REVIEW ARTICLE

Occupational Heat Stress, DNA damage and Heat Shock Protein

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Abstract

Changing climatic scenario and raising temperature is likely to subject millions of working population across the globe to heat stress at their workplaces. Several epidemiological studies, including our own other studies, stand proof of the adverse effects of heat stress on the health of the workers. Heat stress imposes a strain on the physiology of workers exposed to heat stress that invokes physiological responses that includes induction of DNA damage and changes in the Heat Shock Protein (HSP) levels in blood. We conducted an extensive review and examined published data linking the relationship between occupational heat stress, changes in gene expression and HSPs induced by the DNA damage. Though the evidence for the mechanistic pathway is limited, reviewed literature shows strong evidence for the association between occupational heat stress, DNA damage and HSPs. We conclude that occupational heat stress is a significant risk factor and understanding its association with DNA damage will give key insights in how preventive interventions can be adopted to protect the working population from further adverse effects of occupational heat exposures.

***Keywords*:** occupational heat stress, literature review, DNA damage, micronuclei, HSP

1.1 Introduction:

Globally, rise in temperature had paved the way for health threats for millions of people [1,2]. Excess heat exposures are not only an environmental threat but also an occupational hazard for millions of workers exposed to high heat conditions, especially in tropical settings [3]. The workers with prolonged heat exposures in many jobs, both outdoors or in hot indoor environments, are subjected to heat stress with consequent heat strain symptoms [4]. Heat stress, a proven environmental and occupational hazard is exposure to high heat environments and physical exertion [3], while "heat strain" is the physiological responses of the body to exposures to heat stress and "heat stroke" is a condition caused by your body overheating which may lead to unconsciousness and death [5,4].

Workers are subjected to high air temperatures & humidity, radiant heat sources, direct physical contact with hot objects, and/or strenuous physical activities in many jobs. Workers in iron and steel industries, foundries, smelters, brick and ceramic industries, glass and rubber producing industries, boiler rooms of electrical utilities, bakeries, food canneries, commercial kitchens, laundries, chemical plants, outdoor and underground mines, and outdoor workers exposed to direct sunlight are subjected to high heat exposures on a day-to-day basis and have high potential for heat-related illness (HRIs) [6,7,8]. The heat exposures will also exacerbate preexisting chronic health conditions, such as respiratory, cardiovascular diseases and kidney diseases [9,10,11]. Workers in mines, especially those who work in deep mines – geothermal gradients and equipment contribute to high heat and humidity exposures that can adverse health effects [12]. Humidity in workplaces also contributes to heat stress and workers whose work entails wearing insulated clothing can encounter higher heat stress due to additional insulation for evaporative cooling; these include firemen, soldiers, asbestos workers, sandblasters, tank cleaners [13].

1.2 Heat Stress and Physiological Responses:

Chronic heat exposures beyond safe limits and heat stress for a physically active individual will affect a person physiologically [4]. A balance exists between heat gain and heat loss from the body, called the thermal balance to maintain the body's normal core body temperature around 37°C [4]. The existence of an important link between the environment, work rate, metabolic load, and core temperature has been evidenced in occupational studies [14]. Heavy workload in hot working environments creates substantial surplus heat inside the body, and when the air temperature exceeds 37°C, evaporation of sweat becomes the only mechanism to cool the body, but sweat evaporation is strongly influenced by high air humidity and clothing [15]. If there is any alteration in this thermal balance it leads to adverse health impacts like heat stroke which is very fatal [16]. In such conditions, sweat output often exceeds water intake, resulting in a body water deficit (hypohydration) and electrolyte losses [17]. Several researches have shown that dehydration rises heat strain [18] and has the potential to increase the risk of

developing heat illness [19,20]. Heat stress and dehydration in combination heavy workload and lack of adequate fluid intake can have adverse health and performance consequences that can leave the workers vulnerable to HRIs [21].

Increased metabolic work load may cause cardio vascular strain during high ambient temperatures can lead to progressive increases in body heat content and if left unchecked may lead to heat related illnesses [14]. Heart rate is an important parameter in evaluating the exertion required by physical labor in working conditions [22]. Continuous exposure to excessive heat may cause profound increase in heart rate which may lead to sympathovagal imbalance if not treated appropriately. An average heart rate for the working people should be less than 100bpm. OSHA Technical Manual, (1999) recommended that if the heart rate exceeds 110 bpm, the next period of work shift should be shorten by one third and the rest period should be maintain [23].

