkenaan seorang pesakit lelaki yang mempunyai X-CGD manakala abangnya adalah pembawa X-CGD dan mempunyai Sindrom Klinefelter. Flow sitometri digunakan untuk mengkaji ‘respiratory burst’ dan pengekspresan protein gp91-phox dalam neutrofil manakala kajian genetik dilakukan melalui RT-PCR, PCR dan analisis kromosom X ‘short tandem repeats’. Asai Dihydrorhodamine (DHR) mendedahkan bahawa neutrofil pesakit gagal untuk menghasilkan ‘respiratory burst’, manakala ibu dan abangnya menunjukkan respon bimodal. Pengekspresan protein gp91-phox mencerminkan penemuan DHR. Pesakit menunjukkan perubahan di nukleotida 676 pada gen *CYBB* dari C ke T. Perubahan yang serupa dilihat pada DNA genomik pesakit. Ibu dan abang pesakit adalah heterozigot di mana mereka mempunyai nukleotida C dan T pada kedudukan tersebut. Perubahan c.676C>T adalah mutasi ‘nonsense’ yang membawa kepada penamatan pra-matang protein gp91-phox. Abang pesakit dikariotip sebagai 47, XXY dan analisis kromosom X menunjukkan bahawa kedua-dua kromosom X diwarisi daripada ibunya. Kajian ini adalah yang pertama untuk melaporkan kejadian X-CGD dan Sindrom Klinefelter dalam seseorang individu dan oleh itu berhujah supaya ahli keluarga untuk turut disertakan dalam penyiasatan PID.

Kata-kata kunci: Penyakit Imunodefisiensi Primer, Chronic Granulomatous Disease, Sindrom Klinefelter, NADPH-oxidase, protein gp91-phox

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LIST OF SYMBOLS AND ABBREVIATIONS

Arg	Arginine
BCG	*Bacillus Calmette–Guérin*
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CGD	Chronic Granulomatous Disease
CYBA	Cytochrome b-245, alpha polypeptide
CYBB	Cytochrome b-245, beta polypeptide
DHR	Dihydrorhodamine
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
GVHD	Graft-versus-host-disease
HLA	Human leukocyte antigen
Ig	Immunoglobulin
LAD	Leukocyte Adhesion Deficiency
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibilty complex
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
NCF	Neutrophil cytosolic factor
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
Phox	Phagocyte oxidase
PID	Primary immunodeficiency disease

PMA	Phorbol myristate acetate
RNA	Ribonucleic acid
ROI	Reactive oxygen intermediate
rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
SH3	Src-homology 3
SI	Stimulation index
STR	Short tandem repeat
WBC	White blood cell
g	Gravity force
β-ME	Beta mercaptoethanol