EFEITO GENOTÓXICO E MUTAGÊNICO CAUSADO PELA EXPOSIÇÃO OCUPACIONAL À MISTURA DE METAIS EM SOLDADORES: ELUCIDAÇÃO DOS MECANISMOS CELULARES E MOLECULARES

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Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do grau de **Doutor em Ciências (Genética e Biologia Molecular).**

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Porto Alegre, junho de 2021.

INSTITUIÇÕES E FONTES FINANCEIRAS

Esta tese é produto de um projeto financiado pelo Departamento Administrativo de Ciencia, Tecnologia e Innovación - Colciencias, Colômbia, subvenção número 657/125365741083/2014, Universidad Simón Bolivar, Colômbia e Universidade Federal do Rio Grande do Sul, Brasil.

DEDICATORIA

Para minha amada Delsy Del Cristo, seu apoio incondicional permanente tem sido um fator determinante na minha vida acadêmica.

Para meu F1: Milton Fabricio, Juan Camilo e Rossana Paola

Para meu F2: Valentina, Antonella, David e Pedro.

Para meu irmão Guillermo *in memoriam* que infundiu em mim o espírito acadêmico.

AGRADECIMENTOS

Agradeço ao meu orientador, prof. Dr. João Antonio Pêgas Henriques, querido amigo, com quem depois de muito tempo compartilhando publicações, assistência a congressos e intercâmbios científicos, insistiu na ideia de que eu deveria tirar alguns créditos que estavam faltando para o doutorado, e foi assim como comecei esta maravilhosa odisseia cheia de belas e gratificantes surpresas e experiências, que certamente se tornarão saudades. Obrigado por ser meu grande amigo, grande mestre.

À minha co-orientadora, Profa. Dr. Juliana da Silva, por sua grande contribuição cientifica ao longo da minha tese, mas sobretudo por o grande laço de amizade que nos une há muito tempo, por isso tenho um imenso apreço e imensa admiração. Obrigado por todo o seu apoio e críticas construtivas.

Ao Elmo, por ser o amigo que está sempre pronto para prestar uma colaboração oportuna e precisa, sua bondade para com todos o torna uma pessoa incomparável. Obrigado Elmo por ser meu amigo.

Para Grethel e Jaime, meus antigos alunos, meus colegas, meus colaboradores no laboratório onde deram seus primeiros passos como pesquisadores; agora, como doutores, eles se tornaram o grande apoio que me permitiu levar esta tese a bom termo, obrigado por serem meus amigos, obrigado por seu apoio irrestrito, obrigado por ter colocado a cota de cientificidade na construção desta tese.

Para Yurina, Marcos, Ibeth, Marthica y Lyda, por seu grande espírito de colaboração e apoio contínuo em diversas atividades.

A Antonio por seu apoio no desenvolvimento dos temas da biologia molecular e estatística, respectivamente.

À Universidade Simón Bolívar e a Universidade Federal do Rio Grande do Sul por seu apoio financeiro que permite um termo feliz neste Doutorado.

