CHARACTERIZATION OF CELL-FREE DNA IN THE BLOODSTREAM OF NORMAL INDIVIDUALS AND THOSE WITH INFLAMMATION

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THOSE WITH INFLAMMATION

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NOMENCLATURE

Q-TAT	Quantitative Template Amplification Technology
PCR	Polymerase Chain Reaction
nDNA	Nuclear DNA
mtDNA	Mitochondrial DNA
cfDNA	cell-free DNA
CRP	C - reactive protein
ng	nanogram
pg	picogram
µl	Microliters
DNA	Deoxyribonucleic Acid
RFU	Relative Fluorescent Unit
STR	Short Tandem Repeat
SRY	region that determines sex on the Y chromosome
Amelogenin	gene that determines sex

AMEL X	Amelogenin X
AMEL Y	Amelogenin Y
HPRT	hypoxanthine phosphoribosyl transferase
Bp	base pair

CHAPTER I

INTRODUCTION

Since the discovery of cell-free DNA floating in the plasma of all humans (Mandel, 1948), scientists have been trying to explain how DNA ends up liberated from cells into a soluble form and to find a diagnostic use for this occurrence. In the years since the discovery of cell-free DNA, there still has been no agreement on the pathway of DNA liberation. Scientists have proposed the following mechanisms underlying the presence of cell-free DNA in plasma: apoptosis, necrosis, terminal differentiation and active secretion (Lichtenstein, Melkonyan, Tomei, & Umansky, 2001). There are many arguments for and against each proposed mechanism for DNA liberation.

While the exact method of DNA liberation is still unclear, the usefulness of cell-free DNA for diagnostic purposes has not remained in question. In recent years there have been many diagnostic uses for the levels of cell-free DNA in patient plasma. Increased levels of cell-free DNA in patients have been used to help diagnose different types of cancer (Sozzi *et al.*, 2003). Another use for cell-free DNA is for genetic testing for unborn fetuses and other problems associated with pregnancy (Lo, 2000), due to the fact that fetal DNA is present in maternal plasma.

There have also been several studies linking the levels of cell-free DNA to different disease states that are associated with inflammation. High levels of cell-free DNA have been linked to physical over training (Fatouros *et al.*, 2006) and to more serious conditions such as septic shock (Moreira, Prieto, Rodriguez, & Alvarez, 2010)." The studies by Fatouros *et al.* (2006) and Moreira et al (2010) compared levels of cell-free DNA in normal healthy patients and in patients with known disease states, measured by currently accepted methods.

In the study conducted by Fatouros *et al.* (2006), cell-free DNA (cf-DNA) was measured to identify a potential link between cfDNA and cell breakdown caused by overtraining. The study was conducted using 17 healthy males undergoing an increasingly strenuous exercise regimen. The experiment concluded that there was no correlation between cfDNA and the currently used clinical markers for inflammation and cell breakdown (CRP, Creatine Kinase, and Uric Acid). The authors did conclude that there was a connection between cfDNA and overtraining and that further study was warranted (Fatouros, *et al.*, 2006).

The study by Moreira *et al.* focused on cfDNA levels of patients with a varying degree of fever, ranging from localized infections to complete septic shock. The study was conducted using 110 patients who were diagnosed at the hospital with fever, and a control group. Patients were split into four groups based on their grade of fever/infection:

1. Fever of unknown origin
2. Localized infection
3. Sepsis
4. Septic Shock

The purpose of the study was to compare the usefulness of cfDNA as an indicator of the severity of infection as compared to C-Reactive Protein (CRP) and Procalcitonin (PCT) which are the currently used clinical indicators for inflammation. Not only did the authors conclude that there was a definite correlation between the level of sepsis and the level of cfDNA, the study showed that cfDNA was as good of indicator as PCT and better than CRP for monitoring sepsis (Moreira, *et al.*, 2010).

There are a few problems with the previously mentioned studies linking cell-free DNA to different inflammatory states. The Fatouros study was conducted with a smaller than optimal population. The second and larger problem is that both studies focused on only a few of the many known reasons for inflammation (overtraining and sepsis). Unfortunately, due to the common nature of inflammation, it is of very little diagnostic value without other indicators. There are only a few different methods currently used to measure the amount of inflammation in sick patients, the most common being the Erythrocyte Sedimentation Rate and the level of C-reactive protein.

The purpose of this study was to determine the link, if any, between the levels of cell-free DNA in patient plasma and their level of inflammation. By measuring the levels of cell-free DNA in healthy patients and patients with elevated levels of C-reactive protein, which is known to be elevated in inflammation, any link between the concentration of cell-free DNA in patient plasma and the level of inflammation could be investigated.

CHAPTER II

LITERATURE REVIEW

HOW DNA BECOMES CELL-FREE:

The location of DNA found in eukaryotes is in the nucleus of cells. In 1948, P. Mandel discovered that DNA also exists in human plasma (Mandel, 1948). The method of how, exactly, the DNA gets out of the cell and into the plasma of an individual is still a matter of scholarly debate. Currently, four bodily processes are proposed to result in DNA ending up in the plasma of an individual: apoptosis, necrosis, active secretion and "terminal differentiation" (Lichtenstein, *et al.*, 2001).

Apoptosis refers to the process that occurs when a cell reaches its designated lifespan and dies. Every day in the human body more than 1 trillion cells undergo mitosis. To maintain "tissue homeostasis", approximately the same amount of cells must die each day (Lichtenstein, *et al.*, 2001). Usually in apoptosis, the DNA is cut into fragments. However, Lichtenstein noted that some DNA "escaped" from being fragmented and was present in the plasma in high molecular weight form.

The theory that apoptosis is the major contributor to the amount of cell-free DNA in plasma is contested in a more recent paper by Van der Vaart and Pretorius (2008). These authors, citing several studies dating as far back as the 1970s, make the claim that apoptosis is 'not responsible for the free DNA observed in plasma' (Van Der Vaart & Pretorius, 2008). Van der Vaart and Pretorius (2008) also make the argument that all apoptotic cells are 'rapidly ingested' by macrophages and that their DNA is digested by DNase II, found in lysosomes. They do concede; however, that some forms of apoptosis carried out by macrophages with impaired phagocytic function could cause some higher molecular weight DNA to escape.

Necrosis is another postulated mechanism for release of DNA into plasma and refers to cell death caused by injury. Necrotic cells are dealt with in much the same way by the body as apoptotic cells. They are broken down and their DNA is often fragmented by proteases and nucleases. Though it is possible that, as has been suggested for apoptotic cells, some DNA might escape, necrosis is not generally present in healthy individuals. Cell-free DNA is, however, found in both healthy (Mandel, 1948) and sick individuals (Moreira, *et al.*, 2010). Both Lichtenstein *et al.*, (Lichtenstein, *et al.*, 2001) and Van der Vaart and Pretorius (2008), concede that it is not probable that necrosis is a significant source of cell-free DNA

Active secretion of DNA from living cells is yet another postulated mechanism for DNA to end up in the plasma. An experiment in the 1970s brought to light evidence showing that lymphocytes release DNA until a "certain concentration was reached (Anker, Stroun, & Maurice, 1975)". The experiment by Anker *et al.* (1975) was conducted by extracting 10^6 lymphocytes/ml from human blood and placing them in

sterile culture medium, and incubating them at 37°C for varying amounts of time. At 2 hour, 4 hours, 6 hours and 8 hours the lymphocyte suspension was centrifuged and 25ml of the supernatant was removed to be tested for DNA concentration. The remaining lymphocyte suspension was then re-suspended in 25ml of fresh medium. DNA was then quantified in the harvested medium and found to contain an average of 22-23µg/culture. The authors maintain that active DNA secretion is the best explanation for the cell-free DNA, as once the concentration of 22-23µg/culture was reached it didn't continue to increase. Also of note was that even with cells dying the concentration of cfDNA didn't increase. However, the exact nature of this secretion of DNA was not fully explained by Anker et al (1975). Due to the limited understanding of how lymphocytes secrete DNA, scientists are reluctant to accept active secretion as the mechanism underlying cell-free DNA.

