ANALISIS DNA TULANG DAN GIGI PADA LOKUS *SHORT TANDEM REPEAT-COMBINED DNA INDEX SYSTEM* (STR-CODIS), *Y-CHROMOSOME STRs & MITOCHONDRIA DNA* (*mtDNA*) AKIBAT EFEK PAPARAN PANAS SUHU TINGGI

YUDIANTO, AHMAD
Promotor : **Prof.Dr.Med.HM.Soekry Erfan Kusuma,dr.,SpF(K).,DFM**
BONE AND DENTAL DNA ; CHROMOSOME
KKA KK Dis K 30/12 Yud a

ABSTRACT

ANALYSIS OF HIGH-TEMPERATURE EXPOSED BONE AND DENTAL DNA OF LOCI STR CODIS, Y-CHROMOSOME STRs AND mtDNA

Ahmad Yudianto

High-temperature exposure is one of factors for DNA degradation. Bone and dental tissues are among materials most resistant to this DNA degradation. Bone and teeth are the most solid of human body due to its containing hydroxyapatite and extracellular matrices that provides protection for DNA (nuclear DNA and mtDNA). To date, DNA degradation due to the effect of high-temperature exposure on samples of bone and dental DNA in forensic identification has been extensively unknown.

The purpose of the present research was to analyze and elucidate DNA loci detectable subsequent to high-temperature exposure to samples of bone and dental DNA on the basis of loci STR CODIS, Y-STRs, and mtDNA.

Laboratory experimental in order to analyze DNA degradation of bone and dental materials due to effect of high-temperature exposures (500°C, 750°C, 1000°C, and 1250°C for 20, 30, and 40 minutes) on the basis of the loci STR and *mini*-STR CODIS (CSF1PO, D18S51, D21S11, FGA, D8S1179, D5S820, D7S820, D13S317, D16S539), Y-STRs (DYS19, DYS389, DYS390) and 143-bp and 126-bp mtDNA. Samples consisted of 24 ribs and 24 molars from 7 cadavers.

Teeth were more resistant than bones in protecting DNA from high-temperature exposure. This could be seen in the number of presentation positively detected from loci STR, Y-STRs and mtDNA. Loci of STR CODIS of bone materials detected by standard primer were D3S1358, D16S539 (1250°C-20') and CSF1PO (500°C-40'); those of dental materials were D7S820, D8S1179 (1250°C-40'), D3S1358 (1250°C-20'), D13S317 (1000°C-40'), D16S539 (750°C-40'), CSF1PO (750°C-20'). Loci of STR CODIS of bone materials detected by mini

primer were D16S539 (750°C-40'), CSF1PO, D12S137 (500°C-40'), and D3S358 (500°C-30'); those of dental materials were CSF1PO (1250°C-40'), D16S539 (1000°C-20'), D13S317 (750°C-40'), D3S1358 (750°C-20'), D5S818, D7S820, D8S1179, D18S51 (500°C-40'). The detected locus of Y-STRs of bone materials was DYS389I (1250°C-20'); that of dental materials was DYS389I (1250°C-40'). mtDNA was detected at 143 bp (750°C-40' for bone materials and 1250°C-30' for dental materials) and at 126 bp (750°C-40' for bone materials and 1000°C-30' for dental material).
Undetected mini primer on DNA amplification of high-temperature exposed bones and teeth might be due to complete degradation resulting in DNA fragments to lose their annealing sites of primer. Successful detection of those loci at the maximum exposure of the research was supported by differences in amplicon products, GC content. In publication, The ratio of GC content for CSF1PO was 42.6%, D8S1179 was 30.9% and D7S820 was 28.6%, and had power discriminant of different. In conclusion, dental materials that remained capable of detection were loci D7S820 and D8S1179 with standard primer, CSF1PO with mini primer and DYS389I at the maximum temperature exposure (1250°C for 40 minutes) of the research.

Keywords: High-temperature exposure, STR-mini STR CODIS, Y-STRs, mtDNA, bone and dental DNA.

