RELATIONSHIP BETWEEN PSYCHOLOGICAL STRESS AND OXIDATIVE STRESS IN VICTIMS OF MOTOR VEHICLE ACCIDENTS

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Current biobehavioral models of cancer focus primarily on stress-induced failures of immune surveillance as a principle mechanism of cancer progression. However, efforts to identify specific immune mechanisms altered by stress and underlying or affecting cancer course have met with limited success. Although there is clear evidence that stress alters fundamental immune processes, it is not clear whether stress-related changes in immune system activity are of sufficient type or magnitude for cancer to develop or progress (Cohen & Rabin, 1998). Therefore, new innovative approaches are needed to determine if stress is mechanistically linked to cancer. Measuring associations between stress and intermediate endpoints mechanistically linked to carcinogenesis may further understanding of the cancer process and provide insight for intervention. These endpoints include stress-induced alterations in DNA damage and repair (Forlenza & Baum, 2001). The present research measured the urinary concentration of the mutagenic oxidative lesion 8-hydroxy-2′-deoxyguanosine (8-OHdG) in adult victims of motor vehicle accidents (MVA) and controls. Overnight urine samples (approximately 15 hours) were collected within 1 month of the MVA and again 3 months after the accident. The primary hypothesis is that victims of MVAs will have higher concentration of urinary 8-OHdG compared to controls. Further, reported stress experience will be significantly related to urinary concentration of oxidative DNA damage products. Results showed that people in the MVA

group had significantly more distressing somatic symptoms, poorer concentration, significantly more fear and significantly more intrusive thoughts than people in the control group at month 1. Further, these intrusions were related to an objective rating of their injury severity at month 1. Additionally, people in the MVA group had significantly more intrusive thoughts than people in the control group at month 3. There were no group differences in the urinary concentration of 8-OHdG 1 month or 3 months following the MVA and self-reported measures of distress were unrelated to urinary levels of 8-OHdG. Reasons for the lack of association are discussed.

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INTRODUCTION

Specific Aims

Current biobehavioral models of cancer focus primarily on stress-induced failures of immune surveillance as a principle mechanism of cancer progression (e.g. Andersen et al., 1994). These models most often suggest that psychosocial factors such as chronic stress, depression, or lack of social support contribute to cancer initiation or progression by suppressing NK cell numbers or lytic function. However, results from research examining links between stress and cancer course are equivocal and efforts to identify specific immune mechanisms altered by stress and affecting cancer course have met with limited success.

Although there is clear evidence that stress alters fundamental immune processes, it is not clear whether stress-related suppression of immune system activity are of sufficient type or magnitude for cancer to develop or progress (Cohen & Rabin, 1998). Additionally, models which propose that decreased immune surveillance leads to oncogenesis fail to account for the strong relationship between chronic immune activation or inflammation and subsequent tumor development (for review see Balkwill & Mantovani, 2001; O'Byrne & Dalgleish, 2001). Therefore, new innovative approaches are needed to determine if stress is mechanistically linked to cancer.

One strategy entails measuring associations between stress and intermediate endpoints mechanistically linked to carcinogenesis. These endpoints include alterations in DNA damage and repair (Forlenza & Baum, 2000). This approach would address discrepancies between the

timing of stress measurements and the onset of disease as well as connect stress to processes involved in cancer etiology and progression. This approach may further understanding of relationships between biobehavioral variables and the cancer process providing insight for future preventive intervention.

Validation of this model necessitates testing of hypotheses in stressed populations not confounded by cancer or cancer treatment. For this purpose, the present research measured the urinary concentration of the mutagenic lesion 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, in adult victims of motor vehicle accidents (MVA) and controls. Motor vehicle accidents were used as a model of severe stress because MVAs are a frequent and common stressful event with over 6 million police-reported motor vehicle traffic crashes in the year 2000 (NATHA, 2000). MVAs are the leading cause of death for every age from 3 through 33 years and over 3 million people per year have been injured in MVAs since 1990 (NATHA, 2000). A recent review of psychiatric morbidity associated with MVA support the conclusion that experiencing an MVA results in serious distress with a significant number of people meeting criteria for posttraumatic stress disorder (PTSD) and major depression (Blanchard, & Veazey, 2001). The common and distressing nature of this event makes it likely that results will generalize to other individuals experiencing stress and positive results would provide proof-of-principle that stressful experience is linked to fundamental processes of carcinogenesis.

In this study, DNA damage was measured by 8-OHdG, a pre-mutagenic lesion resulting from oxygen free radical attack on DNA. If unrepaired, this lesion causes G → T and C → A transversion mutations (Cheng et al., 1991). If these mutations occur in crucial replication or repair genes, cancer may develop. Oxidative modification of DNA was measured by a

competitive enzyme-linked immunosorbent assay (ELISA) that permits the quantitative measurement of 8-hydroxy-2′-deoxyguanosine in urine using monoclonal antibodies.

Preliminary studies in both animals (Adachi, Kawamura, & Takemoto, 1993; Liu et al., 1996) and humans (Irie et al., 2001a; Irie et al., 2001b) demonstrate that stress is related to oxidative damage of DNA as measured by 8-OHdG from nuclear DNA. By examining urinary levels of 8-OHdG in MVA victims, this study aims to investigate the molecular correlates of this severe, traumatic stress. This study extends earlier findings by examining oxidative damage in a more severely stressed population. If results suggest increased oxidative DNA modification in MVA victims relative to controls, they would underscore the need for precise mechanism-based prevention strategies and targeted interventions in this population. This information would also add to existing knowledge regarding stress and cancer by identifying alternative molecular processes associated with psychosocial factors and disease etiology and progression. The hypotheses and specific aims of the proposed research were:

Hypothesis 1: People who experienced an MVA will report more symptom distress and intrusive thoughts one month and three months after the MVA than a hospital-based control group.

Aim 1: Measure symptom distress (SCL-90-R) and intrusive thoughts (IES) in a sample of adult MVA victims and in controls.

Hypothesis 2: People who experienced an MVA will have higher urinary concentration of oxidative damage products one month and three months after the MVA than a control group.

Aim 2: Measure 8-OHdG in overnight urine samples from a sample of adult MVA victims and in controls.

Hypothesis 3: Symptom distress and intrusive thoughts will be significantly related to urinary concentration of oxidative DNA damage products one month and three months following the MVA only in those people who report continued symptom distress and intrusive thoughts.

Aim 3: Correlate measures of symptom distress and intrusive thoughts with measures of 8-OHdG.

Background and Significance

Considerable interest in biobehavioral pathways linking stress and cancer has increased research examining psychological adjustment, biological responses, and cancer outcomes. However, research examining the affects of psychosocial variables such as stress or depression on the etiology or progression of cancer continues to report mixed effects (Cassileth, 1996; Fox, 1995; Hilakivi-Clark, Rowland, Clarke, & Lippman, 1994; Garssen, & Goodkin, 1999; McGee, Williams, & Elwood, 1996; Spiegel & Kato, 1996). Interpretation of findings is problematic because of discrepancies between the timing of stress measurements and the development of clinically relevant tumors. For example, Roberts et al., (1996) found no differences in the number of reported stressful life events between breast cancer patients and randomly selected population based controls for the five years prior to diagnosis. Interpretation of these results is problematic because the proximal measurement of stress fails to acknowledge that the transformation of a normal cell into a metastatic one is a multi-step process that occurs over many years or decades (Vogelstein & Kinzler, 1993). If stress has effects on tumor induction or progression, these effects likely begin long before the tumor becomes clinically apparent. A short-term, retrospective measurement strategy will miss detecting a relationship between stress and cancer incidence because the time frames are incompatible.

Efforts to identify specific immune mechanisms altered by stress and underlying or affecting cancer course have likewise met with limited success. For example, natural killer cell activity (NKCA) was assessed in malignant melanoma patients participating in a randomized, six week structured psychoeducational intervention (Fawzy et al., 1990). As predicted, patients in the intervention group exhibited significant improvement in NKCA at six-month follow-up. However, changes in NKCA resulting from the intervention were not related to lower recurrence rates (Fawzy et al., 1993). Thus, it is not clear whether stress-related changes in immune system activity are of sufficient type or magnitude for cancer to develop or progress (Cohen & Rabin, 1998).

As immune surveillance is a central mechanism in biobehavioral models of cancer (e.g. Andersen et al, 1994), it is imperative to recognize that, to date, there are no unambiguous data suggesting that immune surveillance is a meaningful mechanism in the protection of humans from common spontaneous neoplastic disease. For example, while immunosuppressed people with advanced HIV disease suffer from the emergence of virally mediated malignancies (Schecter, 2001), they rarely develop common cancers of the lung, breast, prostate, or colon (Goedert, 1998). Rather, they develop Kaposi's sarcoma, a defining feature of clinical AIDS rarely diagnosed in non-AIDS populations. Cancer incidence in transplant patients who are pharmacologically immunosuppressed ranges from 4% to 18% with an average of only 6% (Penn, 1993). Further, the increased incidence is largely attributable to leukemias and lymphomas that may result from ingestion of immunosuppressive agents (e.g. cyclophosphamide, an alkylating agent) that are mutagenic. Finally, models suggesting that cancer is initiated by immune suppression or that failures in immune-surveillance allow cancer to emerge cannot account for the strong relationship between chronic inflammation and subsequent

tumor development (for review see Balkwill & Mantovani, 2001; O'Byrne & Dalgleish, 2001). These data argue against a significant role for suppression of the immune system in the emergence of the more common cancers such as breast, prostate, and lung (Forlenza, 2001) and suggest that alternative mechanisms be explored.

Tumors arise from the clonal expansion of cells that have accumulated numerous somatic mutations in important regulatory and repair genes. Subsequent to these mutations, the cells acquire behaviors that render them independent of stimulatory growth factors and insensitive to growth inhibitory signals (Hanahan & Weinberg, 2000 for review). Additionally, they may acquire other characteristics such as the capacity to evade apoptosis, to initiate and sustain the process of angiogenesis, to replicate without limit, and to invade tissue and metastasize (Hanahan & Weinberg, 2000). Importantly, mutations may happen in any order and mutated cells may or may not progress to a metastatic phenotype. All of these processes occur within the cell nucleus and it is currently unclear how or when components of the immune system might recognize these processes. It is therefore surprising that little attention has been directed at whether stress or other biobehavioral variables can modulate the development or accumulation of somatic mutation and genomic instability. It is possible that a sharper focus on the relationships between behavioral and mutational processes will explain some of the variance in disease outcomes (Forlenza & Baum, 2000). To determine if stress influences the etiology or progression of cancer, researchers first need to demonstrate associations between stress and basic mechanisms of mutagenesis and carcinogenesis such as alterations in DNA damage and repair (see Figure1).