Studies have shown that change in specific gravity of urine on exposure to high ambient temperature and heavy workload has significant correlation with total body water changes [24]. A study by Baker *et al.* (2009) [24] and Oppliger *et al.* (2005) [25] have shown that USG >1.020 leads to 3 % reduction in body mass Candas *et al.* (1986) [26] reported that significant hypohydration was associated with depression of sweating which was directly associated with hypo hydration. Increased dehydration disturbs the homeostasis of the body leading to decreased skin blood flow, elevated CBT, and decreased sweat rate leading to impaired to tolerance to work resulting in increased risks of heat injuries [27]. Due to heat stress and strain, the individuals exposed to high temperature at their workplace suffer from a range of heat-related illnesses (HRIs) such as heat rashes, cramps, fatigue, syncope and even heat stroke that may lead to death [28]. It is apparent and has been reported commonly is the fact that with exercise there is an increase in body temperature, leading to profuse sweating that triggers the cell chaperones and hormones [29].

There is emerging evidence that chronic or repeated episodes of heat stress accompanied by water and solute loss (by dehydration or volume depletion) can cause repeated subclinical ischemic kidney injury, which over time may lead to permanent kidney damage and CKD [30,31]. These repeated episodes of injury, which can be combined with some acute clinical episode [32] eventually result in abnormal repair mechanisms [33] leading to renal fibrosis, vascular rarefication, and glomerulosclerosis [34]. Rhabdomyolysis, hyperosmolarity, hyperthermia, and extracellular volume depletion [33]. Many scientists now believe that heat-stress nephropathy, resulting from extreme occupational heat stress and repeated dehydration, is central in the pathophysiology of Mesoamerican nephropathy [30,35, 36, 33, 31]. Whether heat stress causes CKD directly or in combination with other factors is an intriguing hypothesis; however, it remains unproved [37,38,39].

1.3 Heat Stress and Cell Responses:

Experimental studies with humans with no exercise in a heated environmental chamber showed that 30 minutes of heat stress increased the body temperature & heart rate and decreased the blood pressure of the individual. In addition, norepinephrine and prolactin increases were observed in the the plasma [29]. Mixed exposures occur most often in occupational settings and there is often a synergistic interaction of these substances in the presence of heat [40] and exercise induced dehydration has been associated with enhanced oxidative stress in humans [41]. Oxidative stress is defined as the damage caused to biomolecules [29] by the imbalance between pro oxidative molecules overlapping antioxidative molecules, the increase in reactive oxygen species (ROS) or decrease in antioxidant levels that could happen after exposures to heat stress; however, the exact mechanism is still unknown [42]. Some animal and human studies concluded that oxidative stress is the main factor responsible for damage caused by heat stress [43]. Studies quoted that oxidative stress to be one of the major mechanisms for thermal damage of spermatogenic cells that leads to apoptosis and DNA strand breaks [44,45]. The oxidative imbalance may occur after the reestablishment of the normal temperature and tissue reperfusion and such situation has been described in studies where suppression of testicular function under heat stress led to a decrease in fertility in human affected by varicocele [46]. Possible consequences of oxidative stress could be DNA damage followed by repair or defective repair, apoptosis/necrosis with consequent DNA sequence alterations with adverse clinical endpoints such as oncological, genetic, fertility and immunological health issues [47,48,49]. Premutagenic changes caused by oxidative DNA damage include a range of strand breaks and instability formed directly or by repair processes [50]. Although all four bases are modified by ROS, mutations are generally related to modification of GC base pair as the AT base pair rarely leads to mutations [51]. Many of these factors significantly increase the potential for changes in the DNA structure that leads to the generation of free radicals and induction of different proteins, including heat shock proteins [52].