The last source of cell-free DNA proposed is terminal differentiation. Terminal differentiation refers to cells, like red blood cells, losing their nuclei as part of the final stage in their maturation. Usually the nuclei of these cells are digested in much the same way as cells undergoing apoptosis. Like all the other theories of how DNA ends up in plasma, lots of investigators contend that terminal differentiation does not play a significant role in the amount of DNA found in plasma. Those against terminal differentiation as a source of cell-free DNA cite the lack of differentiation in tumors. Often, individuals with cancer will have a significantly higher amount of cell-free DNA in their plasma.

Even though the pathway is not entirely understood as to how DNA is liberated from the nuclei of cells, cell free DNA can still be useful. Just as the principle of

inheritance was demonstrated by Mendel and his pea plants but not completely understood until many decades later, the fact that nuclear DNA ends up in plasma is useful even though there is not a clear understanding of how it got there.

<u>Diagnostic uses for cell-free DNA:</u>

Cancer Detection:

One of the great health concerns of the last few decades is cancer. Cancer comes in many forms and is frequently fatal. Many forms of cancer are, if they are found early enough, very treatable and show excellent recovery rates. The key to lowering the mortality rate for these types of cancer is early detection. Sozzi et al (2003). showed that the levels of cell-free DNA in plasma in patients with certain types of cancer are greatly increased (Sozzi, *et al.*, 2003). Sozzi *et al.* (2003) demonstrated an average of eight times the level of cell-free DNA was present in patients with certain types of lung cancer as compared to control individuals, known to be heavy smokers but without cancer. The Sozzi et.al. study (2003) not only showed a correlation between cell-free DNA levels and lung cancer but also demonstrated a dramatic reduction in the amount of cell-free DNA in response to therapeutic treatment. Quantifying the amount of cell-free DNA in those suspected of having cancer, especially those at high risk of certain types of cancer, offers a less invasive test that may be informative in the earliest stages of cancer.

Fetal DNA detection:

An ever-present question in prenatal testing is how to develop tests that are less invasive and ultimately safer for both mother and child. The use of cell-free DNA from the fetus, present in maternal plasma, has been instrumental for the diagnosis of some

pre-natal conditions. Y.M. Dennis Lo (Lo, 2000) stated that testing the fetal DNA in maternal plasma can be used for, 'the prenatal diagnosis of fetal rhesus D status, sex-linked disorders, and other paternally inherited genetic disorders. Abnormal fetal DNA concentrations in maternal plasma and serum have been found in common pregnancy-associated disorders, including preterm labor and preeclampsia, as well as in pregnancies complicated by fetal trisomy 21 (Lo, 2000)'. As sampling cells from a fetus in utero carries a risk, testing of the mother's plasma for cell-free DNA originating from the fetus is very advantageous.

Inflammation:

One of the most widespread symptoms accompanying illness is inflammation. Inflammation can be caused by a multitude of problems ranging from overly intense physical training (Fatouros, *et al.*, 2006), to life threatening conditions such as sepsis or septic shock (Moreira, *et al.*, 2010). Because of the wide ranging implications of inflammation, the need to distinguish between the causes quickly and accurately is very important. Bacterial cultures, though the gold standard to determine the identity of the organism causing sepsis, are slow. Cultures often take days to grow in culture medium. C-reactive protein, a marker of inflammation without infection, and Procalcitonin which is associated with inflammation occurring as a result of infection, are currently the methods used to detect inflammation and investigate its source. The quantification of cell-free DNA was shown to differentiate between localized inflammation and septic shock, with levels increasing "proportional to the severity of the infection (higher increases in sepsis than in localized infections, and in septic shock than in sepsis)" (Moreira, *et al.*, 2010). Moreira *et al.* (2010), also demonstrated that the levels of cell-free

DNA were a better predictor than C-reactive protein and at least on the same level as the procalcitonin in measuring the level of infection.

C-REACTIVE PROTEIN

Overview:

C - reactive protein (CRP) is an ‘acute phase reactant’ found in the serum of humans and other species. It was first discovered in the 1930’s by William S. Tillett and Thomas Francis in their paper *SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS* (Tillett & Francis, 1930). It wasn’t until much later in the 1990s that it was realized that CRP was an excellent non-specific indicator of inflammation. It is thought to be more specific than other methods to measure inflammation such as the erythrocyte sedimentation rate (ESR). CRP is produced in the hepatocytes of the liver and has a half-life in the plasma of approximately 19 hours (Pepys & Hirschfield, 2003).

Structure:

CRP is a pentatrexin; it is one of two ‘calcium dependent ligand-binding plasma proteins’ (Pepys & Hirschfield, 2003). CRP has a pentameric structure consisting of five identical sub units, shown in the figure below.

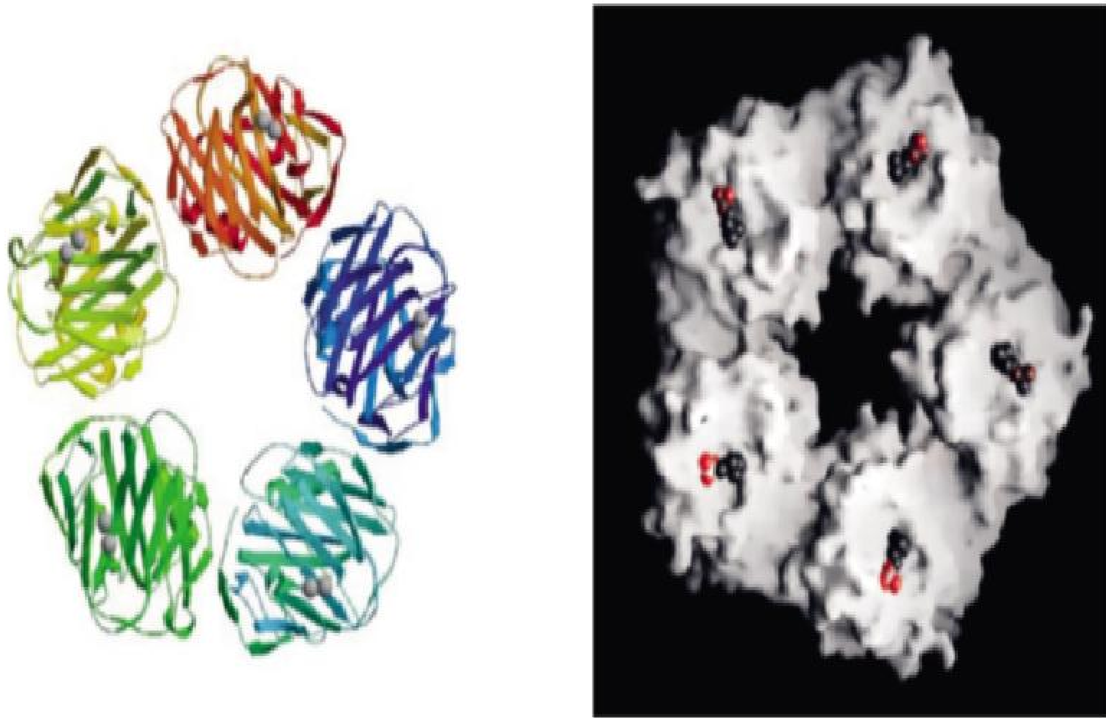


Figure 1. Two computer renderings of the structure of CRP. (Thompson, Pepys, & Wood, 1999)

Clinical Findings and Significance:

While there is no standardized set of methodologies used in all laboratories to quantify CRP, the most commonly accepted unit of measure in the clinical laboratory is in mg/dL. The normal range of CRP circulating in the blood at any given time in a healthy individual should fall below 1.0 mg/dL. It should be noted; however, that normal ranges do tend to fluctuate slightly, depending on the testing population and the testing method employed.

