RINGKASAN

Analisis Efek Paparan Panas Suhu Ekstrim Tinggi terhadap DNA yang berasal dari Tulang dan Gigi

Penelitian Biologi Molekuler di Bidang Kedokteran Forensik

Kejadian *Mass Disaster* (Bencana massal) tidak diragukan semakin meningkat jumlah dan skalanya, sehubungan dengan pertumbuhan populasi dunia (Winne, 2001). Tragedi pemboman gedung WTC di New York, serta ledakan bom di negara kita tepatnya di Legian Kuta Bali, yang menelan 182 korban meninggal tahun 2002, menyebabkan korban tidak jarang sulit untuk diidentifikasi. Belum lagi kasus kriminalitas seperti pembunuhan, perkosaan dengan pembunuhan yang semakin hari semakin meningkat pula kuantitas maupun kualitasnya. Upaya untuk menghilangkan jejak, seperti dengan memotong–motong korban menjadi beberapa bagian, ataupun dengan membakar korban yang membawa akibat bagi derajat kerusakan tubuh korban yang hebat, menjadi bukti dari semakin kompleksnya persoalan yang dihadapi oleh ahli forensik berkaitan dengan identifikasi korban, dalam upaya membantu penegakan hukum .(Noto Soehardjo, 1999).

Pada identifikasi korban yang telah membusuk ataupun hangus terbakar, seringkali identifikasi forensik tidak dapat ditegakkan melalui identifikasi asosiatif, ataupun identifikasi konvensional lainnya. Sehingga dibutuhkan cara identifikasi forensik lainnya, yang salah satunya adalah melalui analisis DNA(Kusuma, 2004)

Namun demikian Ahli DNA forensik seringkali dihadapkan pada kondisi spesimen yang kurang baik, seperti halnya spesimen tidak dalam kondisi segar atau *fresh* untuk dilakukan DNA typing (degraded DNA), sehingga jumlah atau kadar DNA yang diperoleh tidak mencukupi bagi sebuah DNA profiling. (Notosoehardjo, 1999). Kenyataan ini membawa ahli DNA forensik untuk mencari bahan analisis DNA forensik yang tahan terhadap faktor dekomposisi (Stimson, 1997). Salah satu alternatif bahan analisis DNA yang masih memungkinkan untuk digunakan sebagai sediaan DNA profiling pada kondisi *degraded DNA* adalah tulang dan gigi.

Tujuan penelitian ini adalah meneliti efek paparan panas suhu ekstrim tinggi terhadap integritas DNA yang berasal dari tulang dan gigi melalui pemeriksaan DNA atau *DNA profiling* pada beberapa lokus DNA inti, yakni lokus TH01, lokus TP0X, lokus VWA dan gene amelogenin untuk penentuan jenis kelamin, serta DNA mitokondria pada produk amplifikasi 143 bp pada daerah hipervariabel 1, dengan menggunakan primer standar maupun dengan menggunakan primer mini.

Hasil penelitian ini menunjukkan bahwa DNA yang berasal dari tulang dan gigi tetap tidak mengalami degradasi hingga suhu 350 ºC, sehingga dapat digunakan sebagai bahan pemeriksaan DNA sebagaimana tersebut di atas. Degradasi DNA tulang dan gigi ditemukan pada efek paparan panas 550ºC-950ºC. Meski demikian pada penelitian ini ditemukan bahwa tidak terdapat masalah yang serius berkaitan dengan pemeriksaan DNA atau DNA profiling

untuk kepentingan idenfikasi DNA forensik dengan menggunakan 3 lokus STR *(Short Tandem Repeat)* dan amelogenin pada suhu hingga 750 ºC, baik dengan menggunakan primer standar (hingga 550 ºC) atau menggunakan primer mini (hingga 750 ºC).

Pada suhu 950 ºC hanya lokus DNA mitokondria pada produk amplifikasi 143 pb yang dapat diamplifikasi, ketika melalui DNA inti tidak memungkinkan dilakukan amplifikasi, disebabkan oleh kondisi DNA inti yang sudah mengalami degradasi. Hal ini memberikan harapan bagi sebuah upaya identifikasi berkaitan dengan identifikasi individu dengan menggunakan DNA mitokondria, dengan berdasarkan penurunan sifat individu secara maternal.