Males exposed to high occupational heat exposures, such as bakers and welders, have reported fertility issues [53] and also by professional drivers who jobs that involve sitting for long periods in a sedentary position, such as professional drivers. The vital role played by the regulation of body temperature in sperm production and even slight increases in temperatures may cause fertility problems leading to premature death of embryos, low sperm has been demonstrated [54]. The testicular function is temperature dependant and is kept between 2 and 8°C below core body temperature in most mammals [56,57] and in man, increase in testicular/scrotal temperature may occur due to various reasons including occupational exposures, posture and clothing [58, 59]. Mieusset *et al.* concluded from his study that above normal scrotal temperatures in men have resulted in increased rates of sub or infertility and their ejaculates contained an increased incidence of abnormal and immature spermatozoa [58]. Durairajanayam

et al reviewed the effects of heat stress and believed that thermoregulatory failure due to various factors, including occupational heat stress as a causative factor for compromised sperm quality and risk of infertility [60].

1.4 Heat Stress and Gene Response:

It is well-known that direct heat exposure to cells causes' protein degradation and DNA damage, which can lead to genetic alteration and cell death, but very little is known about the impacts of heat-induction on the surrounding tissue. Pursche et al., [61] reported an effect called ''active thermal bystander effect'' (ATBE), caused by the ''bystander'' cells that share the medium with heat-exposed cells exhibit DNA damage, apoptosis, and loss of viability even in the absence of any direct heating, heat diffusion, or cell-to-cell contact. Significant ATBE was induced when fibroblasts were exposed for 10 minutes to a temperature range of 44-50°C which seemed to be an active process [61]. Harrouk et al., believed that during fertilization if a sperm is exposed to DNA damaging agents, it can potentially alter the expression in the one cell stage [62]. Rockett et al. (2001) [55] showed that the expression of a number of DNA repair genes (Ogg1, Xpg and Rad54) were all down-regulated to investigate the changes in global gene expression following heat stress at 43°C. When rats were heat stressed, decreased expression of polyADP Ribose polymerase (PARP) that are involved in detection of strand breaks in both the base and nucleotide excision repair pathways was observed [63, 64]. Nezhad et al. evaluated the response indicators of oxidative and the amount of DNA damage in Sertoli cells exposed to heat stress. The results showed that applying the heat stress caused an increase in the number of damaged cells from DNA and this increase was significant in the 39°C and 42 °C exposed groups compared with the control group [65]. Cryptorchidism-induced heat stress resulted in decreased expression of repairing agents that are involved in the final stages of DNA repair such as DNA polymerase b and DNA ligase III [63].

Feng *et al.* concluded from his in vitro studies that high temperatures induced micronuclei in human lymphocytes [66] that was further enhanced when combined with cigarette smoke. Micronucleus frequency as a marker of genomic damage was elevated in a temperature-dependent and statistically significant manner in a study conducted in human keratinocyte cell line [67]. Minisini *et al.* believed that DNA alterations could represent a common signal for the induction of stress protein synthesis during heat shock or exposure to reactive oxygen species in the humans based on their experiments with premonocytic line U937 while measuring, in parallel, DNA damage (both strand breaks and fragmentation) and HSP synthesis (by biometabolic labeling and Western blotting) after exposure to heat shock, hydrogen peroxide, bleomycin, cadmium or erythrophagocytosis [68]. Reviews conducted on current evidence available about heat-induced DNA damage and its relationship with HSP70 levels suggest the use of HSP70 as a biomarker for DNA damage caused by to heat stress [69]. Kanitdze *et al.* (2016) deduced from their experimental studies that heat stress directly results in the formation of various DNA damage and double strand

breaks depending on the cell type and strength of the heat stress [70].

1.5 Heat Stress and Heat Shock Protein

Cellular stress responses to heat shock such as unfolded protein, DNA damage, and oxidative stress responses form an integral part of physiology that are done by nature either to ensure the cell's survival or to eliminate damaged/unwanted cells [71]. Yan *et al.*, believed that heat-shock proteins (HSPs) played a critical role in cells when exposed to thermal stress by obtaining thermotolerance, therefore, protect the cells from stress-induce cellular damage and the HSPs are also believed to prevent protein aggregation and help transport repair proteins and serve as molecular chaperones. [72].