Cell Free DNA Quantitation

With all the possible diagnostic uses of cell-free DNA the focus of this study was to analyze the correlation (if any) between the levels of cell-free DNA and levels of inflammation as measured by CRP levels. Using the Q-TAT method of DNA quantification, developed by Dr. Allen and various others at Oklahoma State University Center for Health Sciences (OSU-CHS), the concentration of cf-DNA (both nuclear and mitochondrial DNA) was measured in samples of a control group and our test group with known C-reactive protein levels. The cf-DNA could then be compared with the CRP level, to determine if a correlation indeed existed.

CHAPTER III

METHODS

The purpose of this study was to determine if there is a link between the levels of cell-free DNA in patient plasma and their state of inflammation as reflected in CRP level. This correlation, or lack thereof, can be shown by measuring the levels of cell-free DNA in healthy patients and patients with elevated levels of C-reactive protein, the indicator chosen to reflect the degree of inflammation present.

I. Subjects/Participants

For the study, de-identified samples were taken from a control "healthy" population. The control population consisted of the plasma obtained from approximately 100 small segments from units of donated blood obtained from the Memorial Blood Centers of Minnesota. Blood donors are required to be relatively healthy on the day of the donation to be eligible to give blood, making them suitable candidates to use as a baseline. Generally, this screening is accomplished by giving all donors an extensive questionnaire about their medical history and general health status on the day of donation. Patients that have been sick recently, that have certain chronic diseases, or that are "high risk" to have life threatening diseases are not allowed to donate.

The experimental population consisted of 77 plasma/serum samples obtained from Regional Medical Laboratory in Tulsa, Oklahoma. These samples all had the level of C-reactive protein in their serum/plasma tested by Regional Medical Laboratory on the OLYMPUS 5400 clinical chemistry analyzer (Beckman Coulter, Brea, CA). The OLYMPUS 5400 measures CRP by photo-turbidimetry, or the change in light that is scattered as CRP complexes with reactants in the test and then compares results from unknown with a known calibration curve. The level of C-reactive protein in this group varied from a range of CRP levels that were clinically normal (≈50%) and levels above the diagnostic threshold and ranging to extremely high levels (≈50%).

II. QTAT--Quantitative Template Amplification Technology

Background:

Dr. Robert Allen and various graduate students developed the Quantitative Template Amplification Technology (Q-TAT) Assay at OSU-CHS. The Q-TAT methodology was developed to cost effectively quantitate DNA in a given sample. Q-TAT is currently being used by the Tulsa Police Department to quantitate their DNA samples. The Q-TAT assay was originally published in 2006 in the Journal of Forensic Science (Wilson, 2006). Q-TAT is a cost effective alternative to other DNA quantitation methods for several reasons, including the fact the assay is compatible with existing instrumentation present in the DNA typing laboratory.

Subsequent enhancements to the Q-TAT assay have included adding primers that will quantify and characterize mitochondrial DNA (Vandegrift, 2010) and use of the

assay to characterize the integrity of both nuclear and mitochondrial DNA recovered from biological samples (Vandegrift 2010 and Smith, 2011). The primer sequences for the Q-TAT assay can be found in TABLE 1 below. The change of fluorescent dye from ROX to NED was made to facilitate the use of Q-TAT on multi-capillary electrophoresis instruments such as the ABI 3130XL.

Primer Name	Forward Sequence	Reverse Sequence	Fluorescent Label	Product Size
AMEL	5' – ACC TCA TCC TGG GCA CCC TGG – 3'	5' – AGG CTT GAG GCC AAC CAT CAG – 3'	6-FAM	AMEL-X: 210 bp AMEL-Y: 216 bp
SRY	5' – ACG AAA GCC ACA CAC TCA AGA AT – 3'	5' – CTA CAG CTT TGT CCA GTG GC – 3'	6-FAM	110 bp
HPRT	5' – TTA GTG AAA CTG GAA AAG CAA – 3'	5' – TGA TAA TTT TAC TGG CGA TGT –3'	6-FAM	99 bp
Primer Name	**Forward Sequence**	**Reverse Sequence**	**Fluorescent Label**	**Product Size**
mt97	5' – AGC TCT CCA TGC ATT TGG –3'	5' – AGA CAG ATA CTG CGA CAT A – 3'	NED	97 bp
mt287	5' – CAC CAT GAA TAT TGT ACG GT – 3'	5' – CAA GGG ACC CCT ATC TGA –3'	NED	287 bp
pRL	5' – AAG GTG GTA AAC CTG ACG TTG – 3'	5' – TTC ATC AGG TGC ATC TTC TTG – 3'	6-FAM	200 bp

Table 1. The 5 Q-TAT primers, their forward and reverse sequences, the fluorescent dye used to identify them in the capillary electrophoresis instrument and their size in base pairs.

Quantitation using Q-TAT relies on comparing fluorescence in PCR products amplified from samples with unknown quantities of DNA to that in PCR products produced from intact genomic DNA of known concentration (Allen and Fuller, 2006, Wilson et.al. 2010, Vandegrift 2010). The DNA sample used to produce the standard curve contains DNA at 100ng/µl. This sample is serially diluted two fold to 31. 25 pg/ul and then the series of diluted samples (31.25-1000 pg) is amplified with the Q-TAT assay and products are analyzed using an ABI 3130XL genetic analyzer (Applied Biosystems, Inc., Foster City, CA).

After being subjected to 30 rounds of PCR, one microliter aliquots of amplicon from each sample was placed on the ABI 3130XL genetic analyzer. Products were separated by size electrophoretically and fluorescence was quantified in each product. The ABI 3130XL produces electropherograms of the data as seen in FIGURE 2.