Penelitian ini juga berhasil menemukan bahwa DNA yang berasal dari gigi memiliki resistensi yang lebih tinggi dibandingkan dengan DNA yang berasal dari tulang, meski secara statistik tidak ditemukan perbedaan yang signifikan berkaitan dengan temuan tersebut.

SUMMARY

The analysis of extreme heat exposure effect on bone and teeth DNA

Degraded DNA due to heat exposure is a classical problem not infrequently found during investigation by forensic experts, particularly in such cases of mass disasters involving fires or crime investigations where in an effort to eliminate evidence, the victim is burned as to become unidentifiable. It would thus be of concern for forensic experts in their effort to identify victims or persons exposed to fires using DNA analysis (DNA profiling).

Mass disasters and criminal cases involving burning are undoubtedly increasing, in which the victim were burned beyond recognition such as that what happenend during the WTC tragedy and Bali bombing. In such cases, conventional associative identification methods cannot be used, and victim's identification can only be done using DNA analysis.

In badly burned victims, bone and teeth will be the only material available for DNA analysis, bone and teeth being the hardest part of human body, and thus relatively resistant to heat exposure. However, until recently there were few, if any, specific studies on the effect of extreme heat exposure on the integrity of DNA, either nuclear DNA (nu DNA) or mitochondrial DNA (mtDNA) extracted from tissue.

The aim of this work is to study the effect of extreme heat exposure on bone and teeth's DNA (nuDNA and mtDNA) that will be use for forensic DNA analysis.For this purpose, rib bones and teeth samples were collected from 5 bodies assigned for forensic investigation to the Department of Forensic Medicine, Airlangga University, School of Medicine Surabaya. The samples were divided into 2 groups: group I (experimental group) and group II (control group). Group I was then divided into 8 subgroups each incinerated in porcelain oven at 350°C, 550 °C, 750°C, and 950°C for 20 and 30 minutes respectively. Group II as a control group was not exposed to heat.

The DNA from both groups were then extracted and used for DNA amplification using Polymerase Chain Reaction (PCR). The DNA fragments amplified were 3 Short Tandem Repeat (STR) loci (TPOX, TH01, and VWA), the Amelogenin locus and the Hypervariable 1 (HV1) locus of mtDNA. The Amelogenin gene locus was included as it is used in DNA profiling to determine sex, while the mtDNA HV1 locus was included in case the nuDNA is completely destroyed, mtDNA being present in a great number of copies, not all of which will be destroyed. The primers used are standard primers and mini primers giving a shorter amplicon, having the possibility of being still amplified when DNA is being more heavily degraded.

The primers used were as follow:

Standard primers

TPOX (Promega, Gen Bank Accession M68651):
5'ACT GGC ACA GAA CAG CGA TCT AGG 3' (24 nt)
5'GGA GGA ACT GGG AAC CAC ACA GGT 3'(24 nt)

TH01 (Promega, Gen Bank Accession D00269)
5'CTG GGC ACG TGA GGG CAG CGT CT (23 nt)
5'TGC CGG AAG TCC ATC CTC ACA GTC (24 nt)

VWA (Promega, Gen Bank Accession M25858)
5' CCT AGT GGA TGA TAA GAA TAA TCA GTA TG 3' (29 nt)
5'GGA CAG ATG ATA ATT ACA TAG GAT GGA TGG 3' (30 nt)
Amelogenin (Promega primer, X=212 bp, Y=218 bp):
5'-CTGATGGTTGGCCTCAAGCCTGTG-3'
5'-GGAAGTTAAGAGATTCATTAACTTGACTG-3'
mtDNA (Indra mito, nt 15978-16255, 278 bp):
5'-CACCATTAGCACCCAAAGCT-3'
5'-CTTTGGAGTTGCAGTTGAT-3'
Mini Primer (Butler, 2003)
TPOX (Gen Bank Accession M68651)
5'- CTTAGGGAACCCTCACTGAATG -3'
5'- GTCCTTGTCAGCGTTTATTTGC -3'
TH01 (Gen Bank Accession D00269)
5' CCTGTTCCTCCCTTATTTCCC-3'
5' GGGAACACAGACTCCATGGTG-3'
vWA (Gen Bank Accession M25858)
5' AATAATCAGTATGTGACTTGGATTGA 3'
5' ATAGGATGGATGGATAGATGGA3'
Amelogenin (Promega primer):
5'CCCTGGGCTCTGTAAAGAATAGTG 3' (X=106 bp)
5'ATCAGAGCTTAAACTGGGAAGCTG 3'(Y=112 bp)
Mito mini primer 143 bp (nt16268-16410) (AFDIL primer, Edson et al, 2004)
5'- CAC TAG GAT ACC AAC AAA CC- 3'
5'- GAG GAT GGT GGT CAA GGG AC-3'