SUMÁRIO

LISTA DE ABREVIAÇÕES

APOP: Apoptotic cells

IARC: Agência Internacional de Pesquisa do Câncer

PIXE: Emissão de Raio X Induzida por Partículas

***XRCC1*:** Complemento Cruzado de Reparo de Raios X gen 1

***XRCC3*:** Complemento de Reparo de Raios X do Gen 3

OGG1: Gen 8-oxoguanina DNA glycosylase-1

***GSTM1*:** Glutathione S-Transferase Mu 1

***GSTT1*:** Glutathione S-Transferase Theta 1

CC: Cromatina Condensada

MN: Micronúcleos

NPB: Pontes nucleoplasmáticas

NBUD: Brotação nuclear

NECR: Células necróticas

CBMN-Cyt: ensaio de micronúcleo bloqueador de citocinese

SEM: Microscópio Eletrônico de Varredura

TEM: Microscópio eletrônico de transmissão

DNA: Ácido desoxirribinucleico

ROS: Espécies reativas de oxigênio

Redox: Redução-oxidação

U-Cd: Cádmio urinário

Cyto-B: Citocinasina-B

qPCR: Reação de polimerase quantitativa

SASP: Fenótipo Secreto Associado à Senescencia

Tandem TTAGG: Telômeros são sequências repetitivas de DNA

Lariat-Like: Acredita-se que o complexo Shelterin estabiliza uma estrutura

CYP: Citocromo 450

RESUMO

Durante as atividades de soldagem, muitos compostos são libertados, e vários destes causam stress oxidativo e inflamação, e alguns são considerados cancerígenos. De fato, a Agência Internacional de Pesquisa em Câncer (IARC) estabeleceu que os fumos de solda são cancerígenos para os seres humanos. A exposição aos gases de soldagem no local de trabalho é comum em vários setores do mundo. Estes gases contêm partículas de consistência metálica, que são liberados devido às altas temperaturas de fusão e ficam suspensos no ar na forma de óxidos metálicos. Esta exposição pode causar doença pulmonar crônica, doenças neurológicas e até o câncer. Partindo do pressuposto de que a exposição ocupacional é um fator de risco epidemiológico relevante, a IARC alerta sobre os riscos para os soldadores. Assim, o objetivo deste trabalho foi avaliar a exposição ocupacional destes trabalhadores utilizando biomarcadores de genotoxicidade e a influência dos polimorfismos nos genes de reparação *XRCC1* e *XRCC3* e de metabolismo *OGG1*, *GSTM1* e *GSTT1*. Além disto, buscou-se correlacionar os efeitos com os elementos inorgânicos no sangue e na urina, bem como a análise elementar por microscopia eletrônica dos metais encontrados no ambiente de trabalho (SEM-EDS). Os resultados do presente estudo mostraram um aumento significativo na frequência de micronúcleos (MN), pontes nucleoplasmáticas (NPB), broto nuclear (NBUD), células necróticas (NECR) e apoptóticas (APOP), pelo teste de micronúcleos com bloqueio de citocinese (CBMN-Cyt), bem como aumento no comprimento do telômero (TL) dos indivíduos expostos. O genótipo *XRCC1 399 Gln/-* demonstrou ser um genótipo de risco na formação de NPB, e *XRCC1 194 Trp/-* na morte celular. Indivíduos expostos com o *XRCC3 241 Thr/-* demonstraram aumento na frequência de MN, mostrando ser um genótipo de risco para este biomarcador. Além disto, os indivíduos expostos com o genótipo nulo *GSTM1* demonstraram uma frequência significativamente maior de MN e NECR do que os não-nulos; e o genótipo nulo *GSTT1* demonstrou uma frequência significativamente maior de APOP do que o não nulo. Também neste estudo, quanto aos indivíduos não expostos, ambos os genótipos nulos demonstraram ser um fator de risco para

NPB e APOP. Pelo método PIXE, foram observadas maiores concentrações de Cr, Fe e Cu na urina, e Cr, Fe, Mg, Al, S e Mn no sangue do grupo exposto. Além disso, foi observada uma correlação significativa entre MN e idade e entre NPB e tempo de serviço. A análise de composição das partículas mediante SEM-EDS revelou Fe, Mn e Si associados a partículas menores que 2 μm; essas partículas têm a capacidade de aderir umas às outras e formar aglomerados. Em conclusão, a mistura de componentes que são gerados durante a soldagem, constituída por gases como ozônio, dióxido de nitrogênio, monóxido de carbono e fumos metálicos, incluindo Fe, Mn, Si, leva a danos significativos no DNA e processos de morte celular que podem ser influenciados por variações individuais de metabolismo e reparo do DNA. Portanto, é muito importante uma vigilância biológica efetiva desses trabalhadores, levando-se em consideração o entendimento dos mecanismos envolvidos no desenvolvimento das patologias ocupacionais típicas desses indivíduos.

Palavras-chave: Genotoxicidade, citotoxicidade, risco ocupacional, soldagem, danos ao DNA, alongamento telômero.

ABSTRACT

During welding activities many compounds are released, several of these cause oxidative stress and inflammation and some are considered carcinogenic, in fact the International Agency for Research on Cancer has established that welding fumes are carcinogenic to humans. Exposure to welding gases in the workplace is common in many sectors of the world. These gases contain particles of metallic consistency and are released due to high melting temperatures and become suspended in the air in the form of metal oxides. This exposure can cause chronic lung disease, neurological disorders, and even cancer. Based on the assumption that occupational exposure is a relevant epidemiological risk factor, the International Agency for Research on Cancer (IARC) warns about the risks for welders. Thus, the objective of this study was to evaluate the occupational exposure of these workers through genotoxicity biomarkers and the influence of polymorphisms in the repair genes XRCC1 and XRCC3 and metabolism genes OGG1, GSTM1, and GSTT1. In addition, we sought to correlate the effects with inorganic elements in blood and urine, as well as elemental analysis by electron microscopy of metals found in the work environment (SEM-EDS). The results of the present study showed a significant increase in the frequency of micronuclei (MN), nucleoplasmic bridges (NPB), nuclear budding (NBUD), necrotic cells (NECR) and apoptotic cells (APOP) by the cytokinesis-blocking micronucleus test (CBMN-Cyt), as well as increase in telomere length (TL) of the exposed individuals. The XRCC1 399 Gln/- genotype was shown to be a risk genotype in NPB formation, and XRCC1 194 Trp/- in cell death. Individuals exposed with XRCC3 241 Thr/- demonstrated increased frequency of MN, showing it to be a risk genotype for this biomarker. In addition to this, exposed individuals with the GSTM1 null genotype demonstrated a significantly higher frequency of MN and NECR than the non-null; and the GSTT1 null genotype demonstrated a significantly higher frequency of APOP than the non-null. Also in this study as for unexposed individuals, both null genotypes were shown to be a risk factor for NPB and APOP. By the PIXE method, the highest concentrations of Cr, Fe and Cu were observed in the urine, and Cr, Fe, Mg, Al, S and Mn in the blood of the

exposed group. In addition, a significant correlation was observed between MN and age and between NPB and length of service. Particle composition analysis by SEM-EDS revealed Fe, Mn and Si, associated with particles smaller than 2 μm; these particles have the ability to adhere to each other and form agglomerates. In conclusion, the mixture of components that are generated during welding constitutes gases such as ozone, nitrogen dioxide, carbon monoxide, and metal fumes, including Fe, Mn, Si, leads to significant DNA damage and cell death processes that can be influenced by individual variations in DNA metabolism and repair. Therefore, an effective biological surveillance of these workers is very important, taking into consideration the understanding of the mechanisms involved in the development of occupational pathologies typical of these individuals.

Keywords: Genotoxicity, cytotoxicity, occupational risk, welding, DNA damage, telomere lengthening.