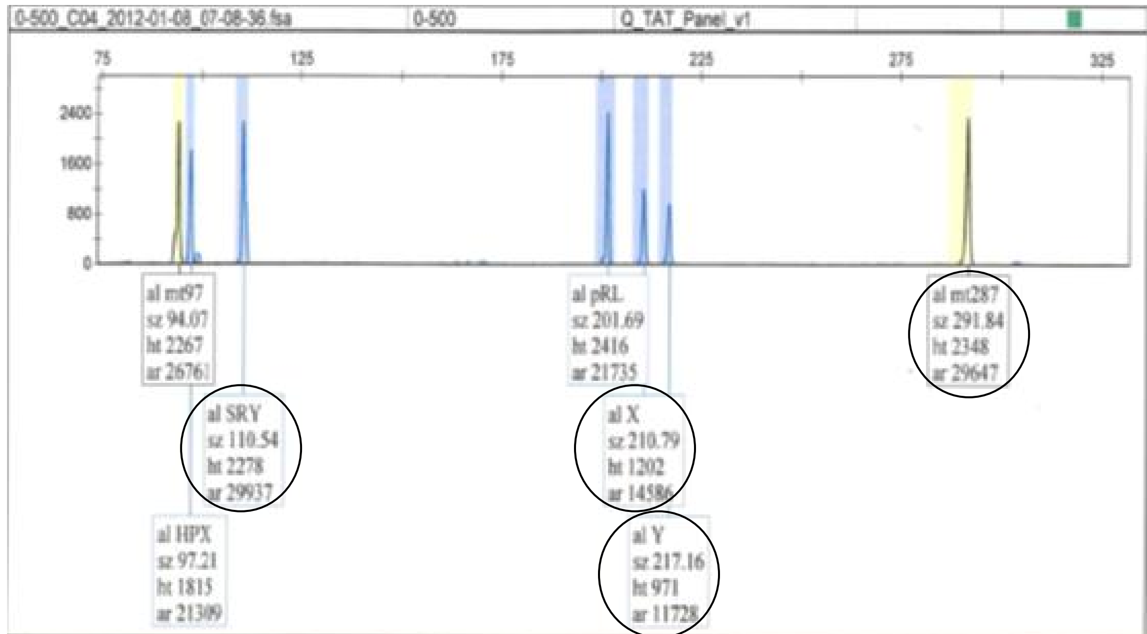


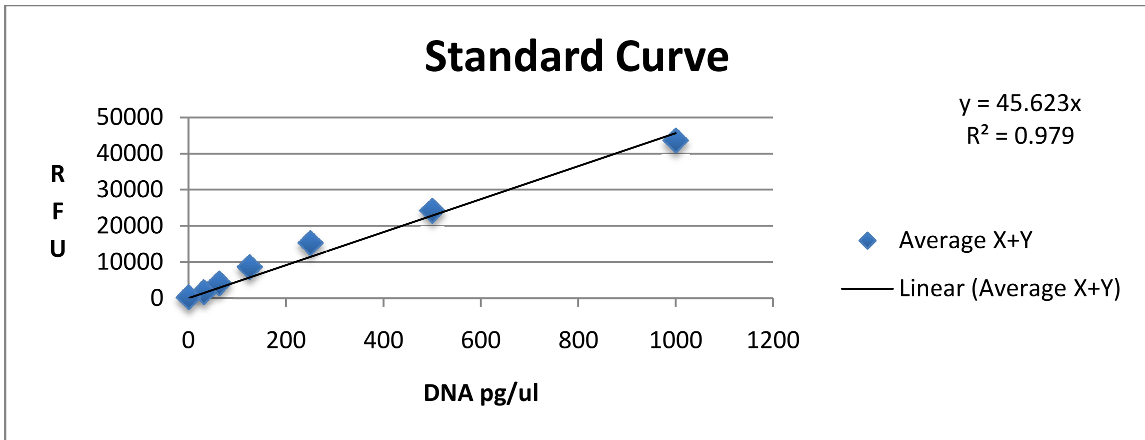
Figure 2. An electropherogram produced by the ABI 3130XL for a standard sample known to contain 500 pg/µl of DNA. The four circled boxes are the markers used to construct the standard curve for total DNA (alX+alY in pg/µl), male DNA (SRY in pg/µl), and mtDNA (mt297 in cell equivalents).

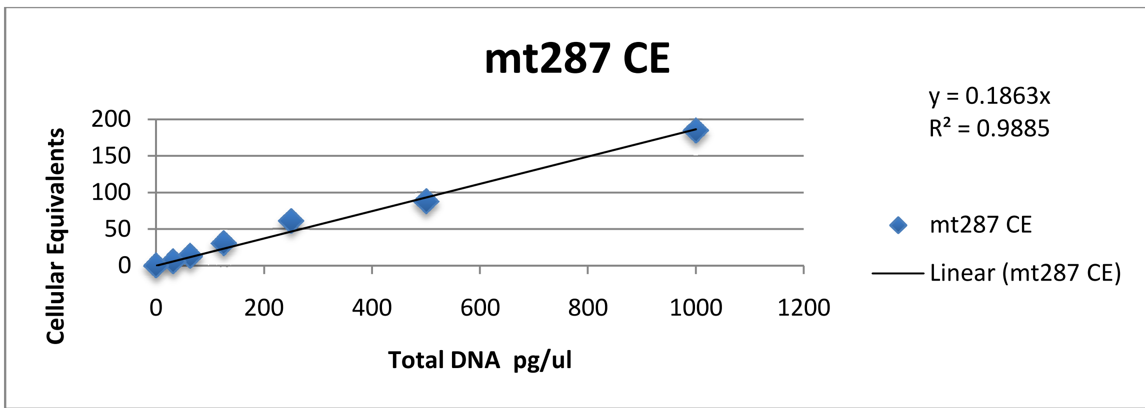
Each peak seen on the electropherogram represents a specific PCR product amplified from a genomic DNA target. The four targets that are circled (X, Y, mt287 and SRY) are of the most importance, while HPX and mt97 are included to show the possible presence of degraded DNA. The pRL peak is an internal control used in Q-TAT. pRL is a commercially available recombinant plasmid harboring the luciferase gene from *Renilla rentiformis*, otherwise known as the sea pansy. The pRL plasmid is used to confirm the lack of PCR inhibitors as it is extremely sensitive and will only amplify in the absence of inhibitors.

Each amplicon seen in the electropherogram is labeled with several data: the identity of the amplicon (the locus is preceded by the abbreviation “al”), the size of the

amplicon is given in base pairs, the peak height of fluorescence in a peak (in relative fluorescence units, or RFU), and the area of fluorescence under the peak are all shown. To construct the standard curve, the area of fluorescence contained within each peak was used as it is a more stable indicator than is the peak height. The area of RFU for each amplicon in a run can be plotted for each dilution of standard DNA. The standard curve used for this study represented the average fluorescence at each dilution of DNA from 13 different runs. Separate standard curves were prepared for total human DNA (using amelogenin X + Y fluorescence), male DNA (using SRY fluorescence), and mitochondrial DNA (mtDNA) (using mt297 fluorescence) (FIGURE 3).

The most important standard curve is for total nDNA. This standard curve is obtained by adding together the area of the Amelogenin X and Y targets. A standard curve can also be constructed for mtDNA. However, as no set of standardized concentration of pure mtDNA is readily available for use producing a standard curve, and mtDNA only makes up approximately .002% of all DNA extracted from a human cell (Vandegrift, 2010) the standard curve for mtDNA is constructed using cellular equivalents (CE). Each cellular equivalent of mtDNA equals the amount of mt287 amplicon produced from 6 pg of input genomic DNA recovered from blood (Vandegrift, 2010). The total DNA in a given sample is obtained by comparing the RFU of the sample to the Amel. X+Y standard curve, to produce the amount of nDNA in pg. The mt287 cellular equivalents are determined by using that same number and dividing by six. FIGURE 3 and TABLES 2 are examples of the data needed for the construction of a standard curve and the standard curves themselves:





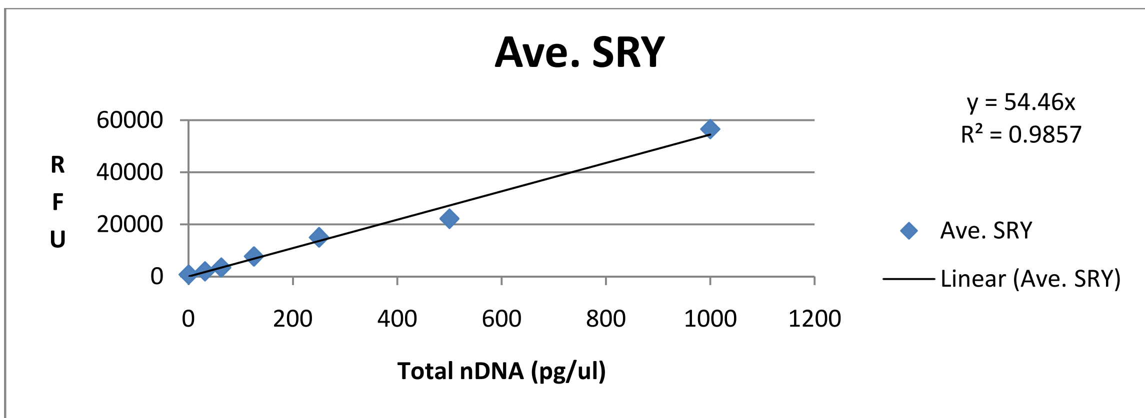


Figure 3. Standard curves for Amel X+Y, SRY, and mtDNA. Each standard curve was obtained from the averaging of 13 separate runs.