The results of this study were as follows:

1. Teeth DNA was found to be more resistant to heat exposure as compared to bone DNA, althouh overall no significant difference was found in the effect of heat effect of heat exposure on teeth and bone DNA.
2. The amount of detected DNA in bone and teeth exposed to heat was found not to differ significantly to that found in bone and teeth not exposed to heat (control) up to 350 ºC and 30 minute exposure, while the amount of detected DNA decreased gradually on exposure to higher temperature (550 ºC-950 ºC).
3. There was basically no serious problems with forensic DNA identification involving bone and teeth DNA exposed to heat up to 750 ºC and 30 minute exposure using 3 STR (TPOX,TH01, VWA) and Amelogenin loci: up to 550 ºC and 30 minute exposure standard primers could be used, while on exposure to higher temperature up to 750 ºC and 30 minute exposure mini primers should be used.

4. mtDNA can still be amplified using mini primers up to 950 ºC and 30 minute exposure, where other loci failed to amplify giving the chance to still identify the unknown person if mtDNA of that person's mother is available by sequencing the 143 bp amplicon.

In conclusion, it was found in this study that the identity of an unknown person can still be reasonably identified by using bone or teeth DNA exposed to extremely high temperature up to around 1000 ºC(950 ºC). However it should be mentioned that exposure was limited to only 30 minute, whether the identity of unknown person exposed to higher temperature and longer duration can still be revealed will require further studies

ABSTRACT

The analysis of extreme heat exposure effect on bone and teeth DNA

In Forensic Medicine, there are cases where identity of an unknown person using conventional assosiative identification method is not feasible. These are cases in which the unknown person's body is badly burned such as the case of WTC tragedy and Bali bombing or criminal cases, in which the victim'body is burned to eliminate evidence. In such cases the only method to reveal the identity of that person is by DNA profiling. In badly burned victims, bones and teeth are often the only tissue available for DNA analysis. Furthermore, there will also be the problem of DNA degradation due to heat exposure.

The aim of this study is to investigate whether the identification by DNA profiling is still possible under those conditions. For this purpose, rib bones and teeth DNA were collected from 5 bodies assigned for forensic investigation. The samples were then divided into 2 groups: experimental group (Group I) and the control group (Group II). Group I further divided into 8 sub groups, each of which was exposed to high temperatures at 350°C, 550 °C, 750 °C, 950 °C for 20 and 30 minutes respectively, while group II was not exposed to heat.The DNA from both groups were then extracted and used for DNA amplification using polymerase chain reaction (PCR). The DNA fragments amplified were 3 short tandem repeat (STR) loci:TPOX, TH01,vWA, the amelogenin gene locus and the hypervariable 1(HV1)locus of mitochondrial DNA (mtDNA). The amelogenin gene locus was included to determine sex while the HV1 mtDNA locus was included in case nuclear DNA (nuDNA) is completely destroyed, mtDNA being present in a great number of copies some of which will be hopefully spared. The primer used are standard primer and mini primer, giving shorter amplicons thus having the possibility of still being amplified when DNA was heavily degraded.

The results of this study revealed that bones and teeth DNA remain intact up to 350 °C being further and further degraded on exposure to higher temperature (550 °C-950 °C). Further it was found that there were no serious problem with the use of DNA profiling to 750 °C, using either standard primers (up to 550 °C) or mini primers (up to 750 °C). At 950 °C, only the mtDNA locus can be amplified, suggesting that at this temperature nuclear DNA is heavily or completely degraded as to be impossible to amplify even using mini primers. At this temperature (950 °C) identification is still possible by sequencing the mtDNA 143 bp amplicon and comparing to that of the victim's mother or next of kin supplied with other available information associated with the victim. Finally, it was also found that while teeth DNA is more heat resistant as compared to bone DNA, no significant difference of the results were found whether teeth or bone DNA is used for DNA profiling.

Keywords: DNA profiling, bone and teeth DNA, heat exposure, degraded DNA, criminal cases, mass disasters.