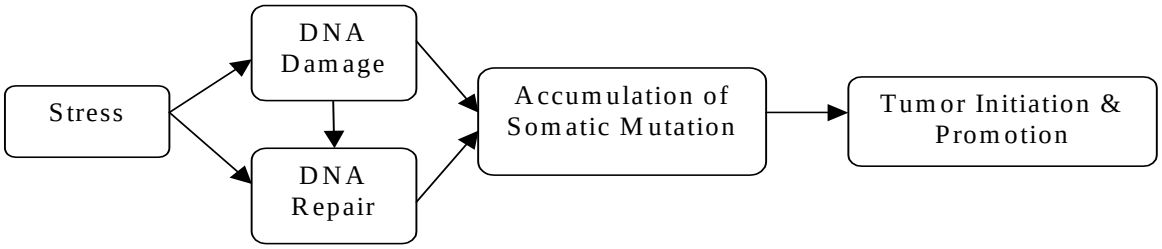

Figure 1: ***An alternative biobehavioral model of cancer stress depicting potential pathways whereby stress may influence mechanisms of DNA damage and repair leading to the accumulation of somatic mutation, genomic instability, and tumor initiation or progression.***

Stress and DNA Repair

Initial examination of this model investigated the relationship linking transient exam stress and levels of nucleotide excision repair (NER) of DNA exogenously damaged with UV light (Forlenza, Latimer, & Baum, 2000). Briefly, NER was measured by unscheduled DNA synthesis (UDS) in blood samples collected from students following their return from spring or summer break and again immediately prior to final or board exams. Based on Kiecolt-Glaser et al., (1985) who found that repair of X-ray damaged DNA was slower in highly distressed psychiatric inpatients, we expected that exam stress would inhibit DNA repair in our student sample. Contrary to prediction, results showed significantly increased levels of NER during the more stressful exam period. Cohen et al, (2000) replicated these findings using the host cell reactivation assay (HCR), an alternative method for measuring NER. Further analyses revealed

that mean levels of NER during the higher stress period were twice the mean levels of NER during the lower stress period *prior* to exogenous damage. That is, repair during stress was significantly increased before cells were exposed to UV light as part of the assay procedure. Although not measured directly, we hypothesized that significant increases in endogenous damage could be driving the observed increases in repair. Because oxidative damage to DNA is ubiquitous and because oxidative lesions are repaired in part by NER mechanisms (e.g. Dianov, et al., 1998), we further hypothesized that oxidative damage to DNA was the likely source of this endogenous damage. Some suggest that endogenous DNA damage in general, and oxidative damage in particular may contribute to genomic instability and a mutator phenotype in tumors (Jackson & Loeb, 2001).

Oxidative Damage and Repair

Oxidative damage to DNA is one of the most common forms of DNA damage (Bohr, 1995) and results from biochemical interactions between various reactive oxygen species (ROS) and nuclear or mitochondrial DNA. Although oxidation leads to the alteration of coding sequences or functional properties of DNA and is thought to contribute to normal aging, mutagenesis, and carcinogenesis (Loft & Poulsen, 1996; Dreher & Junod, 1996; Ames, Shigenaga, & Gold, 1993), current biobehavioral models of cancer have not examined oxidative damage as a plausible mechanism linking stress and mutagenesis, the driving force behind cancer onset or progression.

Mechanisms of oxidative DNA damage and repair are complex. Generation of reactive oxygen species (ROS) is intrinsic to aerobic life and is important in the homeostatic regulation and microbial defense of aerobic organisms. To protect against excessive damage by ROS, aerobic organisms evolved intricate systems of enzymatic antioxidant defense. Should these systems fail, evolved DNA repair mechanisms protect against the propagation of mutations into

future generations of cells. Yet, despite elaborate protection, oxidative damage to DNA is pervasive and mutation occurs.

Mitochondrial Sources of ROS

The major source of endogenous ROS under physiological conditions is mitochondrial production of superoxide (Cadenas & Davies, 2000). Mitochondria produce energy in the form of adenosine triphosphate (ATP) by cytochrome oxidase, an enzyme that transfers four electrons from glucose to an oxygen molecule. The result is two molecules of water and thirty-six molecules of high-energy ATP. However, approximately 1% to 2% of the total electron flux through the mitochondria leaks from the respiratory transport chain and binds to oxygen forming superoxide radicals. Superoxide is not only capable of oxidizing mitochondrial and nuclear DNA, but will increase rates of lipid peroxidation and inactivate numerous enzymes in important biosynthetic pathways (McCord, 2000).

In addition to generation of superoxide, the mitochondria are a significant source of hydrogen peroxide in both the mitochondria and the cytosol (Cadenas & Davies, 2000). This occurs through the oxidative removal of amino groups from biogenic amines (e.g. cathecholamines) by the enzyme monoamine oxidase located in the outer membrane of mitochondria. The relatively long life of hydrogen peroxide and its ability to diffuse long distances contributes to mitochondrial DNA base damage as well as mitochondrial DNA strand breaks.

Superoxide & peroxynitrite from activated immune cells

Activated immune cells are also important sources of ROS. Phagocytes produce superoxide, hydrogen peroxide, nitric oxide (NO), and peroxynitrite ($ONOO^-$) as part of cytotoxic host responses against invading pathogens (Babior & Andreoli, 2000). Several

inducible enzymes generate these ROS in response to antigenic stimulation or cytokine signaling. For example, the enzyme NADPH oxidase produces superoxide from oxygen and NADPH while superoxide dismutase (SOD) converts superoxide into hydrogen peroxide. Nitric oxide synthase produces nitric oxide (NO) from the amino acid arginine, oxygen, and NADPH. Importantly, these ROS may react with each other forming additional radicals. Superoxide and nitric oxide often react together to form peroxynitrite (Kawanishi, Hiraku, & Oikawa, 2001) a potent and long-lived oxidant that is capable of diffusing ten thousand times further than the hydroxyl radical (Aust & Eveleigh, 1999) adding to its potential for damage. Importantly, peroxynitrite forms lesions in DNA similar to hydroxyl radicals (Liebler, Aust, Wilson, & Copeland, 1998).

Once stimulated, phagocytes will release ROS into extracellular spaces in a process termed oxidative burst. The ROS are anti-microbial; they damage lipid membranes and protein structures thus destroying the antigen-bearing cell. However, oxidative damage is not limited to microbial targets and extensive host tissue damage may result (e.g. Simic, 1994). Research suggests that ROS from activated phagocytes can induce strand breaks in neighboring cells (Shacter et al., 1988), damage DNA bases (Jackson et al., 1989) and produce malignant transformations in mice (Weitzman et al., 1985).

Based on the above discussion, it is clear that ROS are inadvertently generated through normal metabolic processes and purposefully generated as part of a coordinated immune response to tissue damage and infection. Regardless of source, excessive production of ROS can overwhelm defensive and repair mechanisms resulting in DNA damage and genetic mutation. Some suggest that the transition metal-catalyzed formation of hydroxyl radicals and peroxynitrite formed during inflammation are the greatest contributors to the oxidation of DNA (Aust &

Eveleigh, 1999). Despite this, ROS are essential for homeostasis and are important mechanisms in cellular proliferation, signal transduction, gene expression, and host defense against microbial infection (Finkel & Holbrook, 2000).

Oxidative DNA Damage Results From Interactions between DNA and ROS

The interaction of ROS with DNA leads to the formation of numerous oxidative damage products (see Aust & Eveleigh, 1999 for review) most notably 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-OHdG). This lesion exists in two tautomeric forms: 8-oxo-2′-deoxyguanosine (8-oxo-dG) is the keto form and 8-hydroxy-2′-deoxyguanosine (8-OHdG) is the enol form (Evans, 2000). Both tautomers are present under physiological conditions. Numerous animal studies have shown that levels of 8-OHdG increase in target tissues following treatment with carcinogenic chemicals and tumor promoters while control animals treated with non-carcinogenic chemicals do not show increased formation of 8-OHdG or tumors (Floyd, 1990; Kasai, 1997).

Unrepaired 8-OHdG leads to Mutation

8-OHdG is formed by the oxidation of the eighth carbon of the guanine base. Oxidation at the C-8 position of guanine alters its base-pairing properties increasing the probability of miscoding by DNA polymerase (Liebler, Aust, Wilson, & Copeland, 1998). Further, the importance of this lesion stems from the fact that it is both abundant in DNA and it is mutagenic causing G → T and C → A transversion mutations (Cheng, Cahill, Kasai, Nishimura, & Loeb, 1992; Moriya, 1993; Le Page et al., 1995). This mutation pattern results from treatment with superoxide and hydrogen peroxide (reviewed in Wang, Kreutzer, & Essigmann, 1998) and is common in *p53*, a known tumor suppressor gene (Greenblatt, Bennett, Hollstein, & Harris, 1994;

Hollstein, Sidransky, Vogelstein, & Harris, 1991). Finally, 8-OHdG can mispair with any base and causes misinsertions at adjacent pyrimidines (Kuchino, et al., 1987). This research suggests that 8-OHdG lesions present in DNA during replication will result in mutation.

It is also possible for oxidative damage to occur to the bases in cytosolic nucleotide pools. If guanine is oxidized to 8-OHdGTP, DNA polymerase may erroneously incorporate this damage product into a new replication strand of DNA. Misincorporation across from an adenine in the template strand leads to AT → CG transversion mutations (Wang, Kreutzer, & Essigmann, 1998). If these mutations occur in important cell regulatory genes, such as oncogenes or tumor suppressor genes, tumor initiation will occur.

Additional Consequences of Oxidative DNA Damage

Unrepaired oxidative DNA damage has significant consequences for mutagenesis and carcinogenesis and is believed to contribute to an individuals cancer risk (e.g. Loft & Poulsen, 1996). Oxidative lesions cause deletions, double strand breaks, chromosomal aberrations, micronuclei formation, damage to histones, abnormal signal transduction, and altered gene expression (Simic, 1994). Additionally, oxidative lesions are mitogenic, increasing the probability of mutation through increasing number of replication cycles (Ames, et al 1993).