DNA (pg)	**Average X+Y**	**mt287 CE**	**Ave. SRY**
1000	43553.53846	184.3593284	56517.38462
500	24163.30769	87.869816	22166.69231
250	15220	61.00246333	15016.92308
125	8565.230769	30.56684747	7703.307692
62.5	4076.076923	13.23359731	3454.615385
31.25	1620.538462	5.706186207	1982.846154
0	196.5384615	0	769.6153846

Table 2. The average RFU values for 13 separate runs on the ABI 3130 genetic analyzer.

The Q-TAT assay includes multiple genomic DNA targets enabling total, male, and mitochondrial DNA to be quantified in a single PCR reaction. The Q-TAT assay also incorporates primer sets that amplify products of differing size. Recent work by Smith (2011) has demonstrated that the assay not only quantifies the different types of DNA in a sample, but the ratio of fluorescence contained within large versus small amplicon products from a given genomic DNA sample is a useful indicator of the degree of integrity of the DNA template recovered. Thus, in a sample in which the ratio of fluorescence in SRY or HPT amplicons versus Amel-X + Amel Y differs significantly from a value of about 1.5 (SMITH 2011) demonstrated evidence of DNA degradation.

III. DNA Preparation

Plasma from healthy individuals and from those whose CRP levels were known was aliquoted into 100ul samples. DNA was recovered from these samples by mixing plasma with an equal volume of DNA extraction buffer composed of 40µl of Proteinase K (20 mg/ml in 10mM Tris-Cl, pH8.0 + 0.2M KCl and 10% glycerol), 50 µl of SDS (20% w/v in water) and 910µl of TNE (10mM Tris-Cl pH8.0 + 0.2M NaCl and 0.1 mM EDTA). This digestion buffer promotes the digestion of any leftover cellular components in the plasma/serum that would compromise DNA retrieval. Samples were then mixed by vortex mixer for 1-2 seconds and placed in a 65°C heat block for two hours.

IV. Extraction

Once digestion was complete, an equal volume (200µl) of a 9:1 mixture of phenol to chloroform/isoamyl alcohol (24:1) was added and the samples were extracted by vortex mixing to create an emulsion. The samples were then centrifuged at 10,000 rpm for 1 minute in a microfuge to separate the aqueous and organic phases. The aqueous top phase containing the DNA was then transferred to a new microfuge tube. 300µl of chloroform/isoamyl alcohol mix was the added to the sample and again vortex mixed until cloudy. The samples were centrifuged again at 10,000 rpm again for 1 minute to separate the phases. The aqueous (top) phase is again placed into a new tube before recovery of the DNA occurs.

V. DNA recovery by binding to silica.

Final recovery of clean genomic DNA was accomplished using a ZYMO brand cleaner/concentrator kit (ZYMO RESEARCH, Orange, CA). DNA in the presence of a high salt environment will bind to silica. The Zymo Clean and Concentrator kit contains buffers and microcentrifuge tube inserts containing a silica plug. Thus, the DNA from the specimens can be mixed with a high salt buffer provided with the kit and passed over the silica plug in the tube insert by centrifugation thereby binding the DNA to the silica. Once bound, contaminants present in an extract can be washed through the silica plug using centrifugation leaving clean DNA bound to the silica. Bound DNA is then eluted using 2 X 15ul aliquots of TE-4 (10mM Tris-Cl, pH 8.0 + 0.1 mM EDTA) (warmed to 65°C) with centrifugations between each elution step. Elimination of the high salt environment elutes the DNA from the silica and it collects in the microfuge tube ready for amplification.

VI. Amplification

A reaction mix was prepared consisting of: 1.25 µl of Q-TAT Primers (SEE Table X), 7.5 µl of GoTaq (Promega Corp., Madison, WI), 1.0 µl of patient DNA, 1.0 µl of pRL DNA (Promega Corp., Madison, WI) as an internal control, and 1.75 µl of water. The samples were amplified in a ABI 9700 thermocycler using the cycling conditions described (Allen & Fuller, 2006)and (Wilson, 2006). Briefly, each cycle consisted of 10 seconds at 98°C, 60 seconds at 55°C, and 30 seconds at 72°C. After thirty repetitions, the thermocycler had a 10 minute 60°C hold time. After the final hold time the thermocycler held the specimens indefinitely at room temperature. Once properly amplified, 0.5 µl of DNA were then added to a mixture of 24.5 µl of formamide and 0.5 µl of LIZ 500 size

standard. The samples were then placed on the ABI 3130XL Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) to be analyzed.

VII. Analysis

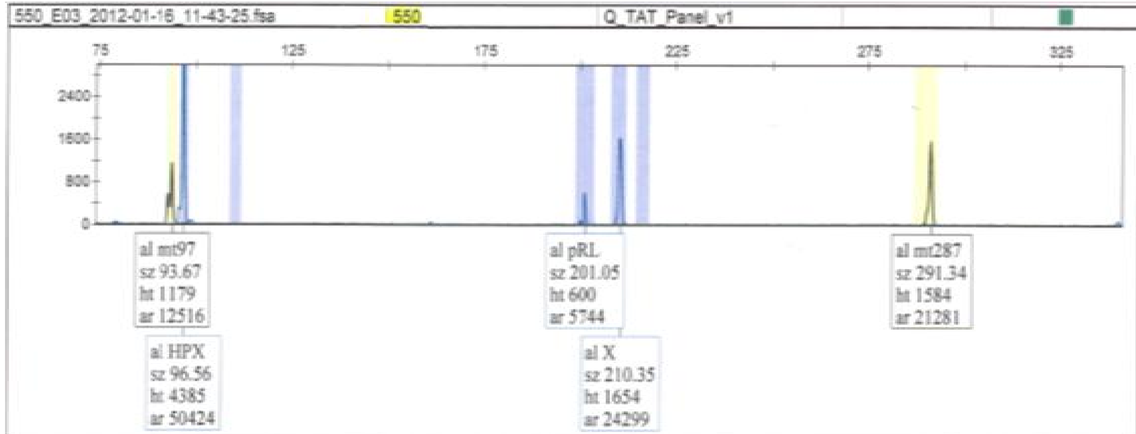


FIGURE 4. Sample electropherogram

After sample data had been collected on the ABI 3130XL (FIGURE 4), the area RFUs for the Amel X and Amel Y genes were added together and the results were then compared to the standard curves to estimate the weight-recovery of cell-free DNA from the sample. The resulting amounts of DNA for the test population was then compared with levels of C-reactive protein that accompany them and plotted on a chart. To determine if indeed there was a significant correlation, the data was then analyzed using different statistical methods. The three comparisons were:

1. Correlations between CRP and cell-free DNA
2. Degradation states in the males of the control group as compared to the CRP normal and CRP high groups.
3. Effects of gender on cell-free DNA concentrations.

CHAPTER IV

RESULTS

Among the group of samples for which CRP levels were known (n=77) 33 were male and 39 were female. DNA from 5 samples was degraded beyond the point where the DNA could amplify properly. The lack of amplification in these 5 samples prevented the gender typing of these patients based on their cfDNA and was not suitable for further analysis. Among the blood donor samples (i.e. "the control group") (n=37) 24 samples were male and 13 were female. One hundred samples were received from MBC, all of which contained approximately 100ul of plasma. DNA was only recovered from 37, for reasons that are unclear but perhaps relate to how long the donated blood had been stored before we received it. Retesting was not possible due to the fact that the specimen was used completely.

Total nuclear DNA (expressed in pg/µl of original plasma) and mtDNA (expressed as cell equivalents/µl of original plasma) were quantified and compared between the CRP tested samples and the normal controls. The Satterthwaite modification of a t-test was used to transform the data using square roots of the means for the two groups.

The results indicated a significant difference in cell-free nDNA and mtDNA concentrations in the plasma of the Control group vs. the CRP- tested group. ($p = 0.0036$) (Table 3).