RINGKASAN

ANALISA DNA TULANG DAN GIGI PADA LOKUS *SHORT TANDEM REPEAT-COMBINED DNA INDEX SYSTEM* (STR-CODIS), Y-*CHROMOSOME* STRs & *Mitochondria* DNA (*mtDNA*) AKIBAT EFEK PAPARAN SUHU TINGGI

AHMAD YUDIANTO

Seringkali proses pemeriksaan analisis DNA dihadapkan pada kondisi bahan atau spesimen DNA tidak dalam kondisi segar atau *fresh* untuk dilakukan DNA *typing* atau dikenal dengan istilah *DNA degraded* (degradasi DNA) (Butler *et al*, 2003). Kondisi degradasi DNA terutama dijumpai pada kasus dengan jenasah terbakar yang hebat. Kondisi spesimen mengalami degradasi DNA akibat paparan suhu tinggi juga merupakan suatu kendala dalam analisis DNA.

Upaya untuk mengatasi identifikasi dengan DNA yang mengalami kerusakan adalah dengan merancang produk amplikon yang lebih pendek dibandingkan dengan yang sering digunakan sebelumnya, yang dapat diperoleh dengan penggunaan *mini primer STR set*. *Mini primer STR* ini pada sampel dalam kondisi DNA yang terdegradasi masih dapat diamplifikasi dengan *Polymerase Chain Reaction* (PCR) sehingga

identifikasi forensik masih dapat dilakukan (Coble & Butler, 2005). *Mini primer* untuk *mtDNA* ditujukan pada daerah *hypervariable* 1 (HV1) ataupun (HV2) *displacement-loop* (d-loop), sehingga didapatkan amplikon dengan ukuran yang lebih pendek (Gabriel *et al*, 2001). Sejauh ini identifikasi forensik molekuler pada kerusakan DNA sebagai efek paparan suhu tinggi pada sampel DNA tulang dan gigi dalam belum banyak diketahui.

Diharapkan dari hasil penelitian yang akan dilakukan ini membantu memecahkan berbagai kasus forensik yang melibatkan pemeriksaan DNA forensik dengan spesimen DNA inti dan mitokondria yang terdegradasi sebagai akibat paparan suhu tinggi, serta dapat memberi informasi ketahanan tulang dan gigi dalam melindungi DNA didalamnya terhadap paparan suhu tinggi.

Penelitian ini bertujuan untuk menganalisis lokus DNA tulang dan gigi yang masih dapat terdeteksi sebagai efek paparan suhu 500^0C, 750^0C, 1000^0C dan 1250^0C selama 20,30 dan 40 menit pada sampel DNA pada identifikasi forensik molekuler, berdasarkan lokus STR CODIS dan *mini primer STR CODIS* (D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 dan D21S11), Y-STRs (DYS19, DYS389 dan DYS390) serta *mtDNA* 143 bp dan 126 bp

Jenis Penelitian ini adalah eksperimental laboratories dengan rancangan penelitian *Randomized post test only control group design.* Sampel penelitian yakni DNA yang berasal dari tulang rusuk dan gigi molar dua yang diambil dari jenasah yang terlantar di Departemen/Instalasi Ilmu Kedokteran Forensik & Medikolegal FK-UNAIR/RSUD dr Soetomo Surabaya dengan melalui uji kelaikan etik. Dari hasil perhitungan diketahui jumlah sampel yang diperlukan 24 tulang rusuk dan 24 gigi molar 2. Sampel berasal dari 7 jenasah.

Dari hasil penelitian, pengukuran berat sampel menunjukkan adanya penurunan 65.1%-91.8% tulang setelah perlakuan berbagai suhu (500^0C, 750^0C, 1000^0C dan 1250^0C) dan waktu (20', 30' dan 40') sedangkan gigi 28,6%-66,7%. Hasil pengukuran kadar DNA dengan menggunakan UV-Spektrofotometer menunjukkan adanya pengaruh perlakuan paparan suhu dan waktu yang bermakna terhadap penurunan kadar DNA tulang (p:0.000) dan gigi (p:0.000).

Hasil deteksi DNA lokus STR CODIS efek paparan suhu tinggi (suhu 500^0C, 750^0C, 1000^0C dan 1250^0C selama 20', 30' dan 40') pada bahan **tulang** dengan *primer* standar, yang masih terdeteksi adalah D3S1358 (41,67%), CSF1PO (16,67%) dan D16S539 (58,53%). Dari ke 3 lokus tersebut, yang menunjukkan adanya pengaruh interaksi bermakna akibat efek perlakuan (suhu dan waktu papaan) hanya lokus CSF1PO (p: 0.018). Pada bahan **gigi** hasil deteksi DNA setelah paparan (suhu 500^0C, 750^0C, 1000^0C dan 1250^0C selama 20', 30' dan 40') adalah D3S1358 (41,67%), CSF1PO (29,17%), D7S820 (62,50%), D8S1179 (66,67%), D13S317 (37,50%), D16S539 (41,67%). Dari ke 6 lokus pada gigi yang menunjukkan adanya pengaruh interaksi bermakna akibat efek perlakuan (suhu dan waktu paparan) hanya lokus CSF1PO (p: 0.037).