INTRODUÇÃO

1 INTRODUÇÃO

Trabalhadores expostos a diferentes atividades relacionadas ao uso e diferentes aplicações na soldagem, tais como reparos industriais, fornos e fundições de metais são propensos a sofrer múltiplas doenças (Balkhyour, 2019), e principalmente aqueles envolvidos em reparos automotivos, estão em risco iminente para a saúde (Cherrie e Levy, 2020; Riccelli et al., 2018). Este último grupo de trabalhadores também está exposto a diferentes tipos de poluentes no local de trabalho, tais como fumos de escape dos mesmos veículos em reparo, exposição direta a solventes orgânicos voláteis (Kurt e Basara, 2020) como hidrocarbonetos policíclicos aromáticos (PAHs), benzeno) Lai et al., 2020) e material particulado (Vinnikov e Tulekov, 2020). Portanto, o efeito dos fumos de solda dependerá dos tipos de solda utilizados, e do tempo de duração do processo (que pode ter um efeito em indivíduos com genótipos favoráveis ou desfavoráveis). Outros fatores físicos que podem estar envolvidos no impacto da exposição são a ventilação adequada do local de trabalho e o uso permanente de equipamentos de proteção individual (Antonini, 2014).

Devido à composição da solda e dos múltiplos elementos metálicos, quando a fusão ocorre por aquecimento e aplicação em superfícies metálicas, surgem nanopartículas que podem alcançar o sistema respiratório e daí passar para a corrente sanguínea (Riccelli et al., 2020). Essas nanopartículas, contidas em misturas complexas, podem contribuir para lesões celulares, que podem posteriormente induzir apoptose e danos ao DNA (Badding et al., 2014). Um exemplo de dano celular é causado pelo Manganês (Mn), o qual é encontrado em eletrodos de solda, e é de preocupação para o eventual desenvolvimento de doenças neurodegenerativas como a doença de Parkinson (Gandour & Krishnan, 2015; Racette et al., 2017) outro metal como o cádmio (Cd), pode gerar disfunção tubular renal (Antonini, 2014).

Estudos epidemiológicos mostram que a exposição prolongada aos fumos de solda está associada aos efeitos da saúde respiratória, incluindo asma, bronquite,

alterações da função pulmonar (Goyal e El-zein, 2020; Sriram et al., 2015; Ding & Wu, 2019; Racette et al., 2017). Arritmias cardíacas, isquemia miocárdica e aterosclerose também foram relatadas (Taj et al., 2021).

A exposição permanente a fumos de solda pode gerar efeitos genotóxicos e citotóxicos, que podem ser evidenciados por danos no DNA, aumento da frequência de micronúcleos (MN) (Aksu et al., 2019), induzção de processos de apoptose e lesões celulares [Aksu et al., 2019], e até mesmo gerar efeitos sobre a estrutura telomérica, comprometendo seu comprimento (Wong et al., 2014). A existência de metais pesados como Cr, Fe; Cu, Mn, Mg, Al no sangue de pessoas expostas a fumos de solda pode induzir a presença de micronúcleos [Aksu et al., 2019]. Além disso, é importante notar que o metabolismo do desequilíbrio pode estar comprometido neste tipo de exposição (Graczyk et al., 2016). Além disso, a ativação de sistemas mediados por glutationa e seus mecanismos genéticos, incluindo os genes GSTM1 e GSTT1, podem ou não ser ativados como consequência da exposição permanente a fumos de solda, todos mediados pela dose recebida de material particulado associado a esta exposição (Pachkowsk et al., 2009).

1.1 Exposição ocupacional dos soldadores

A soldagem é um processo de união de metais pela ação do calor, com ou sem a adição de novo material metálico, dando continuidade aos elementos unidos. É necessário fornecer calor até que o material de enchimento derreta e junte ambas as superfícies, ou o metal das próprias peças. Para que o metal de enchimento seja capaz de soldar corretamente, é necessário que ele "molhe" os metais a serem unidos, o que será verificado sempre que as forças de adesão entre o metal de enchimento e as peças a serem soldadas forem maiores que as forças de coesão entre os átomos do material adicionado (Vacas, 2017).

Em geral, é possível distinguir os seguintes tipos de solda:

- Solda heterogênea: é realizada entre materiais de natureza diferente, com ou sem metal de enchimento, ou entre os mesmos metais, mas com metal de enchimento diferente. Pode ser macio ou duro (Vacas, 2017).

- Soldagem homogênea: os materiais a serem soldados e o metal de enchimento, se houver, são da mesma natureza. Pode ser oxiacetilênico, elétrico (por arco ou por resistência), etc. Se não há metal de enchimento, as soldas homogêneas são chamadas de soldas autógenas (Luan et al., 2020).

A soldagem requer o uso de energia térmica para fundir um metal sendo estas estruturas convertidas em nanopartículas contendo ferro (Fe), Manganês (Mn), Níquel (Ni), cromo (Cr) e outros componentes. A partir daqui, começa a manifestação de riscos à respiração, pois fumos e fumos metálicos são considerados misturas perigosas com possíveis efeitos negativos (Stanislawska et al., 2017). Tais fumos metálicos são produzidos pela fundição, evaporação, transporte e subsequente solidificação de metais, que são liberados devido às altas temperaturas que ocorrem nos eletrodos, deixando em suspensão no ar muitas formas de óxidos como óxidos de alumínio, cádmio, cromo, zinco, cobre, ferro, chumbo, manganês, níquel, titânio e vanádio, entre outros (Krabbe et al, 2019); além disso, gases como ozônio, dióxido de nitrogênio e monóxido de carbono são produzidos pela decomposição dos revestimentos de eletrodos e pela ação dos raios ultravioleta (Mogollón, 2009).