Oxidative damage to DNA is not only important in tumor initiation but figures prominently in all phases of tumor promotion and disease progression (Dreher & Junod, 1996; Loft & Poulsen, 1996). Evidence shows that ROS can differentially induce the proliferation of tumor cells at multiple stages (Dreher, & Junod, 1996). Some suggest that tumors are a significant source of ROS themselves and this may account for many of the characteristics of cancer cells including progressive genomic instability, activation of oncogenes, chemoresistance, & metastases (Toyokuni, Okamoto, Yodoi, & Hiai, 1995). The above-cited literature suggests

that it is reasonable to assume that increased levels of oxidative damage figure prominently in the etiology and progression of many cancers (Marnett, 2000).

Studies Showing Increased Oxidative Damage in Cancer Patients

Research shows significantly higher levels of oxidative damage in white blood cells of breast cancer patients prior to treatment compared to healthy women without a personal or family history of cancer (Djuric et al., 2001; Djuric et al., 1996) and significantly higher levels of 8-OHdG in malignant breast tumor tissue compared to normal breast tissue from reduction mammoplasty (Musarrat, Arezina-Wilson, & Wani, 1996). Similarly, there are significantly higher levels of 8-OHdG in prostate tumor cell compared to normal prostate cells and a significant increase in the proportion of 8-OHdG in prostate tumor cells with age (Malins, et al., 2001). Erhola et al., (1997) showed that lung cancer patients with small cell carcinoma (SCC) had significantly higher urinary levels of 8-OHdG compared to non-SCC-patients and healthy matched controls prior to treatment. Further, they found significant associations between urinary levels of 8-OHdG and radiotherapy, chemotherapy, and response to treatment in SCC patients. Similarly, Tagesson, Kallberg, Klintenberg, & Starkhammer (1995) found increased urinary 8-OHdG in 24 hour urine samples from breast cancer patients, malignant lymphoma patients, and head and neck cancer patients compared to healthy people. Additionally, although cancer therapies induced the highest amount of damage, urinary levels of 8-OHdG were increased in cancer patients prior to initiation of treatment compared to healthy controls.

Although studies showing increasing oxidative modification in cancer patients do not address the source or significance of the oxidative damage (i.e. is oxidative damage a cause or result of cancer or cancer-related treatment), they highlight that these patients are at increasing risk of further mutation from oxidative damage. If psychological stress contributes to or

increases this oxidative damage burden then efforts to decrease stress or block DNA oxidation are warranted. The known mutagenic and carcinogenic potential of oxidative damage in general and 8-OHdG in particular, suggests that measurement of 8-OHdG can be important biomarker in biobehavioral studies of cancer.

Clinical Implications

While it is generally accepted that in vivo measurement of 8-OHdG is a reliable biomarker of oxidative damage to DNA, measures have not yet been unequivocally linked with clinical outcomes (Collins, Cadet, Epe, & Gedik, 1997). It would therefore be premature to suggest that an increase in steady-state levels of 8-OHdG inevitably leads to an increase in an individual's cancer risk. However, biomarkers such as 8-OHdG need not directly predict an increase in disease risk in humans, but rather should reflect biological processes clearly linked to the process of carcinogenesis (Rothman, Stewart, & Schulte, 1995). Further, demonstration of associations between stress and a valid biomarker of DNA damage that is clearly mutagenic provides proof of principle that stress is linked to the fundamental processes of carcinogenesis. This may be most important for people with advanced disease when tumors are most unstable. If stress directly or indirectly increases oxidative damage in stable genomes of healthy people (i.e. has little immediate consequence), then stress reduction and prevention of oxidative damage may be vital in slowing the accumulation of mutation in patients with gnomically unstable tumors. Further research examining the role of oxidative damage in mutagenesis and carcinogenesis is needed before conclusions can be drawn or preventive strategies developed.

Stress and Oxidative DNA Damage

Six studies have directly examined the contribution of stress to oxidative damage of DNA (Table 1). Three of the studies have been conducted with rats (Adachi, Kawamura, & Takemoto, 1993; Irie et al., 2000; Liu et al., 1996) and three have been conducted with humans (Irie et al., 2001a; Irie et al., 2001b; Nakajima Takeuchi, Takeshita, & Morimoto, 1996). Other studies (e.g. Forlenza, Latimer, and Baum, 2000; Cohen et al., 2000) did not measure DNA damage directly, but rather examined effects of stress on measures of DNA repair. Based on the findings of increased DNA repair during stress, it is hypothesized that stress will increase levels of oxidative DNA damage.

Animal Studies

Lui et al., (1996) showed that 8 hours of immobilization stress in rats led to significant increases in DNA damage (8-OHdG) in the cerebral cortex. Other brain areas showed non-significant increases. There were no increases in oxidative DNA damage in the liver or kidney. Adachi, Kawamura, and Takemoto (1993) measured 8-OH-dG in rats exposed to psychologically conditioned stress. Experimental animals were placed in a 30-chamber communication box along with animals that were intermittently shocked. While the experimental animals were not shocked, they were exposed to visual, auditory, and olfactory signals from the shocked animals.

Table 1: ***Studies Examining Psychological Stress and Oxidative Damage to DNA***

Authors	Tissue Sample	Subjects	Independent Variable	Dependant Variable	Outcome
Adachi, et al., (1993)	Cellular DNA from liver	Male Sprague-Dawley rats	Conditioned emotional stimuli (CES)	8-OH-dG by HPLC-EC	Significantly higher damage in rats exposed to CES for 5 hr 2x, 3x & 4x ($p < .01$). No effect of single exposure of 5 hr CES or 10 hr CES
Irie et al., (2001a)	Cellular DNA from PBL	N = 362 white & blue collar workers Male (n=276) Female 9 (=86)	Mood (POMS scores), coping, job stress	8-OH-dG by HPLC-EC	Positive correlation b/w damage & negative mood scores ($p < .01$) & wishful thinking ($p < .05$) in females Positive correlation b/w damage & mean # hours worked/ week ($p < .01$) & self-blame ($p < .05$) in males Mean damage in white collar males higher than blue collar males ($p < .04$)
Irie, et al., (2000)	Cellular DNA from kidney	Male Wistar rats	Conditioned taste aversion	8-OH-dG by HPLC-EC	Significantly higher damage in animals conditioned w/ saccharine & Fe-NTA compared to unconditioned rats ($p < .02$) Significantly higher damage in conditioned rats showing painful response during conditioning compared to conditioned rats w/o pain ($p < .004$)
Irie, et al., (2001b)	Cellular DNA from PBL	N = 54 workers Male (n=27) Female (n=27)	Perceived workload, psychological stress	8-OH-dG by HPLC-EC	Positive correlation b/w DNA damage & perceived workload ($p=.01$), perceived psychological stress ($p=.007$), in females. No relation b/w DNA damage & psychological factors in males
Lui et al., (1996)	Cellular DNA from brain	Male Sprague-Dawley rats	Immobilization stress	8-OH-dG by HPLC-EC	Significant increase in DNA damage in cerebral cortex of immobilized rats ($p<.05$). No effect of immobilization stress on DNA damage in kidney or liver
Nakajima et al, (1996)	Cellular DNA from PBL	N = 92	Age, BMI, health practices, and rating of mental stress	8-OH-dG/ 10^6 dG by HPLC	No relationship b/w DNA damage & age, BMI, or health practices including mental stress

Results showed that levels of 8-OH-dG in the livers of rats exposed to a single bout of conditioned emotional stress (CES) for 5 hours or 10 hours did not differ from non-shocked controls. However, rats exposed to a second, third, or fourth bout of CES had significantly higher levels of 8-OH-dG than controls. Levels of oxidative damage returned to control levels within one hour of CES. Differences in 8-OH-dG were found in the kidney after the forth exposure to CES only if the organ was harvested immediately after exposure. No differences were found in the lung.

These controlled experiments offer suggestive evidence that psychological stress can induce oxidative damage to DNA at least in some tissues. Tissue-specific differences in antioxidant defenses or oxidative damage repair may account for differential effects of stress in different tissues. Damage was not immediately apparent and this may mean that the effects of acute and chronic stress differ. One possibility is that earlier stress episodes act to prime sources of ROS that would subsequently increase future exposure to ROS. For example, Kihara et al., (1992) showed that stimulated neutrophils released more superoxide the day before a stressful national board exam than one month before. Similarly, Kang and McCarthy (1994) demonstrated that stimulated neutrophils from rats exposed to an open-field stress task release significantly more superoxide than non-stressed rats. These data support the hypothesis that stress contributes to increased oxidative damage by increasing production and exposure to ROS.

Conditioning experiments offer evidence that psychological mechanisms can mediate the effects of environmental experiences on physiological processes. Irie et al., (2000) showed that levels of 8-OH-dG in young rat kidneys could be classically conditioned with a conditioned taste aversion paradigm. Using ferric nitrilotriacetate (Fe-NTA), an iron chelator that induces free radical reactions and 8-OH-dG lesions in the kidney, (Umemura, et al., 1990; Yamaguchi, et al.,

1996) as the unconditional stimulus (US) and saccharin as the conditional stimulus (CS), two trials of CS – US pairing, but not one trial, led to significantly higher levels of 8-OH-dG in the kidneys of conditioned rats on exposure to the CS. If rats were grouped according to the expression of pain behaviors after one trial only, rats exhibiting pain behaviors had significantly more 8-OH-dG than rats that showed no evidence of pain. Because of the increases in oxidative damage to DNA in the pain group, the authors argued that distress may play a significant role in oxidative damage to DNA by increasing the probability of a conditioned response.