	Mean nDNA (pg/µL)	**Standard Error**	**Standard Deviation**
Control Group	1010.6	317.8	1932.9
CRP Group	22.2875	5.3223	46.7032

Table 3. Comparison of cell free nDNA levels in plasma in normal controls and in samples subjected to CRP testing The t-test performed using the Satterthwaite method on the square root transformation, produced a p value < 0.0001. (Statistical analysis performed by Dr. Mark Payton.)

The plasma samples from the control group showed a significantly broader range of cell free nDNA values than did those of the CRP test group. Cell free DNA recovered from the control group ranged from 79.35pg/µl of nDNA to over 10,000 pg/µl, greater than a one hundredfold change in concentration. Recoveries of cell free DNA from the CRP test group ranged from 0 pg/µl to 297 pg/µl; the highest cell-free nDNA amount in the CRP samples was only about 3 fold more than the lowest of the control group samples. While the range of concentrations of cell free nDNA in the two groups overlaps there are clear differences between them as shown in Table 3 above. The CRP test group was also divided into patients with low levels of CRP ($CRP \leq 1$) and patients with high CRP levels indicative of inflammation ($CRP > 1$). Cell-free nDNA levels were compared between the low and high CRP groups and results are summarized in Table 4.

	Mean nDNA (pg/µL)	Standard Error
Low CRP (≤ 1)	13.3353	4.7798
High CRP (>1)	29.3660	8.6541

Table 4. Comparison of cell free nDNA levels in plasma with low CRP levels and those with high CRP levels. The t-test performed using the Satterthwaite method on the square root transformation, produced a p = 0.1129. (Statistical analysis performed by Dr. Mark Payton

There was a correlation observed between cell-free nDNA levels and the amount of CRP present in samples (r = 0.32635, p = 0.0038). The difference in the p values shown for the correlation vs. the value shown in Table 4 can be explained by the fact that the correlation is a gradual one across the CRP test group as a whole. This correlation also exists for levels of cell-free mtDNA and levels of CRP (r = 0.28366, p= 0.0124) (Table 6). These results indicate that as the level of CRP increases so does the amount of cell-free DNA (either nDNA or mtDNA).

	Mean mtDNA (CE/µL)	Standard Error	Standard Deviation
Control Group	21.5769	6.5275	39.7052
CRP Group	3.3869	0.9845	8.6387

Table 5. Comparison of cell free mtDNA levels in plasma in normal controls and in samples subjected to CRP testing the t-test performed using the Satterthwaite method and a square root transformation, produced a p value = 0.0004. (Statistical analysis performed by Dr. Mark Payton.)

As the amount of mtDNA can't be directly ascertained (in pg/µl), the amount of mtDNA was calculated using cellular equivalents (CE) per microliter, as mentioned in the Methods Section. The statistical analysis of cell free mtDNA in the blood donors and in the samples subjected to CRP testing is shown in Table 5. The CRP group showed samples with a range of concentrations from 0 CE/µl to 57.45 CE/µl. The control group showed a range almost three times larger than did the CRP test group in terms of mtDNA, ranging from 0 CE/µl to 217.2 CE/µl. The ranges of the concentrations are quite similar as shown by the standard error and the standard deviation.

As with the nDNA analysis, mtDNA concentrations were quantified within the CRP test group, which was divided into low CRP and high CRP patients as discussed previously and results are shown in TABLE 6. The statistical analysis of the data shown in Table 6 showed there was a significant difference in the levels of cell-free mtDNA in the low CRP and high CRP group (p=0.0232)

	Mean mtDNA (CE/µL)	**Standard Error**
Low CRP (≤ 1)	1.1194	0.4114
High CRP (>1)	5.1797	1.6917

Table 6. Comparison of cell free mtDNA levels in plasma with low CRP levels and those with high CRP levels. The t-test performed using the Satterthwaite method on the square root transformation, produced a p = 0.0232. (Statistical analysis performed by Dr. Mark Payton

Degradation

As mentioned in the Methods section, the SRY and HPRT amplicons can be used to assess the amount of degradation present in both nDNA and mtDNA (VanDegrift, 2010; Smith 2011). The ratio of RFU in high versus low molecular weight amplicons amplified in Q-TAT, provides an assessment of degradation of the chromosomal DNA template. Degradation of nDNA is deduced from the concentration estimates for nDNA calculated using the Amel-X/Y amplicons divided by the concentration estimate for nDNA produced using the SRY locus as shown in Figure 5. Since the SRY locus is approximately half the size (110 bp) of the Amel X or Y loci (210 and 216 bp) it will amplify more readily in partially degraded DNA because of the smaller template requirement.

$$\text{Degradation Ratio} = \frac{\text{Cell-free DNA by Amel X+Y}}{\text{Cell-free DNA by SRY}}$$

Figure 5. Degradation Ratio Equation

Within the normal control group, 24 of the 25 male samples were analyzed for degradation (one being omitted because of possible contamination). In the CRP test group, nDNA and mtDNA from 28 of the 29 male samples were quantified. One patient was omitted from analysis as SRY failed to amplify though Amel Y was clearly present. This isolated inconsistent result may be due to a single nucleotide polymorphism in the primer binding region that prevents amplification of the SRY template.

It should be noted that 5 patients in the CRP test group contained DNA that was either below detectable limits or too degraded to amplify. That these samples did not contain an inhibitor of PCR was confirmed by the presence of the pRL amplicon in all of the amplifications from these samples.

	Deg. Ratio Mean	Std. Error
Control Group	0.88743	0.09270
High CRP	1.08214	0.32652
Low CRP	0.38449	0.09754

Table 7. The average degradation ratio and standard deviation for the normal and high test groups as well as the control. Control group and high CRP group show no statistical difference. Low CRP group is significantly different from the other two (p vale = 0.05). Statistical analysis performed by Dr. Mark Payton.

The samples from the control group and the samples from the high CRP group show no significant difference in their ratios of fluorescence of high vs. low molecular weight Q-TAT amplicons indicating comparable levels of degradation of cf-DNA. The samples from the group with low CRP levels, however, showed a significantly higher state of cf-DNA degradation than either the control or normal group (p value = 0.05). This shows that patients that have lower CRP levels have more degraded nDNA in their plasma than do patients with higher CRP levels.

Degradation of mtDNA could not be statistically evaluated at this time due to the fact that the mt97 locus amplifies so readily that it would not produce a reliably linear standard curve to estimate the cell equivalents of mtDNA to compare to the mtDNA estimates produced by the mt287 standard curve. However, it is possible to compare the

RFU in the two amplicons to get a general idea of how degradation is affecting cell free mtDNA. Shown in Figure 6 is a comparison of RFU in mt97 and mt287 amplicons.