A soldagem libera substâncias potencialmente perigosas no ambiente de trabalho e sistemas adequados não são utilizados para impedir ou evitar seu transporte e deposição no sistema respiratório (Riccelli et al., 2020). Cada uma destas substâncias pode produzir desconforto e doenças que em muitos casos são irreversíveis, e a situação se agrava se várias delas estiverem no ambiente de trabalho (Haluza et al., 2014). Quando na circulação sistêmica, os gases absorvidos são capazes de atingir o fígado, rim ou cérebro e produzir danos a estes órgãos. Os gases, com diâmetro de partícula inferior a 0,003 mm, podem alcançar o alvéolo e o depósito, gerando fibrose intersticial e, eventualmente, pneumoconiose. Pode levar muito tempo (até 30 anos de exposição) até que tal doença seja diagnosticada.

Outros problemas causados pelos fumos metálicos incluem a febre e lesões dos soldadores (Hamacher et al., 2018).

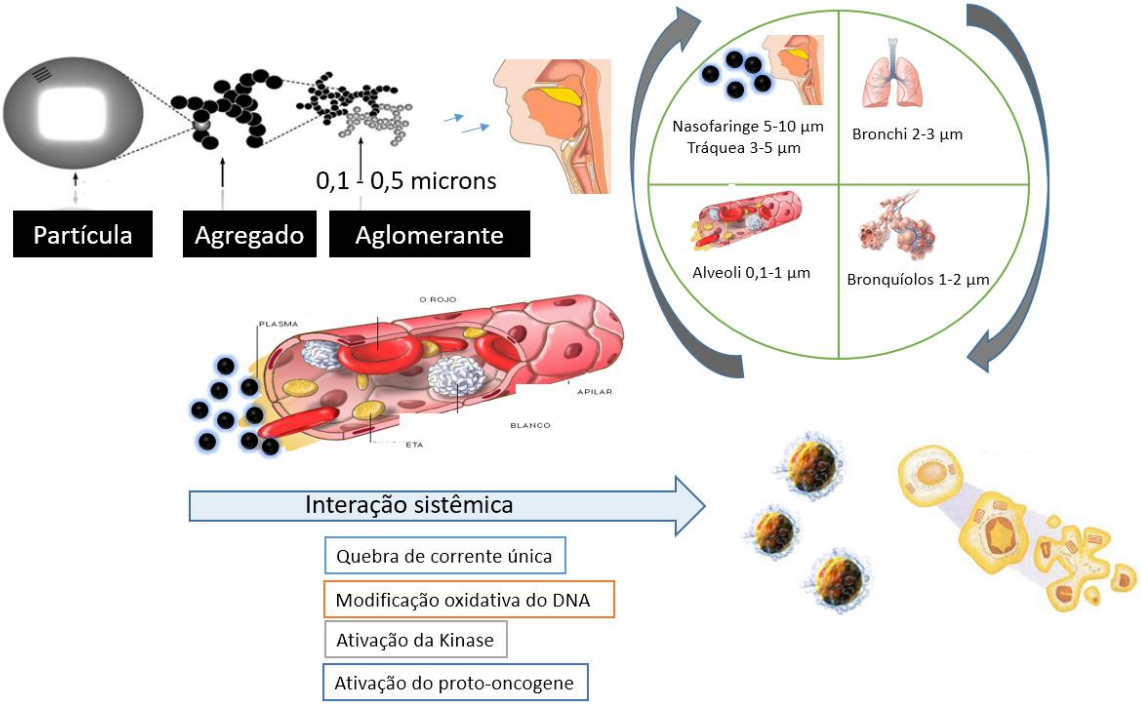

Figura 1. Micropartículas, fumos de solda e efeito sistêmico sobre as células, quebra de DNA de fios simples e duplos, danos oxidativos, ativação da cinase, processos oncogenéticos da exposição a micropartículas geradas pela exposição a fumos de solda.

1.2 Metais relacionados com solda e danos oxidativos ao DNA

A soldagem de gás elétrico é utilizada principalmente nos principais processos industriais (Cohen et al., 1998; Kumar e Singh., 2019). Ela pode produzir gases perigosos (uma mistura complexa de gases e óxidos ou sais metálicos) que podem ser prejudiciais à saúde de um soldador (Pires et al., 2006).

Estudos epidemiológicos relataram um risco elevado significativo de câncer de pulmão entre os soldadores (Moulin, 1997). Foram descritos vários mecanismos de indução de genotoxicidade, mutagenicidade e carcinogenicidade de metais, incluindo danos diretos ao DNA (Ó'Brien et al., 2003), geração de espécies reativas de oxigênio (Hengstler et al, 2003; Antonini et al., 2005), inibição da reparação do DNA (Hartwig, 1998), esgotamento da glutationa (Stohs & Bagchi, 1995; Karmakar et al., 1998) e efeitos sobre a metilação do DNA (Lee et al., 1995). É provável que a maioria desses mecanismos estejam inter-relacionados e podem, em última instância, causar anormalidades da função gênica e transformação neoplásica (Ianmarcovai et al., 2005).

Os metais podem interagir diretamente com o DNA e na replicação do DNA, resultando em danos ao DNA, modificações na base de DNA, purinificação, ligações cruzadas inter e intra-moleculares no DNA e proteínas, rearranjos cromossômicos, indução de quebras de uma fita, modificação oxidativa do DNA, mudanças de sequência, ativação de proto-oncogenes e inativação de genes supressores do tumor, mutações pontuais e aberrações cromossômicas nas células, produzidas principalmente pela indução de alterações e modificações oxidativas (Cooke et al., 2003).