Human Studies

Irie and colleagues have conducted two studies specifically designed to examine the relationship of psychological factors to oxidative DNA damage. In the first study, (Irie et al., 2001a) levels of 8-OH-dG from peripheral blood lymphocytes (PBL) were measured in a large sample of workers (276 males and 86 females). Analyses were run separately in the male and female groups controlling for age, body mass index, cigarette use, and alcohol consumption. Positive relationships were found between levels of 8-OH-dG and the tension-anxiety, depression-rejection, anger-hostility, fatigue, and confusion subscales of the Profile of Mood States (POMS) in female subjects. Although no analyses were reported for the total mood disturbance score of the POMS, these data argue for a relationship between general negative affect and oxidative DNA damage. Additionally, women reporting use of wishful thinking as a coping strategy were more likely to have higher levels of 8-OH-dG than women who did not use wishful thinking. None of the POMS scores were related to oxidative DNA damage in men. However, self-blame and loss of a family member within the past year were significantly related to levels of 8-OHdG in the men as was the average number of hours worked per week. Additionally, males in white-collar jobs showed significantly higher levels of 8-OHdG than

males in blue-collar jobs. No explanation is given for the observed gender differences although they may reflect gender differences in the perception or reporting of stress among Japanese people. The second study (Irie, Asami, Nagata, Miyata, & Kasai, 2001b) investigated the cross-sectional relationships between work-related emotional factors and blood measures of 8-OH-dG in 54 non-smoking and non-drinking Japanese workers (27 males and 27 females). Positive correlations were found between DNA damage and perceived workload, perceived psychological stress, and the perceived impossibility of alleviating stress in females. Again, there were no associations found between DNA damage and job-related stress in males.

Finally, Nakajima (1996) found no association between reported levels of mental stress (much vs. normal or little) and levels of 8-OHdG in healthy male workers. No information is provided about the questionnaire used to measure mental stress and it is assumed that categories were based on single-item responses making interpretation problematic. These studies represent preliminary findings regarding relationships between psychological stress and oxidative damage of DNA.

METHODS

Overview

The present research further investigated stress-related oxidative damage by examining oxidative damage in significantly distressed people. This prospective study measured symptom distress, intrusive thoughts, and urinary excretion of 8-OHdG in a sample of adult victims of MVAs and controls. Overnight urine samples (approximately 15 hours) were collected within 1 month of the MVA and again 3 months after the accident. The prospective design allowed determination of relationships between traumatic stressful experience and oxidative DNA damage over time. This study aimed to provide pilot data and rationale for future intervention

studies aimed at reducing distress and decreasing the potential for DNA modification. Potential confounding variables that have been shown to be related to oxidative damage of DNA (e.g. gender, body mass index, age, physical activity habits, and smoking patterns) were measured and controlled as appropriate in statistical analyses.

The primary hypothesis for this study was that people in the MVA group would have significantly higher levels of urinary 8-OHdG at month 1 than people in the control group. Demonstration of stress-related differences in 8-OHdG requires systematic differences in stressful experience and it is predicted that people in the MVA group will report significantly more distress and intrusive thoughts at month 1 than people in the control group. Reported levels of distress and intrusive thoughts will be significantly related to measured concentrations of 8-OHdG controlling for confounding variables.

We do not expect that exposure to the traumatic stress of an MVA will inevitably lead to increase excretion of 8-OHdG 3 months later. Rather, urinary levels of 8-OHdG will be related to ongoing distress and intrusive thoughts. It is therefore expected that urinary levels of 8-OHdG will be increased only in those people that report persistent distress and intrusions over time. Urinary levels of 8-OHdG from MVA victims who are no longer significantly distressed, or who have low levels of MVA-related intrusive thoughts should not be significantly different than controls.

Participant Recruitment

Participants for this study were adult victims of MVAs and controls. People who had been hospitalized following an MVA were recruited from the regional trauma center of a major metropolitan hospital or from local police reports of serious MVAs. All eligible consecutive admissions to the trauma center where contacted following discharge and asked to participate.

MVA victims were eligible to participate if they were between the ages of 18 and 65 and had experienced a motor vehicle accident requiring hospitalization within the previous 2 weeks. The MVA could have involved a car, truck, or motorcycle and the victim could have been the driver or passenger. MVA victims were ineligible to participate if there was accident-related head trauma, coma, or other organic brain dysfunction as determined by neurological exam, mental status exam, or CAT scan.

Potential control subjects were recruited from the emergency room of a large suburban hospital where they were being treated for minor non-MVA related injuries (e.g. sprains, minor cuts). Control subjects were eligible to participate if they were between the ages of 18 and 65 and were being treated for non-MVA related injuries that did not require hospitalization. Control subjects were ineligible to participate if they had experienced a serious motor vehicle accident within the previous 3 years. Approximately 44% of eligible MVA victims and control subjects agreed to take part in the study.

Eighty six subjects (n = 58 MVA; n = 28 control) completed questionnaires and provided urine samples at both time points. Forty-five percent of the sample was female and the average age was 37 years (range 18 – 64). Thirty percent of the sample described themselves as smokers. Forty percent of the sample were married, forty-two percent were single, and approximately eighteen percent were separated, divorced or widowed. Twenty-nine percent of the sample reported having completed some college and twenty-nine percent competed graduate work. Fifty-six percent of the sample earned less than $40,000 per year while thirty-five percent earned over $50,000 per year (see Table 2).

Procedure

All participants were seen in their home by one of the research staff. During the initial meeting, researchers explained the study purpose and protocol including the rights and responsibilities of the participants. After getting written informed consent, detailed instructions for filling out questionnaires and collecting urine samples were given. Appointments were made for the researcher to return the following day to collect both the questionnaire packet and the overnight urine sample. Participants were paid $25 for each session.

Urine Collection

The concentration of 8-OhdG was measured in 15 hour overnight urine samples. Participants were provided with a resealable collection bucket containing 1 oz. sodium metabisulfite, a non-toxic preservative. They were to collect all voided urine between 6 pm and 9 am the following morning. Urine samples were to be kept refrigerated or on ice. Although collection of 15 hour samples does not permit the calculation of 24 hour excretion rates and limits comparability, this collection timeframe was chosen to maximize collection compliance and minimize loss of data due to incomplete urine samples.

Measures

Variables were divided into 3 categories: background variables, predictor variables, and outcome variables. **Background variables** included anthropomorphic (sex, height, weight, body mass index, basal metabolic rate) and sociodemographic (age, SES) data, indices of smoking habits and physical activity, and family history of cancer. Body mass index (BMI) was calculated reported height and weight by the formula: weight [in pounds] x 704.5 / (Height [in inches]) ^{2}. Basal metabolic rate (BMR) was estimated by the Harris-Benedict formula (Harris &

Benedict, 1919). For males: 66 + (6.3 x body weight in lbs.) + (12.9 x height in inches) - (6.8 x age in years); for females: 655 + (4.3 x weight in lbs.) + (4.7 x height in inches) - (4.7 x age in years). Measurement of background variables was needed for descriptive purposes as well as to ensure group equivalence and to identify potential confounding variables. All subjects completed basic anthropomorphic, sociodemographic, and health habit questionnaires.

Predictor variables included measures of symptom distress, intrusive thoughts, and injury severity (see Appendix A). Symptom distress was measured with the Symptom Checklist 90 Revised (SCL-90-R). This 90-item scale measures emotional, cognitive, and physical symptoms along nine primary symptom dimensions and provides three global indices of distress (Derogatis, 1983). Chronbach's α for the SLC-90-R ranges from .77 for the psychoticism subscale to .90 for the depression subscale. Test-retest reliability ranges from .78 for hostility and .90 for phobic anxiety. The Global Severity Index (GSI) combines information regarding both the number of reported symptoms and the associated perceived distress. The GSI is calculated by summing the subjective distress rating for each of the 90 symptom and dividing this total by 90. The GSI is recommended when a single measure of distress is necessary.

Intrusive thoughts were measured with the Impact of Events Scale (IES; Horowitz, Wilner, & Alvarez, 1979). This 15-item scale assesses the subjective distress associated with the frequency of intrusive thoughts and avoidant behaviors about a researcher-specified event. In this case, the MVA served as the anchoring event. Intrusive thoughts are involuntary thoughts, images, memories, or emotions about past or future events. The unwanted and unpredictable nature of these thoughts makes them distressing and may serve to promote continued stress responding (Baum, Cohen, & Hall, 1993). Research suggests that the IES has good internal consistency across multiple types of traumatic experience (Chronbach's α intrusion = .78;

Chronbach's α avoidance = .82; Horowitz, Wilner, & Alvarez, 1979). Although both the GSI and IES Intrusions score each represent measures of subjective distress, they differ in that the GSI assesses psychophysiological symptoms in general, that is, without regard to a specific event. In contrast, the IES intrusions score assesses distress resulting from thoughts about a specific event. Therefore, the GSI and IES intrusions score represent distinct but related aspects of psychological stress.

Baker's Injury Severity Score (ISS; Baker, & O'Neill, 1976) is derived from the Abbreviated Injury Scale 90 (AIS90; AAAM, 1990), a standardized system for ranking and comparing injuries by severity. The AIS90 is an anatomically based system that classifies injuries according to body region and severity of damage. Injury severity is scored by consensus and ranges from 1 (minor) to 6 (currently untreatable). The ISS is the sum of the squares of the highest AIS score in each of the three most severely injured body regions. Body regions are divided into six sections: head or neck; face; chest, abdominal or pelvic content, extremities or pelvic girdle; and external (body surface regardless of location). Scores can range form 1 to 75. The value of this measure lies in its' anatomical coding system; measures may correlate, but will not be confounded with distress. The ISS was determined at month 1 only.

Outcome Variables

Oxidative modification of DNA was measured using the BIOXYTECH® 8-OHdG-EIA™ kit (Oxis Health Products, Inc.). This kit is a competitive enzyme-linked immunosorbent assay (ELISA) that permits the quantitative measurement of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a premutagenic DNA lesion resulting from attack by reactive oxygen species. Antibody techniques offer a valid and comparatively simple alternative to more technically demanding HPLC-EC or GC-MS techniques for the quantitation of oxidative DNA damage (Cooke, Evans,

Herbert, & Lunec, 2000) and research suggests good correlation between HPLC and ELISA methods (r = .96; Yin et al., 1995).

Briefly, 8-OHdG monoclonal antibodies (50 µl) and urine samples (50 µl) were added to microtiter plates precoated with 8-OHdG and then incubated at 37°C for one hour. The 8-OHdG in the urine samples competes for the monoclonal antibody with the 8-OHdG already bound to the plate. Higher levels of 8-OHdG in the urine sample leads to reduced levels of antibody binding to the plate. Antibodies bound to 8-OHdG in the urine sample were washed out of the plate with 250 µl diluted washing buffer and a second enzyme-labeled antibody (100 µl) was added to the well. The second antibody binds to the 8-OHdG monoclonal antibody attached to the plate. After repeating the plate wash, 100 µl of chromogen was added to each well and incubated in the dark for 15 minutes. The development of color is proportional to the amount of antibody in the plate which in turn, is related to the amount of 8-OHdG in the urine sample. Lower color means higher amounts 8-OHdG. Results are expressed in ng/ml (see Figure 2).