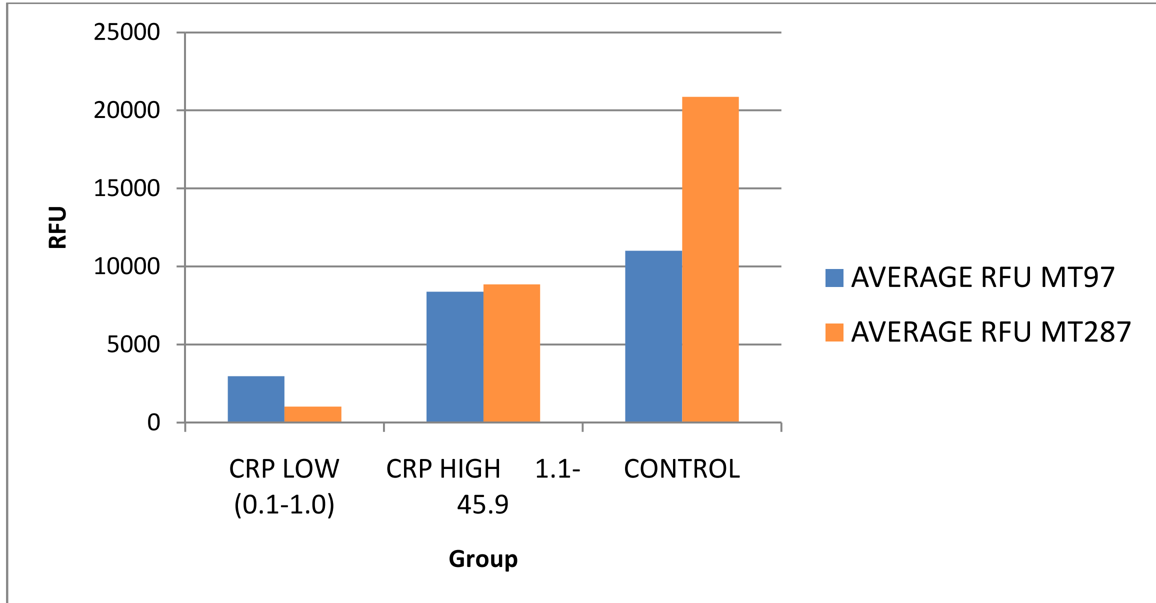


FIGURE 6. Comparison graph of the average RFU for the mt97 and mt287loci.

Figure 6 suggests that the state of the mtDNA in each group is different. In the samples with low CRP levels, mt97 is amplifying at a higher rate than mt287 which would be consistent with a higher state of degradation of the mtDNA in the low CRP group; consistent with results for nDNA. The samples with high levels of CRP show an almost 1:1 ratio between mt97 and mt287 based upon the RFU, indicating that the mtDNA present in these samples is largely intact. The control group shows a much higher RFU for mt287 than does the mt97 locus, an almost 2:1 ratio which cannot be explained at this time.

CF-DNA and Gender

	Gender	Ave. Total DNA X+Y	Std. Error
Control Group	F	842.74	280.784
	M	1120.51	451.579
High CRP	F	28.31	13.001
	M	34.06	11.813
Low CRP	F	15.09	8.544
	M	12.37	4.120

Table 8. Average nDNA and standard error for both genders split up by group. There was no significant difference in any of the groups, when compared by gender (all p values > 0.77). Statistical analysis performed by Dr. Mark Payton.

The sex of the patient doesn't seem to be a factor that effects the concentration of DNA in their plasma, as both males and females show a wide range of different concentrations of both nDNA and mtDNA, in the control group as well as the low CRP group and the high CRP group. Using ANOVA (Analysis of Variance) and assuming a two factor factorial, Table 8 above shows that in all three groupings, males and females showed no significant difference in their respective average levels of nDNA. The p values for the control group, high CRP group and the low CRP group were, 0.7897, 0.7781 and 0.9137 respectively.

Next a comparison of all three groups was made by looking at the genders separately, using the same information from TABLE 6 above and the same ANOVA methods (performed by Dr. Mark Payton) mentioned previously in this section. Results showed no statistical similarity for males or females separately across the three groups (p value < 0.0001). This supports the finding that the three groups (control, low CRP and high CRP) have different levels of cfDNA.

CHAPTER V

DISCUSSION

The purpose of this study was to investigate levels of cfDNA in the plasma of a healthy control group compared with a patient population whose inflammation status is quantified through the plasma CRP clinical test. Blood donors represented the initial "control group" and were chosen because each donor goes through a health questionnaire and is assessed by collection personnel before donating blood. Results from our study however demonstrated that the control group exhibited some of the highest cfDNA levels and generally control samples had more cfDNA than any of the CRP samples. In addition, there was a very wide distribution of cf-DNA levels in the control group. Thus, the blood donor population may not represent a good "control" group for studies such as this one. Among all samples there was no difference between males and females and thus gender does not appear to be a factor affecting circulating cfDNA.

Among the samples with known CRP levels, those exceeding the clinical threshold (CRP >1) that defines an inflamed state were grouped into the "high CRP" group. The remaining samples falling below the clinical CRP threshold (CRP ≤ 1) were the "low CRP" group. Interestingly, the low CRP (lwCRP) group exhibited a tighter range of cfDNA levels for both nDNA and mtDNA levels, and the mean cfDNA was significantly lower than among high CRP samples (hCRP) as well as among the controls.

An explanation for this phenomenon could be selection bias in our choice of a control group. Since the CRP level of the blood donors was unknown, it is possible that individuals within this group could have wide ranging CRP levels on the day of donation. If the inflammation were not debilitating to the individual, they could still probably donate. In contrast, the samples in the low CRP group were specifically selected based upon low, sub-pathologic levels of CRP and thus, if there were a correlation between cfDNA and CRP, that relationship would be apparent in this group.

Using the Q-TAT method, cell free nDNA and mtDNA were quantified in the same PCR reaction. Once DNA amounts in all the samples were estimated, the concentrations were compared using statistical analysis. The amount of cell-free nDNA and mtDNA showed no correlation between the control group and the CRP test group

A correlation between cf-DNA, both nDNA and mtDNA, and the levels of CRP present in the test group was observed. This can be explained by CRPs role in the body. As mentioned previously, CRP not only reacts with phosphocholine in damaged cell membranes but it is also recognized by molecules of the complement pathway which act primarily to destroy foreign cells (bacteria and fungi) but will also destroy damaged and apoptotic cells. This could cause more DNA to be liberated from cells. Due to CRPs role in destroying damaged and apoptotic cells, the physical state of the cf-DNA was analyzed. As the CRP and compliment pathway destroy cells, DNA can be liberated more quickly, however the DNA that is liberated from the cell may not remain intact due to the presence of DNase and other materials in human plasma that degrade DNA. The nature of cell free DNA was characterized in one study as being between 180-10,000 bps in length (Van Der Vaart & Pretorius, 2008). Thus, cfDNA may exist in varying states of

degradation. The Q-TAT assay was used to assess degradation in our samples. The ratio of the estimate of pg of nDNA recovered from a sample and estimated using SRY versus the estimate calculated using Amel-X/Y was used to quantify degradation (Smith 2011). The Amel X/Y locus is approximately twice the size of the SRY locus. In intact DNA the ratio should be approximately 1:1. The control group and the high CRP group showed no statistical difference in the estimated level of nDNA degradation, while the test group with clinically low/normal levels of CRP was significantly different from the other groups and exhibited a greater proportion of degraded DNA. This analysis showed that patients with little to no inflammation have a higher level of degraded cf-DNA than do patients with higher levels of inflammation. This result was somewhat unexpected but may reflect a difference in the balance between cellular destruction (greater in the high CRP group) versus DNA degradation (occurring at equal rates in high and low CRP groups).

Expanding the usefulness of cfDNA

For years it has been known that DNA is present in human plasma free from the cells where it originates. The how and the why of cell-free DNA in plasma has been a source of much debate. The fact that it is present in plasma of all humans and quite often is present in elevated concentrations in patients with certain health conditions, ranging from cancer to pregnancy, means that it can be used as a minimally invasive qualitative test. It is possible that testing for cell-free DNA may become ‘an important and integral aspect of routine molecular testing,(Butt, 2008)’.

C - reactive protein is the most common marker used by doctors to diagnose the presence of inflammation. The problem with CRP is that it is not a very specific marker; it rises due to many different kinds of inflammation from sepsis to heart attacks. Thus selecting our groups for quantitation of cf-DNA may have been based on a somewhat non-specific marker of inflammation. CRP's role in the body is that it helps to activate the compliment pathway to eliminate foreign bodies, damaged cells and apoptotic cells.