The heat shock or stress response is a highly conserved response of all cells, tissues, and organisms on exposure to elevated temperatures, to a variety of environmental stresses, and to pathological stimuli such as infections, fever, inflammation, malignancy, and autoimmunity [73,74] and also enhance the immune system [75]. Intracellular HSPs function as molecular chaperones, supporting protein folding and transport mechanisms under physiological conditions and after physical or chemical stress [76]. Literature reports adverse immunological endpoints (such as cell damage, death and altered function) and provide the temperature at which these effects occur has been studied in many *in vitro* studies of adverse temperature effects, but has limited validation *in vivo* [77]. Extracellular HSPs are involved in the induction of the cellular immune response. In particular, HSP70 genes may play a specific role in controlling cellular responses to stress and apoptosis [78,79]. The cell's protective response mechanism to exposures to sub lethal heat shock by synthesizing heat shock proteins via the heat shock-dependent transcription pathways to resist impacts of higher adverse temperatures [80,81].

Studies show that the among the HSPs that are synthesized during heat stress to protect the cells and DNA from damage and the HSP 70 could prevent the cell damage due to heat stress [82,83]. Though numerous studies have investigated the association between various potential stress factors and synthesis of HSPs [84], only few studies have investigated the variations in the level of HSPs in natural and physical conditions only few studies have investigated the variation of HSP levels under varying natural and physical conditions [85]. HSP 70 is sufficient to protect against most division abnormalities, demonstrating the involvement of HSP 70 in a repair mechanism of heat-damaged mitotic centrosomes [86]. Besides its chaperoning activities, HSP70 also protect the mitochondria and interfering with the stress-induced apoptotic program [87].

The generation of metabolic oxidative stress, indicated by malondialdehyde (MDA) formation, occurs before and concomitantly with lactate dehydrogenase (LDH) leakage [88] that can induce the expression of HSP70 [89]. Since hot environments are the direct cause of acute heat-induced illness [90], HSP70 has also been reported to distribute in the extracellular space as well, suggesting that it may have different functions in different cellular compartments

[91] such as induced HSP 70 acting as a danger signal to the immune system or disease conditions in plasma [92,93,94] and secreted HSP70 playing an important role in bacterial infection [95].

HSPs are induced in response to various stresses including exposure to elevated temperature, and environmental and physiological stresses [73,74]. The capability of exercise in inducing a heat shock response in a number of tissues in animals [96,97,98] and in humans [47]. Cell injury induced by coke oven emissions (COEs) for exposed workers in steel industry includes genotoxicity, oxidative stress, and other cell damages. Though the role of intra- and extracellular HSPs as responsive biomarkers as protection or danger signals remains unknown, the authors concluded that high levels of HSP 70 in lymphocyte and plasma may provide protection and serve as a danger marker respectively [69].

Studies have also reported significant associations between levels of HSP70 expression and DNA damage in peripheral blood lymphocytes [69]. The HSP72 level significantly increased with heat stress (48.7% ± 53.9%). Significant differences in cardiovascular and blood variables were observed between the control and exposure trials (quiet sitting in the heat chamber with no heat stress vs heat exposures [29]. Molecular chaperones synthesized due to intake of toxins or adverse exposures (such as alcohol, other poisons, sunburn, anxiety, etc.) are responsible for the "conformational homeostasis" of cellular proteins and notably, HSP 70 prevents cellular damage including cardiac muscle [99]. Induced heat shock proteins, due to moderate heat treatment, has been found to be beneficial to transplanted organs as it reduces transfer-damage and the risk of organ rejection [100].

Investigations have shown correlations between the aberrant expression of HSPs and disease states, especially HSP70 which are believed to protect many of these systems and organs from the damage of stresses [101] and also can behave as danger markers under certain conditions [102]. Tang-Chun *et al.* (1998) study showed that the increase in plasma of workers in stressful environments had higher levels of most Heat Shock Proteins, when compared to a control group [103]. Pilots exposed to acute heat stress had an increase in lymphocyte DNA damage with the positive antibodies during heat stress to HSPs than those pilots with the negative antibodies [75]. High incidence of antibodies HSP72 and HSP70 was observed in workers working in an extreme heat combined with high carbon monoxide exposures compared to the control group [104]. Results of similar nature were obtained in a study conducted in a group of young person's exercising in a hot environment who had a significantly higher occurrence of antibodies against Hsp71 and Hsp90α among individuals with symptoms of heat-induced illness than the unaffected exercising individuals [105]. Wu *et al.* study clearly suggested that the measurement of antibodies to HSPs may be useful in assessing how individuals are responding to abnormal stress within their living and working environment and may be used as one biomarker to evaluate their susceptibility to heat-induced diseases [105].