Pada penggunaan *mini primer*, hasil deteksi DNA lokus STR CODIS efek perlakuan yang masih terdeteksi pada bahan **tulang** adalah D3S1358 (8,33%), CSF1PO (12,50%), D13S317 (12,50%) dan D16S539 (37,50%), sedangkan pada bahan **gigi** adalah D3S158 (16.67%), FGA (7.12%), CSF1PO (41,67%), D5S818 (7,12%), D7S820 (7,12%), D8S1179 (7,12%), D13S317 (45,63%), D16S539 (29,17%), D18S51 (7,12%). Dari ke 9 lokus STR CODIS pada bahan gigi menunjukkan adanya pengaruh interaksi bermakna akibat efek perlakuan hanya lokus D13S317 (p: 0.033).

Hasil deteksi DNA lokus Y-STRs akibat efek paparan suhu tinggi yang masih terdeteksi pada bahan **tulang** hanya lokus DYS389I (58,30%), sedangkan pada bahan **gigi** adalah DYS19 (8,33%), DYS389I (58,33%) dan DYS390 (4,16%). Dari ke 3 lokus Y-STR bahan gigi menunjukkan adanya pengaruh interaksi bermakna akibat efek perlakuan hanya DYS19 (p: 0.018).

Hasil deteksi *mtDNA* 143 bp akibat efek paparan suhu tinggi yang masih terdeteksi pada bahan **tulang** adalah 25%, sedangkan pada bahan **gigi** adalah 54.50%, sedangkan pada *mtDNA* 126 bp bahan **tulang** adalah 25% dan bahan **gigi** adalah 41,70%.

Hasil uji *Chi Square* pada kekuatan deteksi DNA bahan tulang dan gigi pada lokus STR CODIS dan *mtDNA* dengan dan tanpa perlakuan menunjukkan terdapat perbedaan yang bermakna terhadap deteksi lokus D7S820 (p:0.000), D8S1179 (p:0.000) dan D13S317 (p:0.013) dan deteksi *mtDNA* 143 bp (p:0.006). Hasil uji *Chi Square* pada kekuatan deteksi DNA bahan tulang lokus STR CODIS pada penggunaan *primer* standar dan *mini primer* dengan dan tanpa perlakuan,yang menunjukkan perbedaan bermakna pada tulang hanya lokus D3S1358 (p:0.020), sedangkan pada bahan gigi lokus D7S820 (p:0.001) dan D8S1179 (p:0.000).

Kerusakan DNA yang disebabkan oleh paparan-paparan yang abnormal contohnya suhu yang tinggi, (Watson *et al*, 1987) disebabkan oleh rusaknya *hydrogen bond* DNA yang *irreversible*. Paparan yang ada ini mengakibatkan kerusakan pasangan purin-primidin pada DNA. Pasangan purin-primidin merupakan komponen utama pada struktur DNA, dimana adenin selalu berpasangan timin dan guanin selalu berpasangan sitosin. Efek lingkungan dalam hal ini suhu serta lama paparan dalam penelitian ini terbukti berpengaruh terhadap kadar DNA yang terkandung diukur dengan Spektrofotometri menunjukkan penurunan kadar pada sampel-sampel DNA tulang dan gigi yang cukup signifikan. Adanya penurunan kadar tersebut, bukan merupakan suatu hambatan pemeriksaan DNA lebih lanjut sebab kadar-kadar DNA yang tersisa masih memungkinkan untuk dilakukan pemeriksaan *DNA profiling*. Dikemukan bahwa pemeriksaan *DNA profiling* memerlukan kadar DNA minimal 50 ng (Notosoehardjo,1999). Pada penelitian lain mengemukan bahwa kadar DNA yang dibutuhkan pada pemeriksaan STR berbasis *Polymerase Chain Reaction* (PCR) minimal adalah berkisar antara 0,25-2 ng (Simun *et al*, 2005). Di samping kadar DNA sampel pada pemeriksaan DNA berbasis PCR juga perlu dipertimbangkan kualitas DNA yang mencukupi. Kualitas DNA yang

dimaksud yakni DNA yang digunakan dalam analisis harus dalam kondisi yang tidak terdegradasi. Jika DNA mengalami degradasi parah, maka akan mengakibatkan *primer* tidak dapat menempel (*annealing)* pada target DNA yang akan digandakan.