Urine samples from each subject at each time point were split and run in duplicate. Plates were read by a VERSAmax tunable microplate reader. The concentration of 8-OHdG in each sample was determined by generating standard curves for each lot of assay reagents from standardized samples contained in each ELISA kit. Curve fitting was done with SOFTmax Pro 4.0 formula generating software (Life Science Edition). The average coefficient of variation (CV %) between duplicate samples was 15.3% for time 1 and 14.2% at time 2. The CV% at time 1 and time 2 were not significantly correlated (r = -.02, *n.s.*).

Measurement of the 8-OHdG excreted in urine is most often interpreted as reflective of overall oxidative damage in the whole body (Poulsen, H. E., Priemé, H., & Loft, S., 1998). This

includes not only the nuclear DNA, but also mitochondrial DNA, and DNA from cytoplasmic nucleotide pools (Cooke et al., 2000).

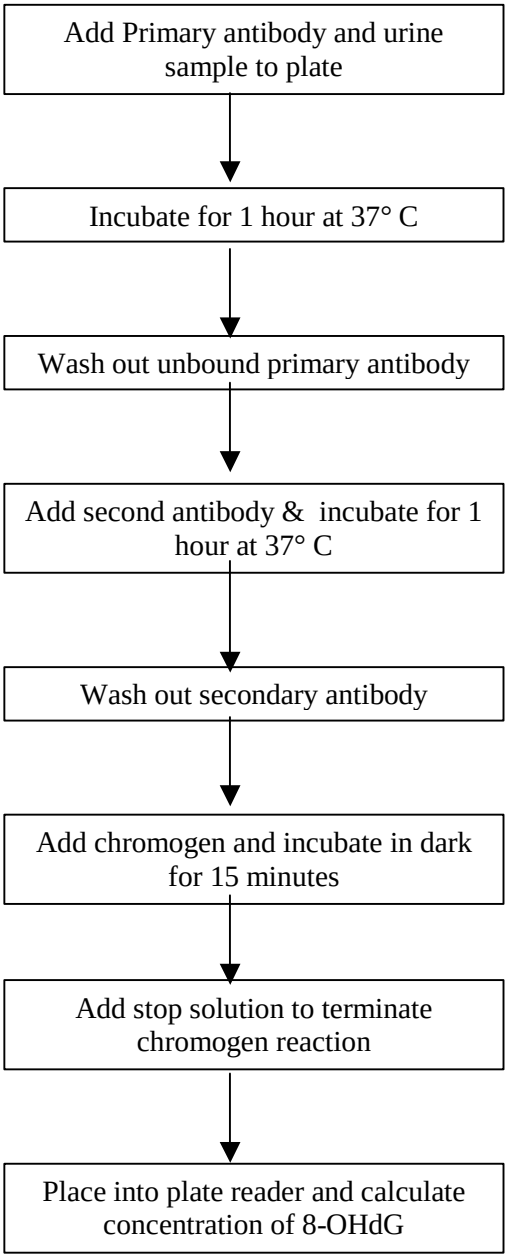

Figure 2: ***Procedural Flow Chart for the Determination of Urinary 8-OHdG by ELISA***

Data Analytic Strategy

Data were analyzed with SPSS version 10. Group equivalence was assessed by analyzing background variables using a series of univariate analysis of variance (ANOVA) for continuous variables and chi square analysis for categorical variables. Because background variables were not necessarily theoretically or conceptually related, multivariate analysis of variance (MANOVA) was not appropriate (Huberty & Morris, 1989). Any background variables significantly different between MVA and control groups at month 1 or month 3 were further examined by correlational analyses to determine the relationship between the background variable and independent or dependant variables. If correlation analyses showed significant correlations between the background variable and independent or dependant variables, the background variable was entered as a covariate in subsequent analyses.

To ensure that the analyzed data reflected reliable measurements of 8-OHdG, participants were selected for analysis at each time point if the CV% of their duplicate urine samples was less than or equal to 20%. However, this strategy resulted in participant samples that differed slightly at 1 month and 3 months post-MVA in terms of participant composition, calling into question whether repeated measures ANOVA was the best way to analyze the data. Sample selection and data analysis had to be as inclusive as possible, using as many subjects' data as possible and maximizing statistical power. Had we used repeated measures ANOVA and analyzed only subjects with small CV% at both time points, we would have lost data from 20 additional participants and been left with a substantially smaller sample size (n = 34 MVA victims and n = 16 controls) and a corresponding loss of power. Therefore, data were analyzed separately for each time point and a confirmatory repeated measures analysis using the smaller sample (Group x Time) yielded identical results.

Analysis for Aim 1:

It was expected that MVA victims would report significantly more symptom distress and intrusive thoughts than control subjects at month 1 and month 3. Group differences in GSI scores and IES intrusion scores were determined by a series of univariate analysis of variance (ANOVA) for month 1 and month 3.

Analysis for Aim 2:

It was expected that MVA victims would have significantly higher concentration of urinary 8-OHdG than control subjects at month 1 and month 3. Group differences in urinary 8-OhdG were determined by ANOVA at month 1 and ANCOVA at month 3 controlling for group differences in estimated body mass index (BMI) and estimated basal metabolic rate (BMR).

Analysis for Aim 3:

It was expected that measures of distress and intrusive thoughts would be significantly correlated with levels of oxidative DNA damage. Pearson product-moment correlations were calculated to determine the relationships among urinary excretion of 8-OHdG, and symptom distress, and intrusive thoughts. Multiple regression was used to analyze interactions.

RESULTS

Of the original 86 subjects there were 49 MVA victims and 21 controls at 1 month post-MVA. There were 43 MVA victims and 21 controls at 3 months post-MVA. The mean CV% at 1 month with the cutoff at 20% was 7.42 (*SD* 5.35). The mean CV% at 3 months with the cutoff for inclusion set at 20% was 7.73 (*SD* 5.64). These CV% fall within the conventional guidelines of less than 10% for establishing the precision of an assay (ESCODD, 2000).

Group Equivalence

Group equivalence was assessed at each time point by analyzing background variables using a series of chi square and univariate analysis of variance (ANOVA). At 1 month, MVA and control groups did not differ in age, sex, marital status, education, income, smoking status, the number of exercise sessions per week, estimated body mass index or estimated basal metabolic rate (see Table 2).

Table 2: ***Group Equivalence on Background Variables at 1 month Post MVA***

Variable	MVA	Control	*p*
N	49	21	
Mean Age (*SD*)	37.7 (14.4)	34.7 (10.9)	*n.s.*
Sex (%)	19 female (38.8 %)	9 female (42.9%)	*n.s.*
	30 male (61.2%)	12 male (57.1%)	
Marital Status			*n.s.*
Single (%)	18 (36.7%)	9 (42.9%)	
Married (%)	18 (36.7%)	10 (47.6%)	
Separated/ Divorced (%)	9 (18.3%)	2 (9.5%)	
Highest Education Levels			*n.s.*
At least some College	24 (54.5%)	11 (55%)	
College Degree or Above	20 (40.8%)	9 (42.8%)	
Income			*n.s.*
Less than $20 K per year	14 (31.1%)	6 (28.6%)	
More than $20 K per year	31 (53.2%)	15 (74.4%)	
Smoking status (%)	13 smokers (27%)	7 smokers (33%)	*n.s.*
Mean # cigarettes per day (*SD*)	17.5 (7.35)	17.00 (13.01)	*n.s.*
Mean # packs per day (*SD*)	1.45 (2.07)	1.67 (2.73)	*n.s.*
Mean Exercise Sessions Per Week (*SD*)	2.41 (2.64)	2.42 (2.06)	*n.s.*
Mean Body mass index (*SD*) (*n* = 61)	24.6 (5.16)	25.2 (3.96)	*n.s.*
Mean Basal Metabolic Rate (*SD*) (*n* = 61)	1726.18 (215.3)	1769.83 (195.9)	*n.s.*

At 3 months, MVA and control groups did not differ on age, sex, marital status, education, income, smoking status, or the number of exercise sessions per week (see Table 3). However, the control group had significantly higher estimated body mass index, $F(1,59) = 4.34$, $p < .05$ and estimated basal metabolic rate, $F(1,59) = 7.63$, $p < .01$ than the MVA group. As expected, BMI and BMR were significantly correlated ($r = 8.08$, $p < .001$). Because of the potential for confounding, BMI and BMR were entered as covariates in subsequent analyses of month 3 data.

Table 3: ***Group Equivalence on Background Variables at 3 months post-MVA***

Variable	MVA	Control	*p*
N	43	21	
Mean Age (*SD*)	36.07 (13.97)	35.29 (10.99)	*n.s.*
Sex (%)	21 female (48.8%)	10 female (47.6%)	*n.s.*
	22 male (51.2%)	11 male (52.4%)	
Marital Status			*n.s.*
Single	18 (43.9%)	10 (47.6%)	
Married	13 (31.7%)	7 (33.3%)	
Separated/ Divorced	10 (24.4%)	4 (19.1%)	
Highest Education Levels			*n.s.*
At least some College	22 (55%)	11 (52.4%)	
College Degree or Above	18 (45%)	10 (47.6%)	
Income			*n.s.*
Less than $20 K per year	15 (36.6%)	6 (28.5%)	
More than $20 K per year	26 (63.5%)	15 (71.4%)	
Smoking status (%)	13 smokers (30.2%)	6 smokers (28.6%)	*n.s.*
Mean # cigarettes per day (*SD*)	15.67 (6.85)	16.33 (13.63)	*n.s.*
Mean packs per day (*SD*)	1.73 (2.72)	1.6 (3.05)	*n.s.*
Mean Number Of Exercise Sessions Per Week (*SD*)	2.80 (2.59)	2.39 (2.25)	*n.s.*
Body mass index (*SD*) (*n* = 61)	23.98 (4.22)	26.39 (4.29)	<.05
Basal Metabolic Rate (*SD*) (*n* = 61)	1687.09 (163.46)	1811.25 (167.63)	<.001

Manipulation Check

To determine whether the MVA group was more distressed than the control group, a series of ANOVAs were conducted on the SCL-90R and the Intrusions subscale of the IES from 1 month and 3 months post-MVA (see Table 4). SCL-90 scores at 1 month were positively skewed and were square root transformed to normalize the distribution for analysis. Reported means and standard deviations are from untransformed data. Results from ANOVA, correlation, and regression analyses reflect square root transformed data but are discussed as untransformed values for ease of interpretation. All analyses were carried out on untransformed and transformed data and the pattern of results did not differ.