A inflamação induzida por partículas nos pulmões causa a liberação de espécies reativas de oxigênio (ROS) por macrófagos. Os metais de transição na superfície das partículas também poderiam gerar ROS pela reação de Fenton e levar à formação de danos oxidativo no DNA (Mukherjee et al., 2004). Outro mecanismo possível envolve vários tipos de oxigênio reativo e várias espécies de radicais livres derivadas de reações redox catalisadas por metal. Os efeitos

patogênicos gerais mediados por metais (aumento da resposta inflamatória, inibição das defesas antioxidantes celulares, peroxidação lipídica, inibição do reparo do DNA) também podem contribuir para mutações, mudanças na expressão gênica e modificação do ciclo celular (Ianmarcovai et al., 2005).

Sob condições de exposição ocupacional contínua, esses mecanismos contribuem para processos carcinogênicos. Foi relatado que dois dos mais potentes metais cancerígenos em humanos são o cromo e o níquel e este fato está relacionado à sua forte capacidade oxidativa (Kasprzak, 1995; Desoize, 2003; Goulart et al., 2005,). Também foram sugeridas capacidades similares para alumínio, cádmio, cobalto, manganês e chumbo (Banasik et al., 2005; Waisberg, et al., 2003; De Boeck et al., 2003; HaMai et al., 2004; Silbergeld, 2003). Entretanto, ficou demonstrado que os danos ao DNA causados pelos fumos de solda diferem da exposição a um único metal devido ao efeito aditivo ou sinérgico de múltiplos compostos genotóxicos no ambiente de trabalho (Yu et al., 2004; Ianmarcovai, et al., 2005).

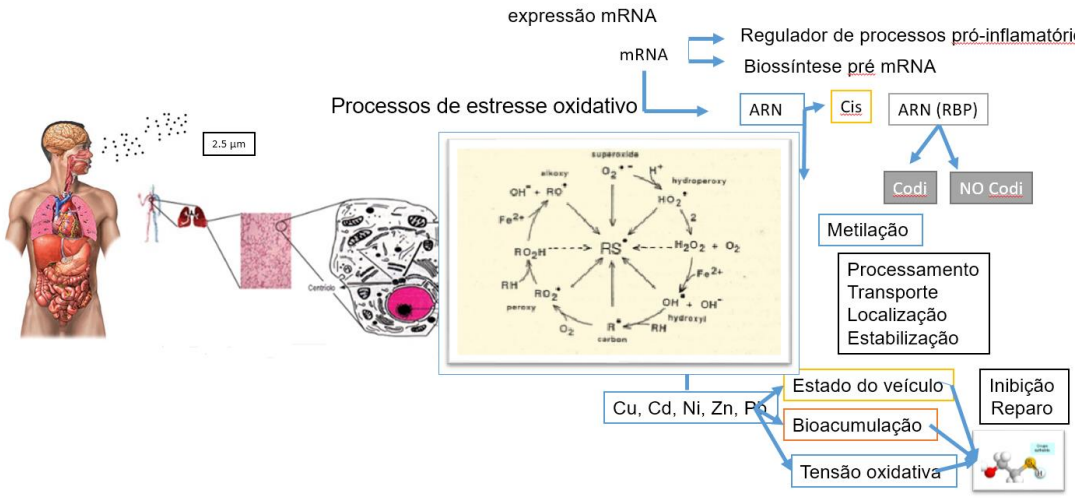

Figura 2. A exposição a partículas geradas pela soldagem desencadeia processos de stress oxidativo intracelular, mediados pela expressão do mRNA, que regulam os processos de transporte, localização e estabilização, bem como a inibição da replicação adequada do ADN, dependendo do feedback dentro dos processos específicos de ADN metilado ou hiper metílico, que pode ser influenciado pela presença de metais respiráveis nos fumos de solda.

Os fumos gerados durante o processo de soldagem possuem pelo menos 13 metais incluindo manganês (Mn), berílio (Be), cádmio (Cd), cromo (Cr), cobalto (Co), cobre (Cu), ferro (Fe), chumbo (Pb), mercúrio (Hg), molibdênio (Mo), níquel (Ni), zinco (Zn), antimônio (Sb) e vanádio (V) (Guojun et al., 2004; Imamoglu et al., 2008).

O níquel, cádmio e cromo VI são três metais que foram classificados como carcinógenos de Classe I pelo IARC nos anos 90, com base em dados de estudos experimentais e epidemiológicos (Beveridge et al., 2008). Estes metais podem interagir diretamente com o DNA, causar alterações na replicação do DNA e danos ao DNA (Iarmarcovai et al., 2005; Snow, 1992). Outro mecanismo é que as espécies de níquel e cromo também podem estimular a resposta imune celular e promover a liberação de espécies reativas de oxigênio (Snow, 1992).

A exposição crônica ao cromo hexavalente solúvel (Cr+6) leva à bronquite, asma, ulceração e perfuração do septo nasal, lesões hepáticas e renais em trabalhadores expostos (Jyh-Larng et al., 2002). Além disso, compostos como o cromo (VI) são descritos na literatura como mutagênicos tanto em células procarióticas quanto em eucarióticas *in vitro*. Existe uma correlação entre o aumento do risco de câncer de pulmão nos soldadores e o aumento da duração do tempo desde a primeira exposição ao Cr+6 contido nos fumos (Cohen et al., 1998).