Pada penelitian ini dilakukan analisis STR, karena pada umumnya sampel-sampel forensik yang akan dilakukan pemeriksaan DNA, 40% sudah mengalami degradasi atau kontaminasi (Notosoehardjo, 1999b). Analisis STR pada DNA yang mempunyai *core sequences* kurang 1 kb (*kilobase*) sangat efektif dengan nilai keberhasilan cukup tinggi, terutama pada DNA yang mengalami degradasi/ terfragmentasi menjadi fragmen yang pendek-pendek. Hal ini dibuktikan dengan terdapat PCR yang positif pada kontrol DNA tak terpapar

Kegagalan deteksi fragmen DNA dengan *primer* STR CODIS pada penelitian dengan paparan suhu tinggi diatas 1000^0C ini, dilanjutkan dengan pemeriksaan menggunakan *mini primer* STR CODIS yang akan mengamplifikasi fragmen DNA yang lebih pendek. Penggunaan *mini primer* ternyata memiliki potensi yang tinggi untuk mendeteksi fragment DNA tersebut. Hal ini memberikan gambaran bahwa paparan suhu tinggi yang merusak DNA dapat menyebabkan kegagalan dalam proses identifikasi secara keseluruhan. Untuk *mtDNA* amplifikasi dengan *mini primer* akan menghasilkan fragmen dengan ukuran daerah HV1 ataupun HV2 lebih pendek (Gabriel, 2001; Butler, 2003).

Kegagalan deteksi dalam pemeriksaan DNA dengan metode PCR dapat disebabkan beberapa faktor (Bartlett dan Stirling, 2003) yaitu sedikitnya jumlah DNA target, DNA target telah mengalami degradasi atau *damage*, enzim DNA *polymerase* yang tidak mencukupi, kurangnya siklus PCR atau adanya inhibitor PCR.

Hasil penelitian ini menunjukkan bahwa terdapat pengaruh paparan suhu tinggi terhadap kadar DNA tulang dan gigi, namun dengan beberapa lokus STR CODIS, Y-STRs dan *mtDNA* masih dideteksi. Lokus DNA yang masih terdeteksi pada paparan maksimal penelitian (1250^0C-40') dengan menggunakan *primer* standar hanya dari bahan **gigi** yakni: D7S820, D8S1179 dan DYS389I serta CSF1PO menggunakan *mini primer*, sehingga keempat lokus tersebut merupakan temuan baru dalam penelitian ini. Letak lokus-lokus tersebut adalah pada autosomal kromosom (STR: D7S820, D8S1179, CSF1PO) dan Y-STRs (DYS389I), sehingga metode pemeriksaan melalui lokus ini sangat potensial dimanfaatkan untuk pemeriksaan identifikasi terutama dalam kondisi sampel terdegradasi akibat efek paparan suhu tinggi.

Dalam hasil penelitian ini gigi, memiliki kekuatan yang lebih kuat karena faktor kandungan *hydroxyapatite* dan kadar '*mineral hard tissue'* yang lebih tinggi pada gigi daripada tulang, sehingga mampu melindungi DNA dalam gigi. Disamping itu pula gigi memiliki mineral sekunder penting yang lebih tinggi dari tulang yakni : *calcite*, *limonite*, *pyrite* dan *vivianite* sehingga gigi memiliki suatu pertahanan atau perlindungan yang lebih kuat terhadap pengaruh-pengaruh dari luar.

Perbedaan lokus STR yang dapat dideteksi efek paparan suhu tinggi pada sampel DNA tulang dan gigi, adalah karena adanya perbedaan *GC content* masing-masing lokus. Menurut Bartlett dan Stirling (2003) serta Muladno (2002), *GC content* atau ikatan guanin sitosin memiliki tingkat kestabilan yang tinggi terhadap factor denaturasi dibandingkan dengan ikatan antara adenin dan timin

Menurut Butler (2003) kegagalan deteksi DNA sampel pada pemeriksaan forensik dapat disebabkan adanya degradasi DNA yaitu keutuhan DNA berkurang sehingga sulit diamplifikasi. Kegagalan amplifikasi DNA dengan *mini primer* DNA tulang maupun gigi yang terpapar suhu tinggi, diakibatkan karena DNA sampel telah mengalami degradasi lebih parah sehingga tidak memungkinkan lagi *mini primer* menempel pada fragmen DNA. *Mini primer* merupakan alternatif sebagai pengganti *primer* standar dalam kondisi DNA mengalami degradasi, dimana *primer* standar pada kondisi tersebut memberikan keberhasilan rendah (Chung ***et al***, 2004; Butler, 2003).