Table 4: ***Mean Scores on Distress Measures at 1 Month and 3 Months by Group***

	MVA Month 1 *M*[a] *(SD)*	Control Month 1 *M*[a] *(SD)*	*F*[b]	MVA Month 3 *M*[a] *(SD)*	Control Month 3 *M*[a] *(SD)*	*F*[b]
Global Severity Index	.62 (.39)	.50 (.40)	1.37	.53 (.52)	.37 (.34)	1.65
IES Intrusion Scale	14.43 (8.8)	6.81 (7.0)	12.42*	10.63 (9.0)	2.05 (4.36)	17.10*
Injury Severity Score	5.55 (5.41)	1.43 (1.08)	12.6*			

Note: * $p < .001$ (two-tailed); [a] untransformed data; [b] transformed data

As expected, people in the MVA group reported significantly more distress regarding somatic symptoms, poorer concentration, and greater phobic axniety at month 1 compared to people in the control group. However, the MVA and control groups did not differ on month 1 depression, interpersonal sensitivity, anxiety, anger, suspicion, alienation or GSI scores.

Comparison with normative data from non-patient cohorts (Derogatis, 1983) shows that the control group for this study was more distressed than expected (Mean GSI $_{control}$ = .50 vs. Mean $_{normative\ cohort}$ = .31). This may account for the lack of differences between the MVA and control group on month 1 GSI scores. IES Intrusion scores were also positively skewed and were square root transformed for analysis. As expected, the MVA group (*M = 14.43, SD = 8.8*) had significantly more intrusions at month 1 than the control group (*M = 6.81, SD = 7.00*), *F(1, 68)* = 12.42, $p < .001$. At month 1, GSI score were significantly correlated with IES intrusions, $r = .62$, $p < .001$. Thus at month 1, people in the MVA group had significant distress that was related to intrusive thoughts regarding the accident.

Month 3 GSI and IES intrusion scores were positively skewed and were square root transformed for analysis. There were no significant differences in SCL-90 (subscales or GSI) scores between the MVA and control groups at month 3. As expected, the MVA group (*M = 10.63, SD = 9.0*) had significantly more intrusive thoughts than the control group (*M = 2.05, SD = 4.36*), *F (1,62)* = 17.10, $p < .001$ (see Table 4). Month 3 GSI and IES intrusion scores were significantly correlated, $r = .46$, $p < .001$.

Table 5: ***Pearson Correlations at Month 1 among Distress Measures, Injury Severity, and Urinary 8-OHdG***

	Global Severity Index	IES Intrusion Scale	Injury Severity Scale
Global Severity Index	-	.62**	.15
IES Intrusion Scale	.62**	-	.33*
Injury Severity Scale	.15	.33*	-
8-OHdG	-.04	-.00	.29*

Note: * p < .01 (two-tailed) ** p < .001 (two-tailed)

Table 6: ***Pearson Correlations at Month 3 among Distress Measures, Injury Severity, and Urinary 8-OHdG***

	Global Severity Index	IES Intrusion Scale	Injury Severity Scale
Global Severity Index	-	.46**	.10
IES Intrusion Scale	.46**	-	.32*
Injury Severity Scale	.10	.32*	-
8-OHdG	-.11	.06	.19

Note: * $p < .05$ (2 tailed) ** $p < .001$ (2-tailed)

Results using BMI and BMR as covariates yielded the same pattern of results. There were no group differences in month 3 GSI scores with BMI and BMR as covariate $F(1,57) = .90$, *n.s.*). However, people in the MVA group reported more distressing intrusive thoughts than people in the control group controlling for group differences in BMI and BMR, $F(1,57) = 15.17$, $p < .001$. Month 3 GSI and IES intrusions were significantly related controlling for BMI and BMR, $r = .44$, $p < .001$. Thus, at month 3, people in the MVA group had significantly more distressing intrusive thoughts than the control group and these intrusive thoughts were related to the severity of their injuries 3 months earlier.

Group Differences in Urinary Excretion of 8-OHdG

Group differences in 8-OHdG were determined by ANOVA at month 1 and ANCOVA at month 3 with BMI and BMR as covariates. Measures of 8-OHdG at month 1 were positively skewed and square root transformed to satisfy assumptions of normality and homogeneity of variance. As shown in Figure 3, there were no group differences in the concentration of 8-OHdG excreted in overnight urine samples at time 1 ($M_{Exp} = 5.69$ *ng/ml,* $SD = 4.73$; $M_{control} = 6.44$ *ng/ml,* $SD = 5.85$, $F(1,68) = .47$, *n.s.*).

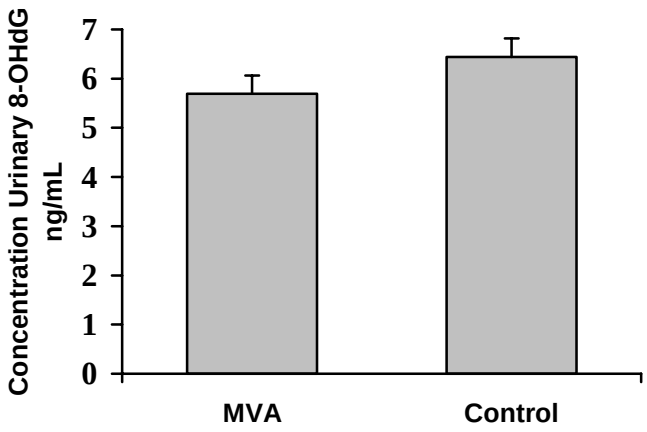

Figure 3: ***Mean Concentration of Urinary 8-OHdG (ng/ml) by Group at Month 1 Post-MVA***

As shown in Figure 4, there were no group differences in the concentration of 8-OHdG in overnight urine samples at month 3 with BMI and BMR as covariates (M_{Exp} *= 5.23 ng/ml, SD = 3.12;* $M_{control}$ *= 5.71 ng/ml, SD = 5.02, F(1,57)* = .21, *n.s.*).

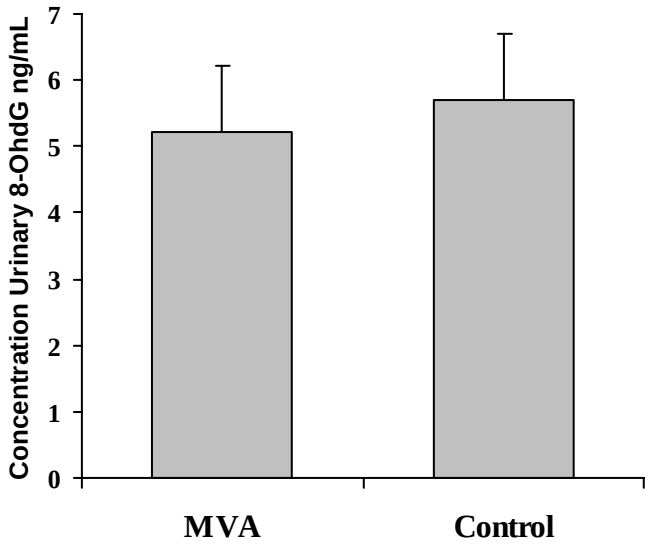

Figure 4: ***Mean Concentration of Urinary 8-OHdG (ng/ml) by Group at Month 3 Post-MVA***

Relationship among Symptom Distress, Intrusive Thoughts, Injury Severity, and Urinary 8-OHdG

As expected, GSI scores were significantly correlated with IES intrusion scores at month 1, $r = .62$, $p < .001$ and month 3, $r = .46$, $p < .001$ but were not correlated with urinary concentrations of 8-OHdG at month 1, $r = -.04$, *n.s.* or month 3, $r = -.11$, *n.s.* Similarly, none of the subscale scores from the SCL-90 were related to the concentration of 8-OHdG at month 1 or month 3. IES intrusions were correlated with the injury severity (ISS) at month 1, $r = .33$, $p < .01$ and month 3, $r = .32$, $p < .05$ but not with urinary concentrations of 8-OHdG at month 1, $r = -.002$, *n.s.* or month 3, $r = .06$, *n.s.* ISS scores were significantly correlated with 8-OHdG at month 1, $r = .29$, $p < .05$ but not at month 3, $r = .19$, *n.s.* (See Tables 5 and 6). Multiple regression analysis revealed that there was no interaction between month 1 IES intrusion scores and ISS in predicting concentration of 8-OHdG at 1 month, $t(61) = -.52$, *n.s.*, or 3 months, $t(57) = .40$, *n.s*.

Partial correlations were calculated between month 3 distress measures and 8-OHdG controlling for BMI and BMR. Results revealed the same pattern as described above. Month 3 GSI, IES intrusions, and ISS scores were not related to urinary measures of 8-OHdG.

Other Group Differences

Gender has been reported to influence urinary levels of 8-OHdG (Loft, Vistisen, et al., 1992). There were no differences in urinary 8-OHdG between men ($M = 6.09$ ng/ml, $SD = 5.00$) and women ($M = 5.65$ ng/ml, $SD = 5.24$) at month 1, $F(1,68) = .30$, *n.s.* in our sample. Similarly, there were no differences in urinary 8-OHdG between men ($M = 6.06$ ng/ml, $SD = 4.26$) and

women (*M = 5.33 ng/ml, SD = 4.15*) at month 3, *F(1,62)* = .64, *n.s.*. Interactions between gender and symptom distress were examined by multiple regression. At month 1, the gender x GSI interaction was not significant, *t(66)* = -.08, *n.s.*). Results are summarized in Table 7. Similarly, the month 1 gender x IES intrusion interaction was not significant, *t(66)* = -.61, *n.s.*. Results are summarized in Table 8.