Two studies mentioned earlier performed by Fatouros *et al.* 2006 and by Moreira *et al.* 2010 focus on the use of cell free DNA and how it compares to currently accepted methods of measuring inflammation. The Fatouros *et al.* 2006 study concluded that in regards to intense training cell-free DNA was a much better indicator of overtraining than was CRP. Cell free DNA increased dramatically during periods of intense training where CRP and other methods took longer to elevate. When comparing cf DNA and CRP for any correlation Fatouros *et al.* concluded that there wasn't any. The results contrast our own in that we did show a correlation. However, in the two studies the reasons for the elevated CRP levels were quite different which could explain the difference.

The Moreira *et al.* 2010 study showed that similarly cell-free DNA was a better indicator of sepsis and septic shock than was CRP. The levels of cf DNA increased dramatically, '10-fold the upper limit of the reference range' (Moreira, *et al.*, 2010) when comparing normal healthy patients to patients suffering from septic shock. Interestingly, Moreira *et al.* noticed a slight correlation between cfDNA, CRP and other tests. The authors do note however that similar studies have been done, some showing correlation between CRP and cfDNA and others showing that no such correlation exists.

Areas for further Study

The goal of all clinical testing is to develop tests that are more accurate, require less sample volume and are cost effective. Cell-free DNA is widely associated with several known health states that are monitored clinically. While this study has shown only a small negative correlation between cfDNA and inflammation, there are many other diseases that there has been no research done about the concentration of cfDNA. If more information was available for various diseases it might be possible that cfDNA could be used as a more efficient clinical test for these diseases. Once the concentration of cf-DNA was established certain disease states could be eliminated. As well, the remaining cleaned DNA could be used with other molecular targets to further the diagnosis.

Conclusion

The analysis of cf-DNA is an area of study with many clinical uses. The goal of this study was to identify the relationship, if any between cf-DNA and levels of inflammation as determined by CRP levels. Even though the control group proved not to be the optimal choice for baseline cf-DNA levels, due to wide fluxuations in cf-DNA concentrations and unknown levels of CRP, useful analysis could still be performed within the CRP known group by splitting up the patients with clinically low CRP from those with clinically high CRP. A correlation was found between CRP and cf-DNA levels, meaning as the CRP level rises so does the level of cf-DNA. In addition the low CRP group exhibited more degraded cf-DNA than did the high CRP group or the control group. Lastly, gender doesn’t appear to affect the concentration of cf-DNA.

REFERENCES

Allen, Robert W., & Fuller, Valerie Mattimore. (2006). Quantitation of Human Genomic DNA Through Amplification of the Amelogenin Locus*. *Journal of Forensic Sciences, 51*(1), 76-81. doi: 10.1111/j.1556-4029.2005.00011.x

Anker, P., Stroun, Maurice, & Maurice, Pierre. (1975). Spontaneous release of DNA by human blood lymphoctyes as shown in an in vitro system. *Cancer Res. , 35*, 2375-2382.

Butt, Asif N., Swaminathan, R. (2008). Overview of Circulating Nucleic Acids in Plasma/Serum Update on Potential Prognostic and Diagnostic Value in Diseases Excluding Fetal Medicine and Oncology. *Annals of the New York Academy of Sciences, 1137*, 236-242.

Fatouros, Ioannis G., Destouni, Aspasia, Margonis, Konstantinos, Jamurtas, Athanasios Z., Vrettou, Christina, Kouretas, Dimitrios, . . . Papassotiriou, Ioannis. (2006). Cell-Free Plasma DNA as a Novel Marker of Aseptic Inflammation Severity Related to Exercise Overtraining. *Clin Chem, 52*(9), 1820-1824. doi: 10.1373/clinchem.2006.070417

Lichtenstein, A. V., Melkonyan, H. S., Tomei, L. D., & Umansky, S. R. (2001). Circulating Nucleic Acids and Apoptosis. *Annals of the New York Academy of Sciences, 945*(1), 239-249. doi: 10.1111/j.1749-6632.2001.tb03892.x

Lo, Y.M. Dennis. (2000). Fetal DNA in Maternal Plasma: Biology and Diagnostic Applications. *Clin Chem, 46*(12), 1903-1906.

Mandel, P., Metais, P. (1948). Les acides nucléiques du plasma sanguin chez l'homme. *C. R. Acad. Sci. Paris, 142*, 241–243.

Moreira, Vanessa Garcia, Prieto, Belen, Rodriguez, Julia San Martin, & Alvarez, Francisco V. (2010). Usefulness of cell-free plasma DNA, procalcitonin and C-reactive protein as markers of infection in febrile patients. *Ann Clin Biochem, 47*(3), 253-258. doi: 10.1258/acb.2010.009173

Pepys, Mark B., & Hirschfield, Gideon M. (2003). C-reactive protein: a critical update. *The Journal of Clinical Investigation, 111*(12), 1805-1812.

Smith, Byron. (2011). *EVALUATING DNA SAMPLE DEGRADATION WITH A QUANTITATIVE GENDER TYPING END-POINT PCR MULTIPLEX.* Oklahoma State University College of Health Sciences, Tulsa.

Sozzi, Gabriella, Conte, Davide, Leon, MariaElena, Cirincione, Rosalia, Roz, Luca, Ratcliffe, Cathy, . . . Pastorino, Ugo. (2003). Quantification of Free Circulating DNA As a Diagnostic Marker in Lung Cancer. *Journal of Clinical Oncology, 21*(21), 3902-3908. doi: 10.1200/jco.2003.02.006

Thompson, Darren, Pepys, Mark B., & Wood, Steve P. (1999). The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure (London, England : 1993), 7*(2), 169-177.

Tillett, William S., & Francis, Thomas. (1930). SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS. *The Journal of Experimental Medicine, 52*(4), 561-571. doi: 10.1084/jem.52.4.561

Van Der Vaart, M., & Pretorius, P. J. (2008). Circulating DNA. *Annals of the New York Academy of Sciences, 1137*(1), 18-26. doi: 10.1196/annals.1448.022

Vandegrift, Emily. (2010). *QUANTITATION AND CHARACTERIZATION OF HUMAN NUCLEAR AND MITOCHONDRIAL DNA WITH PCR AND CAPILLARY ELECTROPHORESIS.* MSFS, Oklahoma State University College of Health Sciences, Tulsa.

Wilson, J., Fuller V., Benson, G., Juroske, D., Duvall, E., Fu, J., Pritchard, J., Allen, R. (2006). Molecular Assay for Screening and Quantifying DNA in Biological Evidence: The Modified Q-TAT Assay. *Journal of Forensic Science*.

VITA

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Scope and Method of Study:

The presence of DNA in the plasma of humans has been known since 1948. The pathway that leads to the liberation of DNA from the cell still remains open to debate. . Scientists have proposed the following mechanisms underlying the presence of cell-free DNA in plasma: apoptosis, necrosis, terminal differentiation and active secretion. While the exact method of DNA liberation is still unclear, the usefulness of cell-free DNA for diagnostic purposes has not remained in question. The purpose of this study was to determine the link, if any, between the levels of cell-free DNA in patient plasma and their level of inflammation. By measuring the levels of cell-free DNA in healthy patients and patients with elevated levels of C-reactive protein, which is known to be elevated in inflammation, any link between the concentration of cell-free DNA in patient plasma and the level of inflammation could be investigated.

Findings and Conclusions:

Using the Q-TAT method of quantitation, nDNA and mtDNA were quantitated simultaneously. The results indicated a significant difference in cell-free nDNA and mtDNA concentrations in the plasma of the control and test groups ($p = 0.0036$). There was a slight correlation observed between cell-free nDNA and the amount of CRP present in samples, possibly explained by the role CRP plays in the complement pathway, destroying foreign bodies and damaged cells. The samples from the group with low CRP levels showed a significantly lower ratio of degradation than either the control or normal group (p value = 0.05). This shows that patients that have lower CRP have more degraded nDNA in their plasma than do patients with higher CRP levels. Gender didn't affect the concentration of nDNA or mtDNA in any of the groups.

ADVISER'S APPROVAL: Dr. Robert Allen