Kesimpulan dalam penelitian pengaruh berbagai paparan suhu ini, adalah hasil amplifikasi lokus STR CODIS pada bahan tulang yang terdeteksi dengan *primer* standar: D3S1358 dan D16S539 (paparan suhu 1250^{0}C-20'); CSF1PO (paparan suhu 500^{0}C-40') dan pada bahan gigi: D7S820 dan D8S1179 (paparan suhu 1250^{0}C-40'); D3S1358 (paparan suhu 1250^{0}C-20'); D13S317 (paparan suhu 1000^{0}C-40'); D16S539 (paparan suhu 750^{0}C-40'); CSF1PO (paparan suhu 750^{0}C-20').

Deteksi lokus STR CODIS pada bahan tulang dengan *mini primer*: D16S539 (paparan suhu 750^{0}C-40'); CSF1PO dan D13S317 (paparan suhu 500^{0}C-40'); D3S1358 (paparan suhu 500^{0}C-30'), sedangkan dari bahan gigi: CSF1PO (paparan suhu 1250^{0}C-40'); D16S539 (paparan suhu 1000^{0}C-20'); D13S317 (paparan suhu 750^{0}C-40'); D3S1358 (paparan suhu 750^{0}C-20'); D5S818, D7S820, D8S1179 dan D18S51 (paparan suhu 500^{0}C-40').

Lokus Y-STRs pada bahan tulang yang terdeteksi: paparan suhu 1250^{0}C-20' dan pada bahan gigi paparan suhu 1250^{0}C-40' adalah DYS389I. Untuk *mtDNA* 143 bp dari bahan tulang masih terdeteksi pada paparan suhu 750^{0}C-40' dan dari bahan gigi pada paparan suhu 1250^{0}C-30', sedangkan *mtDNA* 126 bp dari bahan tulang masih terdeteksi setelah paparan suhu 750^{0}C-40' dan bahan gigi setelah paparan suhu 1000^{0}C-30'

SUMMARY

ANALYSIS OF HIGH-TEMPERATURE EXPOSED BONE AND DENTAL DNA OF LOCI STR CODIS, Y-CHROMOSOME STRs AND mtDNA

Process of DNA analysis is frequently confronted by a condition of non-fresh specimens of DNA examination for DNA typing, a condition being called *degraded DNA* (Butler *et al*. 2003). For the most part, condition of degraded DNA is found in severely burnt human corpses. Degraded DNA due to high-temperature exposure also brings with it a challenge in DNA analysis.

The challenge in the identification of degraded DNA was addressed by the use of shorter amplicons than those commonly used previously, the ones being called *mini STR set*. The mini STR set in samples of degraded DNA remained to be capable of amplification by the use of polymerase chain reaction (PCR), enabling forensic identification (Coble and Butler, 2005). In addition, the size of the region hypervariable 1 (HV1) or hypervariable 2 (HV2) at the displacement loop (d-loop) of mitochondrial DNA was reduced as its amplicons (Gabriel *et al*. 2001). To date, degraded DNA of bone and dental samples due to the effect of high-temperature exposure in molecular forensic identification has been extensively unknown.

Results of the present investigation were expected to be useful in solving forensic cases involving DNA forensic examination for nuclear DNA and mitochondrial DNA degraded by effect of high-temperature exposure and to provide information on bone and dental resistance in providing protection for DNA therein against high-temperature exposure.

The purpose of the present research was to analyze and elucidate DNA loci remained to be capable of detection subsequent to high-temperature exposures of 500°C, 750°C, 1000°C and 1250°C for 20, 30, and 40 minutes on samples of bone and dental DNA in molecular forensic identification on the basis of loci STR CODIS and mini-STR CODIS (CSF1PO, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51 and D21S11), Y-STRs (DYS19, DYS389 and DYS390), 143-bp and 126-bp mtDNA.