Table 7: ***Summary of Multiple Regression Analysis of Gender and GSI Scores Predicting Concentration of Urinary 8-OHdG at Month 1***

Variable	Urinary 8-OHdG				
	B	SE B	β	t	Sig.
Gender	-.005	.698	-.030	-.083	.93
GSI	-.005	.612	-.017	-.094	.93
Gender by GSI	-.007	.889	-.035	-.084	.93

Table 8: ***Summary of Multiple Regression Analysis of Gender and IES Intrusions Predicting Concentration of Urinary 8-OHdG at Month 1***

Variable	Urinary 8-OHdG				
	B	SE B	β	t	Sig.
Gender	.272	.715	.143	.381	.71
IES Intrusions	.004	.098	.066	.443	.66
Gender by intrusions	-.118	.193	-.245	-.612	.54

At month 3, the gender x GSI interaction, *t(60)* = .06, *n.s.* and the gender x IES intrusions interaction, *t(60)* = -.73, *n.s.* were not significant. Results are summarized in Tables 9 and 10 respectively.

Table 9: ***Summary of Multiple Regression Analysis of Gender and GSI Scores Predicting Concentration of Urinary 8-OHdG at Month 3***

Variable	Urinary 8-OHdG				
	B	SE B	β	t	Sig.
Gender	-.153	.506	-.094	-.302	.76
GSI Scores	-.282	.645	-.105	-.437	.66
Gender by GSI	.004	.773	.024	.058	.95

Table 10: ***Summary of Multiple Regression Analysis of Gender and IES Intrusions Predicting Concentration of Urinary 8-OHdG at Month 3***

Variable	Urinary 8-OHdG				
	B	SE B	β	t	Sig.
Gender	-.003	.345	-.016	-.075	.94
IES Intrusions	.102	.097	.220	1.054	.30
Gender by intrusions	.009	.127	-.215	-.734	.47

As previous literature found group differences based on smoking status (Suzuki, et al., 1995; Loft, Vistisen, et al., 1992) group differences in urinary excretion of 8-OHdG were examined between smokers and non-smokers. There were 20 participants that self-identified as smokers and 50 participants that self-identified as non-smokers at month 1. There were 19

participants that self-identified as smokers and 45 participants that self-identified as non-smokers at month 3. There were no group differences between smokers (*M = 5.72, SD = 4.75*) and non-smokers (*M = 6.41, SD = 5.87*) on urinary concentration of 8-OHdG at month 1, *F(1,68)* = .04, *n.s.*) or month 3 (M_{exp} *= 6.19, SD = 4.45;* $M_{control}$ *= 4.55, SD = 3.32*), F*(1,62)* = 2.62, *n.s.*) (see Table 11).

Table 11: ***Mean Concentration of Urinary 8-OHdG at Month 1 and Month 3 by Smoking Status***

		Smokers	Non-Smokers	*F**
Urinary 8-OHdG		*M (SD)*	*M (SD)*	
	Month 1	6.41 (5.87)	5.72 (4.75)	.27
		(*n* = 20)	(*n* = 50)	
	Month 3	4.55(3.32)	6.19 (4.45)	2.10
		(n = 19)	(n = 45)	

* *n.s.*

DISCUSSION

The primary goal of this study was to evaluate associations between measures of psychological distress and urinary markers of oxidative DNA damage with the ultimate intention of developing a mechanistic model of biobehavioral carcinogenesis. As predicted, people experiencing a serious motor vehicle accident had significantly more one month after the MVA compared to people in being treated for minor injuries at a hospital emergency room. People in the MVA group continued to have increased intrusive thoughts relative to controls at month 3. Our results are consistent with previous literature showing that intrusive thoughts are frequent and common following MVAs (Bryant, & Harvey, 1996) and other traumatic events such as

hurricanes (Ironson et al., 1997) or tornados (Steinglass & Gerrity, 1990). Results also showed significant associations between IES intrusion scores and general distress as reflected in GSI scores. This too is consistent with the literature: increased intrusions are strongly associated with distress among cancer survivors (Lewis, et al., 2001; Vickberg, et al., 2000), cancer patients (Epping-Jordan et al., 1999; Baider & DeNour, 1997), and women at risk for ovarian cancer (Schwartz, et al., 1995). Thus, our findings provide further support for intrusions as an important component of psychological stress in response to traumatic events.

Contrary to predication, and despite increases in distressing somatic symptoms, poorer concentration, and increased phobic anxiety, there were no differences in GSI scores between the MVA and control groups. As previously stated, the hospital-based control group was more distressed than expected and this may account for the lack of group differences in general distress. Recruitment of the control group from a hospital emergency room was intended to control for the experience of treatment in a hospital setting while allowing the groups to differ with regard to traumatic experience. It is therefore not surprising that these people were experiencing some distress regarding bodily symptoms. Even minor injuries or illness can be upsetting, particularly if the condition is painful or limits common activities of daily living. A control group chosen from the general population rather than the hospital might have resulted in clearer differences in general distress.

Urinary 8-OHdG was assessed as a marker of oxidative DNA damage, and it was expected that people in the MVA group would have higher concentration of urinary 8-OHdG than people in the control group. Surprisingly, results showed no group differences at month 1 or month 3 in urinary 8-OHdG despite significant group differences on intrusive thoughts, the putative cognitive mechanism driving or sustaining physiological stress responding (Baum,

Cohen, & Hall, 1993). It was expected that physiological responding resulting from increased intrusions would result in increased excretion of 8-OHdG.

There are several possible explanations for the negative finding. First, although the MVA group had increased intrusions, increased symptom distress in the control group may have been associated with increased stress responding which in turn raised levels of 8-OHdG. Because both groups were distressed, stress-related differences in urinary 8-OHdG would be difficult to detect. Alternatively, it may be that associations between psychological stress and 8-OHdG are not detectable in urine. No other studies have looked at associations between urinary 8-OHdG and stress. Irie et al., (2001a; 2001b) found associations between negative mood and 8-OHdG in nuclear DNA from peripheral blood lymphocytes and Adachi et al., (1993), Irie et al., (2000), Lui et al., (1996) found associations between stress and 8-OhdG from DNA taken from cells in the liver, kidney, and brain respectively. For 8-OHdG to be detectable in urine, the damaged guanine base must be recognized in the DNA strand and removed by the enzyme hOGG1 (Dianov, Bischoff, Piotrowski, & Bohr, 1998) and it is tempting to speculate that enzymatic mechanisms responsible for detecting and removing 8-OHdG from the DNA may be inhibited by stress. Research shows that hOGG1 is inhibited *in vitro* by both nitric oxide, and peroxynitrite (Jaiswal et al., 2001), free radicals released by activated phagocytes as part of the cytotoxic host response against invading pathogens (Babior & Andreoli, 2000). Additionally, peroxynitrite forms lesions in DNA similar to hydroxyl radicals (Liebler, Aust, Wilson, & Copeland, 1998). Therefore, it is possible that ROS from phagocytes activated by stress could not only cause oxidative damage to DNA, but could simultaneously act to inhibit repair of 8-OHdG via inhibition of hOGG1. If this is the case, associations between stress and 8-OHdG would be

detectable only in cellular DNA, not in urine. Further, these lesions would persist in the DNA increasing the probability of mutation.

This brings up an important conceptual issue with regard to interpretation of urinary measurement of 8-OHdG. As previously discussed, urinary measurements of 8-OHdG are most often interpreted as measuring whole body oxidative damage (Poulsen, H. E., Priemé, H., & Loft, S., 1998). However, as already stated, for 8-OHdG to be detectable in urine, guanine first has to be oxidatively damaged and then it has to be removed from the DNA, that is, it has to be repaired by hOGG1. If repair is assumed to be both constant and constitutive, then increases in urinary products will reflect increases in damage. The above cited research suggests that the kinetics of DNA repair are not necessarily constant and that repair enzymes may alter their level of repair activity under various conditions. Therefore, urinary measurements of 8-OHdG may confound damage and repair. Higher concentration of 8-OHdG may reflect increases in damage or increases in repair of existing damage. This difference is not simply academic; the first implies negative consequences and an increase in the likelihood of mutagenesis. The second interpretation implies the opposite and risk of mutation should decrease. Future studies may benefit by examining 8-OHdG from cellular DNA, plasma, and urine simultaneously and in conjunction with assays of hOGG1 activity to determine the relationship between oxidative damage and repair under different physiological conditions.

Results failed to support hypothesis 3, that there would be significant associations between measures of distress and urinary markers of oxidative DNA damage. Although GSI and IES intrusion scores were significantly related, neither measure was related to 8-OHdG at month 1 or month 3. Again, this does not necessarily mean that there is no relationship between stress and oxidative DNA damage. Rather, as suggested above, associations between psychological

stress and oxidative DNA damage may not be detectable in urine. Evaluation of relationships between stress and 8-OHdG in cellular DNA may show significant associations.

The associations among intrusive thoughts, injury severity and urinary 8-OHdG are of interest. The month 1 data show that people who were seriously injured in an MVA have distressing intrusions about the MVA and this distress is related to the severity of their injuries. However, the lack of interaction between intrusion and injury severity in predicting urinary 8-OHdG and the significant correlation between injury severity and urinary 8-OHdG suggests that increases in oxidative damage may be mediated by a biological process (e.g. inflammation) associated with both severe injuries and oxidative stress rather than increased stress responding resulting from distressing intrusive thoughts. The lack of association between injury severity and 8-ohdG at month 3 supports this notion as it is reasonable to expect that inflammatory processes resulting from injuries sustained in the MVA should have resolved themselves by three months.

Analyses designed to examine gender differences in oxidative damage found no differences in levels of urinary 8-OHdG between women and men and analyses testing interactions between stress and gender also failed to find differences. These findings are in contrast to those of Irie et al., (2001a) who found positive correlations between negative mood scores on the POMS and cellular levels of 8-OHdG in women. Again, issues regarding measurement of 8-OHdG in urine or DNA are applicable. Additionally, Irie et al., examined components of negative mood which may be related but are distinct from distress arising from bodily symptoms or intrusive thoughts. We originally hypothesized that increases in urinary 8-OHdG would result from increased stress responding driven by distressing intrusions. In contrast, the negative mood described by Irie et al., is not necessarily associated with increased arousal. Similar to Irie et al., we found no relationships between distress and 8-OHdG in men.