HSP70 also play a major role during normal growth and differentiation of many cell types [106,107] and involved in protecting the cells from the thermal stress [108]. It was reported that workers exposed to high heat conditions had high level of DNA damage and high HSP70 levels [42,109] and that HSP 70 was directly linked to molecular recovery that would counter the deleterious effects of the environmental stressors including toxicants [110,49]. The mapping of HSP70 may have significant implications with regard to immune mediated diseases [111,112,43]. Therefore, HSPs may constitute valuable tier-1 biomarkers among the broad-response biomarkers that are being used for preliminary screening of complex environments [113]. A recent study revealed that cells with inactivated HSP70 displayed telomere instability, high frequency of spontaneous chromosomal aberration and maintenance of Genomic stability [114]. In addition, high levels of intracellular HSP70 in lymphocytes may be a danger marker, as observed in late-stage patients with cerebral infarction [93]. The HSP 70 measurement acts as a good biomarker for identifying the risk of disease recurrence in patients with primary tumors [115]. Many recent studies have suggested the possible significance of plasma and lymphocyte HSPs measurements in the understanding of the mechanism of pathogenesis, diagnosis, and prognosis of many diseases [93,94,116,42]. However, only few comparative studies have been carried out to investigate the presence of HSPs in both plasma and lymphocytes either in the individuals with similar exposures to environmental stressors or in patients with the same disease. Because HSP70 can be sensitively induced by a large number of chemicals or metabolites, its presence might be considered an alternative biomarker for exposure to a wide range of pollutants as tested in cultured cells [113]. Though many studies indicate the HSPs association with the risk and outcome of a variety of hypoxia-related diseases [112,43,40], not many studies have explored the association between the HSP70 and the incidence of Heat Related Illnesses in the worker population exposed to occupational heat stress.

The feasibility of using HSP70 as biomarker for genetic damage from heat exposures is an unexplored area in Occupational settings. Though studies have investigated DNA damage and HSP release due to various stressors, very little is known about the expression of HSP70, its relation with genotoxicity caused by environmental stresses, especially heat, and its possible biomedical significance in pathogenesis processes in humans [65]. The question of "Can HSP70 levels in blood can be used as biomarkers to assess the extent DNA damage induced by heat?" is vaguely answered or faced with no definitive answer. With a warming climatic scenario across the globe that will potentially expose millions of workers to high heat working environments, there is an urgent need to understand the effects of heat stress on cell damage and its relationship with HSP levels in blood, so that some interventions could be adopted to protect the workers.

1.6 Conclusion:

Heat-related illness are one of the silent causes of death worldwide and will continue to increase in severity with the rise in global temperatures due to Climate Change [117]. Heat stress not only inhibits DNA repair systems, but can also act as a DNA damaging agent. Cell response to heat stress involves most sub-cellular compartments and metabolic processes. In summary, the research evidence clearly indicates heat stress directly results in the formation of various DNA damage and the type and the fate of the heat stress-induced damage depends on the stage of the cell cycle when the cell is exposed to high temperatures. Genetic damage due to occupational heat exposures have been under addressed at a global level and a complete understanding of HRIs at physiological as well as molecular level is required to facilitate design of more efficient preventive and treatment strategies. Can the HSPs which act as protective chaperones be is an accurately measure the stress response from the heat exposures? If yes, then periodic measurements of HSP as a biomarker for genetic damage for workers engaged in risky high heat operations will allow optimal protective and preventive strategies to be implemented and further damage can be avoided. Understanding the mechanism would aid researchers to determine the magnitudes of the stress response to various temperatures and help delineate the effective use of HSPs as biomarkers to develop targeted surveillance for the high risk workers and interventions including therapies to avert the adverse effects of occupational heat stress.

1.7 Conflict of interest/Funding statement

There is no conflict of interest involving any of the co-authors.

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