A exposição ocupacional ao níquel ocorre predominantemente na maioria dos processos industriais, em particular na soldagem (Kasprzak et al., 2003). Os compostos insolúveis de níquel são fortemente cancerígenos *in vitro* e *in vivo* (Snow, 1992). Os riscos de câncer respiratório estão principalmente relacionados à exposição ao níquel solúvel (Kasprzak et al., 2003). Estudos demonstraram altas concentrações de níquel em amostras de sangue, tecido e urina. Aproximadamente 30% do níquel inalado chega aos pulmões e outros 20% são absorvidos pela circulação, e sabe-se que a Ni2+ tem a capacidade de aumentar a metilação do DNA (Chiang et al., 2009).

O cádmio (Cd) é um componente natural da crosta terrestre. No ambiente de trabalho, os trabalhadores podem ser expostos ao Cd por inalação dos fumos

gerados durante a soldagem de materiais contendo cádmio, ou inalação de partículas de metal, ferrugem ou pó de tinta (Verougstraete et al., 2002). Nos trabalhadores, o cádmio também tem sido associado à função pulmonar prejudicada e é suspeito de causar câncer de pulmão e possivelmente de próstata. Em baixos níveis de exposição, o cádmio urinário (U-Cd) reflete principalmente um efeito sistêmico, enquanto uma situação de exposição aguda sem danos renais pode ser representada em uma única amostragem. O cádmio no sangue reflete principalmente os últimos meses de exposição sob condições de exposição moderadas (Ianmarcovai et al., 2005).

1.3 Ferramentas de avaliação de risco genético: biomarcadores moleculares e genotóxicos

Um método para quantificar a exposição aos xenobióticos e seu possível impacto sobre o organismo é o uso de procedimentos de monitoramento biológico por meio de biomarcadores. Marcadores biológicos ou biomarcadores são as mudanças que podem ser medidas, sejam elas bioquímicas, fisiológicas ou morfológicas, que estão associadas à exposição a um agente tóxico ou qualquer alteração bioquímica precoce, cuja determinação em fluidos biológicos, tecidos ou ar exalado permite avaliar a intensidade da exposição e o risco à saúde (Ramirez, 2006).

1.3.1 Análise do citoma de linfócitos

Atualmente, o Teste de Micronúcleos com Bloqueio de Citocinese (CBMN-Cyt) de linfócitos sanguíneos periféricos tem uma abordagem promissora para medir danos do DNA, efeitos citostáticos e citotoxicidade. Os eventos de dano ao DNA são medidos especificamente em células binucleadas (BN) uma vez divididas e incluem: 1) verificação de frequência de micronúcleos (MNi), que é um biomarcador da quebra cromossômica ou perda do mesmo; 2) medição de pontes

nucleoplasmáticas (NPBs), que é um biomarcador de má reparação de DNA ou fusão das extremidades dos telômeros; e 3) brotos nucleares (NBUDs) que é considerado um biomarcador para a remoção de complexos amplificados de DNA e/ou de reparo de DNA [60].

Os efeitos citostáticos são medidos pela proporção de células mono, bi e multinucleadas, e a citotoxicidade pela proporção de células apoptóticas necrosadas. Este ensaio foi aplicado com sucesso no biomonitoramento humano devido à exposição aos agentes genotóxicos, para avaliar a genotoxicidade *in vitro,* e em outros campos como nutrigenômica e farmacogenômica. Também é considerado um preditor de risco de câncer e sensibilidade à radiação de tecidos normais e tumorais (Fenech, 2007).

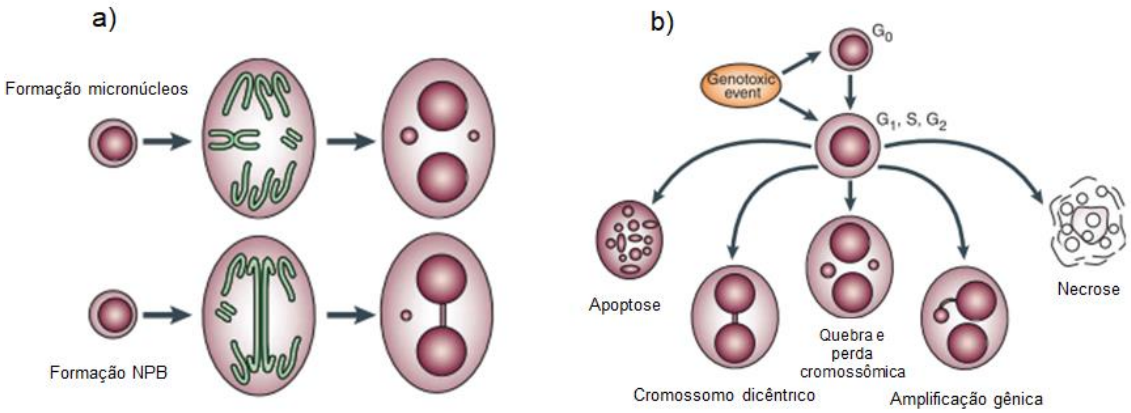

Figura 3. a) Formação de MN e NPB em células submetidas à divisão nuclear. Os MNs são originários de cromossomos inteiros ou de fragmentos de cromossomos acêntricos. Os NPBs têm origem em cromossomos dicêntricos que podem ser causados por reparos inadequados de quebras de DNA de dupla cadeia ou fusões terminais telômeros. b). Os diferentes destinos possíveis das células cultivadas com bloqueio de citocinese após exposição a agentes citotóxicos/genotóxicos. Usando esses biomarcadores no ensaio CBMN, é possível medir a frequência de quebra de cromossomos (MN), perda cromossômica (MN), rearranjo cromossômico, por exemplo, cromossomos dicêntricos (NPB), amplificação de genes (NBUDs), necrose e apoptose. Além disso, os efeitos citostáticos são facilmente estimados a partir da proporção de células mono, bi- e multinucleadas. (Adaptados de Fenech, 2007).