Results showed no differences in urinary 8-OHdG between smokers and non-smokers. These results stand in contrast to Suzuki et al., (1995) and Loft et al., (1992) but are consistent with Irie et al., (2001a) and Nakajima et al., (1996). Results reported in the literature are mixed with five studies reporting higher damage among smokers compared to non-smokers (Asami et al., 1996; Asami et al., 1997; Lodovici, 2000; Loft et al., 1992; Suzuki et al, 1995), two studies reporting lower damage among smokers compared to non-smokers (Besarati Nia, 2001; van Zeeland et al., 1999), and three studies reporting no differences between smokers and non-smokers (Besarati Nia, 2001; Takeuchi, 1994; Zwingman, 1998). The reasons for the discrepant findings among studies are not immediately clear. Tissue samples were from both cellular and urinary DNA and all but one study used HPLC methods that have been shown to be reliable. Small sample sizes are likely an issue especially when samples were not randomly selected. Those studies with negative and null findings tended to have larger samples lessening the probability of type 2 errors.

Results from the present study were compared with published results from similar studies to evaluate comparability between our measured values for urinary 8-OHdG and values cited in the literature. Values for urinary 8-OHdG are often reported as a function of excreted creatinine rather than as a simple concentration. This requires the additional measurement of urinary creatinine as a correction factor for incomplete (i.e. less than 24 hour) urine samples. Because we have no direct measure of urinary creatinine concentration, and our results are reported as a straight concentration in the urine sample, direct comparison was impossible. However, the amount of creatinine (in grams) excreted in 24 hrs can be estimated with the Cockroft-Gault equation (Cockroft & Gault, 1976; see Appendix B for calculations). When our values for urinary 8-OHdG were corrected for creatinine (M = 4.49, SD = 5.93) and contrasted with overall

means (*M* = 30.78, *SD* = 13.97) reported for urinary 8-OHdG determined by ELISA in Erhola, et al., 1997; *M* = 22.6 ng/mg), Honda et al., (2000; *M* = 33.4 ng/mg), Huang, Helzlsouer, & Appel (2000; *M* = 17.8 ng/mL), and Thompson et al., (1999; *M* = 49.3 ng/mg), our values appear substantially lower suggesting the possibility that our samples may have degraded over time with multiple freezing and thawing.

The present study examined differences in urinary 8-OHdG between people that experienced a traumatic MVA and people being treated for minor injuries at a hospital emergency room. Although people in the MVA group reported significantly more intrusive thoughts than people in the control group and these intrusions were significantly related to general distress, there were no group differences in levels of 8-OHdG and no associations between indices of stress and urinary 8-OHdG. Despite the null findings, logical examination of the results suggests that stress effects on 8-OHdG may not be easily detectable in urine. Future studies would benefit by examining associations between indices of stress and 8-OHdG isolated from cellular DNA, plasma, and urine simultaneously.

APPENDICES

Appendix A

Impact of Events Scale

Below is a list of comments made by people after stressful life events. Please check each item indicating how frequently these comments were true for you **DURING THE PAST SEVEN DAYS**. If they did not occur during that time please mark the *"not at all"* column.

Please think of the following event while filling out the questionnaire:

Your Recent Motor Vehicle Accident

	Frequency			
	not at all	rarely	sometimes	often
1. I thought about it when I didn't mean to.				
2. I avoided letting myself get upset when I thought about it or was reminded of it.				
3. I tried to remove it from my memory.				
4. I had trouble falling asleep or staying asleep.				
5. I had waves of strong feelings about it.				
6. I had dreams about it.				
7. I felt as if it hadn't happened or wasn't real.				
8. I tried not to talk about it.				
9. Pictures about it popped into my mind.				
10. I stayed away from reminders of it.				
11. Other things kept making me think about it.				
12. I was aware that I had a lot of feelings about it, but didn't deal with them.				
13. I tried not to think about it.				
14. Any reminder brought back feelings about it.				
15. My feelings about it were kind of numb.				

SCL-90R

Instructions: Below is a list of problems people sometimes have. Please read each one carefully, and circle the number that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU DURING THE PAST 7 DAYS INCLUDING TODAY. Circle only one number for each problem and do not skip any items.

	Not at all	A little bit	Moderately	Quite a bit	Extremely	How much were you distressed by:
1	O	O	O	O	O	Headaches
2	O	O	O	O	O	Nervousness or shakiness inside
3	O	O	O	O	O	Repeated unpleasant thoughts that won't leave your mind
4	O	O	O	O	O	Faintness or dizziness
5	O	O	O	O	O	Loss of sexual interest or pleasure
6	O	O	O	O	O	Feeling critical of others
7	O	O	O	O	O	The idea that someone else can control your thoughts
8	O	O	O	O	O	Feeling others are to blame for most of your troubles
9	O	O	O	O	O	Trouble remembering things
10	O	O	O	O	O	Worried about sloppiness or carelessness
11	O	O	O	O	O	Feeling easily annoyed or irritated
12	O	O	O	O	O	Pains in the heart or chest
13	O	O	O	O	O	Feeling afraid in open spaces or on the streets
14	O	O	O	O	O	Feeling low in energy or slowed down
15	O	O	O	O	O	Thoughts of ending your life
16	O	O	O	O	O	Hearing voices that other people don't hear
17	O	O	O	O	O	Trembling
18	O	O	O	O	O	Feeling that most people cannot be trusted
19	O	O	O	O	O	Poor appetite
20	O	O	O	O	O	Crying easily
21	O	O	O	O	O	Feeling shy or uneasy wit the opposite sex
22	O	O	O	O	O	Feelings of being trapped or caught
23	O	O	O	O	O	Suddenly scared for no reason
24	O	O	O	O	O	Temper outbursts that you could not control
25	O	O	O	O	O	Feeling afraid to go out of your house alone
26	O	O	O	O	O	Blaming yourself for things
27	O	O	O	O	O	Pains in lower back
28	O	O	O	O	O	Feeling blocked in getting things done
29	O	O	O	O	O	Feeling lonely
30	O	O	O	O	O	Feeling blue
31	O	O	O	O	O	Worrying too much about things
32	O	O	O	O	O	Feeling no interest in things
33	O	O	O	O	O	Feeling fearful
34	O	O	O	O	O	Your feelings being easily hurt
35	O	O	O	O	O	Other people being aware of your private thoughts
36	O	O	O	O	O	Feeling other do not understand you or are unsympathetic
37	O	O	O	O	O	Feeling that people are unfriendly or dislike you
38	O	O	O	O	O	Having to do things slowly to insure correctness

39	O	O	O	O	O	Heart pounding or racing
40	O	O	O	O	O	Nausea or upset stomach
41	O	O	O	O	O	Feeling inferior to others
42	O	O	O	O	O	Soreness in your muscles
43	O	O	O	O	O	Feeling that your are watched or talked about by others
44	O	O	O	O	O	Trouble falling asleep
45	O	O	O	O	O	Having to check and double check what you do
46	O	O	O	O	O	Difficulty making decisions
47	O	O	O	O	O	Feeling afraid to travel on busses, subways, or trains
48	O	O	O	O	O	Trouble getting your breath
49	O	O	O	O	O	Hot or cold spells
50	O	O	O	O	O	Having to avoid certain things, places, or activities because they frighten you
51	O	O	O	O	O	Your mind going blank
52	O	O	O	O	O	Numbness or tingling in parts of your body
53	O	O	O	O	O	A lump in your throat
54	O	O	O	O	O	Feeling hopeless about the future
55	O	O	O	O	O	Trouble concentrating
56	O	O	O	O	O	Feeling weak in parts of your body
57	O	O	O	O	O	Feeling tense or keyed up
58	O	O	O	O	O	Heavy feelings in your arms or legs
59	O	O	O	O	O	Thoughts of death or dying
60	O	O	O	O	O	Overeating
61	O	O	O	O	O	Feeling uneasy when people are watching or talking about you
62	O	O	O	O	O	Having thoughts that are not your own
63	O	O	O	O	O	Having urges to beat, injure, or harm someone
64	O	O	O	O	O	Awakening early in the morning
65	O	O	O	O	O	Have to repeat the same actions such as touching, counting, or washing
66	O	O	O	O	O	Sleep that is restless or disturbed
67	O	O	O	O	O	Having urges to break or smash things
68	O	O	O	O	O	Having ides or beliefs that others do not share
69	O	O	O	O	O	Felling very self-conscious with others
70	O	O	O	O	O	Feeling uneasy in crowds, such as shopping or at a movie
71	O	O	O	O	O	Feeling everything is an effort
72	O	O	O	O	O	Spell of terror or panic
73	O	O	O	O	O	Feeling uncomfortable about eating or drinking in public
74	O	O	O	O	O	Getting into frequent arguments
75	O	O	O	O	O	Feeling nervous when you are left alone
76	O	O	O	O	O	Others not giving you proper credit
77	O	O	O	O	O	Feeling lonely even when you are with people
78	O	O	O	O	O	Feeling so restless you couldn’t sit still
79	O	O	O	O	O	Feelings of worthlessness
80	O	O	O	O	O	The feeling that something bad is going to happen to you
81	O	O	O	O	O	Shouting or throwing things
82	O	O	O	O	O	Feeling afraid that you will faint in public
83	O	O	O	O	O	Feeling that people will take advantage of you if you let them

84	O	O	O	O	O	Having thoughts about sex that bother you a lot
85	O	O	O	O	O	The idea that you should be punished for your sins
86	O	O	O	O	O	Thoughts and images of a frightening nature
87	O	O	O	O	O	The idea that something serious is wrong with your body
88	O	O	O	O	O	Never feeling close to another person
89	O	O	O	O	O	Feelings of guilt
90	O	O	O	O	O	The idea that something is wrong with your mind

Injury Severity Score Coding Sheet

ISS Body Region	Injury	AIS Code	Highest AIS	AIS^2
Head/ Neck				
Face				
Chest				
Abdomen				
Extremities				
External				
			ISS =	

Appendix B

Cockroft-Gault Equation (Cockroft & Gault, 1976) to Estimate the Amount of Creatinine Excreted In Urine in 24 Hours

For Men: [(140-age) x (weight in Kg)]/5000

For Women: [(140-age) x (weight in Kg)] x.85/5000

We estimated the amount of excreted creatinine (in grams) in 24 hours and corrected for the shorter (15 hr) sample collection period (multiply by .625). This was then multiplied by 1000 to yield the estimated mg of creatinine excreted in 15 hours. Next, this estimated figure was multiplied by the collected urine volume (in ml) to yield the estimated creatinine mg/ml. Finally, measured values of urinary 8-OHdG (ng/ml) were divided by estimated values of urinary creatinine (mg/ml) to yield estimates of 8-OHdG ng/mg of creatinine. Corrected and uncorrected measures of 8-OHdG were correlated r = .734

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