The present research was of laboratory experimental by means of *randomized post test only control group design*. Sample was DNA originated from ribs and molars of abandoned cadavers in the Department of Forensic Medicine and Medico-legal of Airlangga School of Medicine/Dr. Soetomo General Hospital Surabaya. Sample consisted of 24 ribs and 24 molars from 7 cadavers.

Weighing of sample mass indicated a post-treatment reduction of 65.1% to 91.8% for bone and 28.6% to 66.7% for teeth. Quantification of DNA contents by means of UV-spectrophotometer indicated an effect of treatment (temperature exposure) on a decrease in content of bone (p: 0.000) and dental (p:0.000) DNA.

DNA detection with standard primer of locus STR CODIS for high-temperature exposed (500°C, 750°C, 1000°C, and 1250°C for 20, 30, and 40 minutes) on bone materials indicated loci remaining detectable were D3S1358 (41.67%), CSF1PO (16.67%), and D16S539 (58.53%), Of those three loci, it was only CSF1PO that indicated a presence of a significant ($p = 0.018$) interactional effect of the treatment (temperature and duration exposure). DNA detection for dental material subsequent to exposure (500°C, 750°C, 1000°C, and 1250°C for 20, 30, and 40 minutes) indicated that loci remaining detectable were D3S1358 (41.67%), CSF1PO (29.17%), D7S820 (62.50%), D8S1179 (66.67%), D13S317 (37.50%), D16S539 (41.67%). Of those six loci, it was only CSF1PO that indicated a presence of a significant interactional effect of the treatment.

DNA detection with mini primer of locus STR CODIS for high-temperature exposed bone materials indicated that locus remaining detectable was D3S1358 (8.33%), CSF1PO (12.50%), D13S317 (12.50%) and D16S539 (37.50%); whereas for dental material, it was D3S1358 (16.67%), FGA (7.12%), CSF1PO (41.67%), D5S818 (7.12%), D7S820 (7.12%), D8S1179 (7.12%), D13S317 (45.63%), D16S539 (29.17%) and D18S51 (7.12%). Of those nine loci of STR CODIS for dental materials, it was only D13S317 that indicated a presence of a significant interactional effect of the treatment (p: 0.033).

DNA detection of locus Y-STRs for high-temperature exposed bone materials indicated that only locus remaining detectable was DYS389I (58.30%); whereas for dental materials, it was DYS19 (8.33%), DYS389I (58.33%) and DYS390 (4.16%) . Of those three loci of Y-STRs for dental materials, it was only DYS19 that indicated a presence of a significant interactional effect of the treatment (p: 0.018).

DNA detection of mtDNA 143 bp for high-temperature exposed bone materials indicated that locus remaining detectable was 25%; whereas it was only 54.50% for dental materials. DNA detection of mtDNA 126 bp for high-temperature exposed bone materials indicated that locus remaining detectable was 25%; whereas it was only 41.70% for dental materials.

The Chi-square test of the robustness of DNA detection of bone and dental materials of locus STR CODIS with and without treatment indicated a significant difference for locus D7S820 (p: 0.000), D8S1179 (p: 0.000), D13S317 (p: 0.013) and mtDNA 143 bp (p: 0.006). The Chi-square test of the robustness of DNA detection with standard primer and mini primer of bone materials of locus STR CODIS with and without treatment indicated a significant difference for locus D3S1358 (p: 0.020); whereas it was only locus D7S820 (p: 0.001) and D8S1179 (p: 0.000) for dental materials.

DNA degradation subsequent to abnormal exposures, such as high temperature, results from irreversibly damaged hydrogen bond of DNA. Those exposures lead to damaged purine-pyrimidine pairs of DNA.

Purine-pyrimidine pairs are the main components of DNA structure, in which adenine always pair with thymine and guanine with cytosine. The present research demonstrated environmental effects of temperature and duration of exposure on DNA levels. Spectrophotometry indicated adequately significant decreases in DNA levels of samples of bone and dental materials. Those decreases in DNA levels did not represent an obstacle to further DNA analysis since the remaining DNA levels allowed DNA profiling. It was suggested that DNA profiling requires minimally DNA level of 50 ng (Notosoehardjo, 1999). Other reports indicated that DNA level required for PCR-based STR analysis was minimally in a range of 0.25 to 2.0 ng (Simun et al, 2005). In addition to DNA level of the samples, an adequate quality of DNA was needed to be taken into account. In this case, DNA used in the analysis must not be in a degraded condition. Severely damaged DNA caused primers incapable of annealing the target DNA to be amplified.