1.3.2 Comprimento Telomérico

Os telômeros são estruturas de proteção presentes nas extremidades dos cromossomos, que se tornam mais curtos durante a replicação do DNA e são importantes na estabilidade do genoma. Telômeros encurtam como resultado da

replicação celular, levando à parada permanente do ciclo celular, também conhecida como senescência replicativa (Victorelli e Passos, 2017). A senescência replicativa foi proposta como um biomarcador molecular indicativo de estresses oxidativos acumulados em células (Zglinicki e Martin-Ruiz, 2005). O comprimento menor do telômero tem sido associado a uma variedade de doenças, tais como doenças cardiovasculares e seus fatores de risco associados e doenças pulmonares obstrutivas crônicas (COPD) (Fitzpatrick et al., 2007).

Agora está claro que o impacto da senescência *in vivo* não se limita à perda da capacidade proliferativa. Além da parada do ciclo celular, foi demonstrado que as células senescentes sofrem mudanças dramáticas em termos de expressão gênica, metabolismo, epigenoma e, o que é importante, foi demonstrado que têm um perfil secreto distinto conhecido como o Fenótipo Secreto Associado ao Senescence (SASP) (Coppé et al., 2008). O SASP inclui citocinas pró-inflamatórias, assim como fatores de crescimento e proteínas degradantes de matriz extracelular, e pensa-se que tenha evoluído como uma forma das células senescentes se comunicarem com o sistema imunológico, mas também como um sinal extracelular para promover a regeneração tecidual pela estimulação de células progenitoras próximas (Childs et al., 2015).

Telômeros são sequências repetitivas de DNA (repetições em tandem TTAGGG) associadas a várias proteínas que formam um complexo conhecido comumente o complexo "Shelterin". Acredita-se que o complexo Shelterin estabiliza uma estrutura tipo *lariat-Like* chamada *telomere-loop ou t-loop* com a finalidade de proteger a extremidade exposta dos cromossomos lineares (De Lange, 2005). Os telômeros contêm tanto uma cadeia rica em citosina, quanto uma fita rica em guanina, que contém uma saliência de 3', que compreende repetições de nucleotídeos de uma fita. Acredita-se que a saliência se ligue a uma das regiões de DNA de dupla cadeia e facilite a formação da estrutura t-loop (Griffith et al., 1999). Os telômeros desempenham um papel importante na integridade e estabilidade do genoma, participando do processo de dano/reparação do DNA. A instabilidade telomérica estaria associada a danos acumulados no DNA, especialmente as

mudanças incorporadas devido a diferentes polimorfismos genéticos; como será visto posteriormente, estes polimorfismos poderiam estar presentes em genótipos de pessoas mais suscetíveis ao ataque de contaminantes presentes no ambiente (M'kacher et al., 2020).

OBJETIVOS

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito genotóxico e mutagênico causado pela exposição ocupacional à mistura de metais em soldadores pela análise de citotoxicidade, de dano no DNA, de metais e da influência de polimorfismos em genes de metabolismo e reparação de DNA em uma população de Montería (Colômbia).

2.2 Objetivos específicos

- Avaliar o citoma de linfócitos de sangue periférica de indivíduos expostos ao fumo de solda e comparar com os indivíduos controles.
- Avaliar a integridade dos telômeros de células de sangue periférica dos indivíduos expostos ao fumo de solda e comparar com os indivíduos controles.
- Analisar os níveis de metais no sangue e urina dos indivíduos expostos ao fumo de solda e comparar com os indivíduos controles.
- Verificar a existência de associações dos polimorfismos dos genes de metabolismo GSTM1 e GSTT1 e de reparo OGG1, XRCC1 e XRCC3 com os diferentes biomarcadores do citoma de linfócitos de indivíduos expostos ao fumo de solda.
- Caracterizar o material particulado gerado durante os processos de solda no ambiente de trabalho usando o método de PIXE e microscopia eletrônica de varredura (SEM) e espectroscopia de energia dispersiva de raios-X (EDS).

CAPÍTULO I

3 CAPÍTULO I - *Genotoxic and cytotoxic response in people exposed to welding fumes, cytokinesis-blocking micronucleus cytome assay (CBMN-Cyt), telomere length (TL) analysis of heavy metal concentration in blood and urine.*

APRESENTAÇÃO

Este capítulo corresponde a uma publicação científica na qual, da perspectiva da genotoxicidade e citotoxicidade, é analisado o problema relacionado ao evidente risco ocupacional ao qual os soldadores estão expostos aos fumos emitidos nesta atividade, que foram classificados como cancerígenos pela Agência Internacional de Pesquisa do Câncer (IARC). Os efeitos produzidos no corpo dos trabalhadores foram avaliados pela aplicação de testes específicos que mostram danos celulares e de DNA, expressos no aparecimento de micronúcleos (MN), pontes nucleoplasmáticas, gemas nucleares, células necróticas, células binucleadas e cromatina condensada; também foram avaliados o comprimento do telômero e a concentração de metais e elementos inorgânicos no sangue e na urina.