The present research used STR analysis since 40% of forensic samples for DNA identification were degraded or contaminated (Notosoehardjo, 1999b). STR analysis of DNA with core sequences of less than 1 kb was effective and had an adequately high rate of success, particularly those DNA being degraded into short segments. This was demonstrated by the presence of positive PCR for unexposed DNA controls.

Failure in detecting DNA fragments by the use of primers of STR CODIS exposed to high temperature above 1000°C in the present research warranted analyses by means of mini primers of STR CODIS that would amplify shorter DNA fragments. In fact, the use of mini primers possessed a high potential to detect those DNA fragments. This provided an indication that high-temperature exposure that degraded DNA was capable of leading to failure in the overall identification processes. *mt*DNA amplification by means of mini primers would produce fragments with shorter regional sizes of HV1 or HV2 (Gabriel, 2001; Butler, 2003).

Failure of detection in DNA analysis with PCR was caused by several factors (Bartlett and Stirling, 2003). Those factors were limited amount of target DNA, degraded or damaged target DNA, limited quantity of DNA polymerase, inadequate PCR cycles, and presence of PCR inhibitors.

This research indicated presence of an effect of high-temperature exposure on DNA levels of bone and dental materials, but with several loci of STR CODIS, Y-STRs, and *mt*DNA remained detectable. The DNA loci remaining detectable with the use of standard primers at the maximum exposure of the research (1250°C for 40 min) were only of dental materials, namely D7S820, D8S1179, DYS389I and CSF1PO (with mini primer), which used set so that those four loci represented novel findings in the this research. Sites of those loci were at autosomal chromosomes (STR: D7S820, D8S1179, CSF1PO) and Y-STRs (DYS389I) so that an analytical method through those loci would be

highly potential for the purpose of identifying primarily degraded materials due to an effect of high-temperature exposure.

Findings of the present research indicated that dental materials were more solid due to the fact hydroxyapatite and mineral hard tissue that dental materials had higher contents and levels of tissues than bone materials that were capable of preserving DNA in dental materials. Additionally, teeth had a higher content of an important secondary mineral, calcite, limonite, pyrite and vivianite which constituted a more resistant protection against external influences.

Difference in loci of STR capable of detection in samples of high-temperature exposed bone and dental DNA was due to a difference in GC of individual loci. GC content or guanine-cytosine bonds had high extent of stability to denaturing factors relative to adenine-thymine bonds (Bartlett and Stirling, 2003; Muladno, 2002).

Failure in detection of DNA samples in forensic identification could result from DNA degradation, in which DNA was of less integrity that made it difficult to be amplified (Butler, 2003). Failure in DNA amplification with mini primers of high-temperature exposed bone and dental DNA was caused by the fact that the DNA was severely degraded that made it impossible for mini primers to anneal DNA fragments. Mini primer constituted an alternative to standard primer with regard to degraded DNA, since the use of the latter demonstrated a low rate of success (Chung et al, 2004; Butler, 2003)

In conclusion, amplification with standard primers of loci STR CODIS for bone materials detected locus of D3S1358 and D16S539 (at 1250°C for 20 min), CSF1PO (at 500°C for 40 min) and it was D7S820 and D8S1179 (at 1250°C for 40 min), D3S1358 (at 1250°C for 20 min), D13S317 (at 1000°C for 40 min), D16S539 (at 750°C for 40 min), CSF1PO (at 750°C for 20 min), for dental materials. DNA detection with mini primer of locus STR CODIS for bone materials indicated that locus remaining detectable was D16S539 (at 750°C for 40 min) and it was CSF1PO (at 1250°C for 40 min), D16S539 (at 1000°C for 20 min), D13S317 (at 750°C for 40 min), D3S1358 (at 750°C for 20 min), D5S818, D7S820, D8S1179 and D18S51 (at 500°C for 40 min) for dental materials. DNA detection of locus Y-STR for temperature-exposed bone materials indicated that locus DYS389I remaining detectable was 1250^0C for 20 min and for dental material was 1250^0C for 40 min. *mt*DNA 143 bp of bone material remained detectable at 750°C for 40 min and at 1250^0C for 30 min for dental materials. And *mt*DNA 126 bp of bone material remained detectable at 750°C for 40 min and at 1000^0C for 30 min for dental materials.