Ecotoxicology and Environmental Safety 212 (2021) 111935

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Cytokinesis-block micronucleus cytome (CBMN-CYT) assay biomarkers and telomere length analysis in relation to inorganic elements in individuals exposed to welding fumes

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ARTICLE INFO

Edited by Dr Fernando

Barbosa *Keywords:*

Welding
Inorganic elements
Cytome
Micronuclei
Telomere

ABSTRACT

During the welding activities many compounds are released, several of these cause oxidative stress and inflammation and some are considered carcinogenic, in fact the International Agency for Research on Cancer established that welding fumes are carcinogenic to humans. The aim of the present study was to analyze the cytotoxic and genotoxic potential of exposure to welding fumes and to determine concentrations of metals in blood and urine of occupationally exposed workers. We included 98 welders and 100 non-exposed individuals. Our results show significant increase in the frequency of micronuclei (MN), nucleoplasmic bridges (NPB), nuclear buds (NBUD) and necrotic cells (NECR) in cytokinesis-block micronucleus cytome (CBMN-Cyt) assay, as well as in the telomere length (TL) of the exposed individuals with respect to the non-exposed group. In the analysis of the concentrations of inorganic elements using PIXE method, were found higher concentrations of Cr, Fe and Cu in the urine, and Cr, Fe, Mg, Al, S, and Mn in the blood in the exposed group compared to the non-exposed group. A significant correlation was observed between MN and age and between NPB and years of exposure. Addi-tionally, we found a significant correlation for TL in relation to MN, NPB, age and years of exposure in the exposed group. Interestingly, a significant correlation between MN and the increase in the concentration of Mg, S, Fe and Cu in blood samples of the exposed group, and between MN and Cr, Fe, Ni and Cu in urine. Thus, our findings may be associated with oxidative and inflammatory damage processes generated by the components contained in welding fumes, suggesting a high occupational risk in welding workers.

1. Introduction

Welding requires the use of heat energy to melt a metal and poses health risks due to the metallic fumes and gases produced (Rana et al., 2019b). The fumes are generated by the evaporation and subsequent solidification of the metals that are released due to the high melting temperatures, being suspended in the air in the form of metallic oxides (Honaryar et al., 2019). Depending on the type of welding, aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), titanium (Ti), and vanadium (V) oxides

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https://doi.org/10.1016/j.ecoenv.2021.111935
Received 25 August 2020; Received in revised form 9 December 2020; Accepted 11 January 2021
Available online 9 February 2021

may be present. In addition, gases such as ozone, nitrogen dioxide, and carbon monoxide are generated by the decomposition of electrode coatings and by the action of ultraviolet rays (Honaryar et al., 2019; Michalek et al., 2019). In 2017, the International Agency for Research on Cancer (IARC) listed welding fumes as a carcinogen based on sufficient evidence of lung cancer in humans (IARC, 2018).

The absorbed gases reach the liver (Boyce et al., 2020), kidney (MacLeod et al., 2017), or brain (Lee et al., 2018) through systemic circulation and damage these organs. Metal fumes, with particle di-ameters less than 2.5 μm, can reach and deposit in the alveolus, gener-ating interstitial fibrosis and eventually diseases such as chronic obstructive pulmonary disease (COPD) (Koh et al., 2015), asthma (Wittczak et al., 2012), cancer ('t Mannetje et al., 2012), and Parkinson's and other neurodegenerative disorders (Kenborg et al., 2012).

The use of genetic biomarkers to detect the effects caused by exposure to environmental genotoxins early and efficiently has increased and they have become a robust tool that supports population epidemiolog-ical data in human biomonitoring studies (Aiassa et al., 2019; LaKind et al., 2014). The cytokinesis-block micronucleus cytome (CBMN-Cyt) assay is a widely used assay to evaluate cytotoxicity, DNA damage, and cytostatic effects caused by exposure to genotoxic agents (Fenech et al., 2011).

Telomeres are a structural complex of tandem repeats of DNA (TTAGGG) at the ends of eukaryotic chromosomes and function to protect the stability of chromosomes. Several proteins protect the integrity of telomeres, such as the protein complex shelterin and telo-merase. Telomerase acts as a reverse transcriptase and is responsible for the elongation and maintenance of telomere in mostly germinative and some somatic tissues and in malignant tumors (Blackburn, 2005; Baird et al., 2018). DNA damage, oxidative stress, and inflammatory processes are associated with telomere shortening, presumably due to terminal chromosome deletions. Shorter telomeres are associated with acceler-ated ageing and adverse health outcomes, such as cardiovascular disease and cancer (Kahl et al., 2016; Ma et al., 2020). However, experimental evidence has shown that heavy metal exposure can lead to longer telo-meres, both in vivo (Dioni et al., 2011; Li et al., 2012; Shoeb et al., 2020, 2017) and in vitro (Liu et al., 2004; Ferrario et al., 2009). Mechanisms involved in telomeric shortening and elongation are not understood; both telomeric lengths are related to DNA methylation and the increase or decrease of telomerase activity by genetic and environmental factors that interfere in the telomerase recruitment-signaling pathway and epigenetic modifications (Shoeb et al., 2020).

The aim of this study is to analyze the cytotoxic and genotoxic po-tential of exposure to welding fumes and to determine concentrations of inorganic elements in the blood and urine of exposed individuals. This work represents the first study in Colombia on the effects of exposure to welding fumes and contributes to the state of knowledge about the risk of this occupation worldwide and may eventually contribute to promote the use of biomarkers to assess occupational risk and the implementa-tion of programs of sensitivity and prevention in occupational health.

2. Materials and methods

2.1. Individuals and sampling

The population was composed of workers from the city of Monteria-Cordoba´ and included 198 male volunteers: 98 welders with permanent exposure to welding activities and 100 office workers with no history of exposure but living in the same region. Workers exposed to welding fumes worked from Monday to Saturday with a work shift of 8 h/day.

After a stage of motivation, each individual answered a questionnaire to provide sociodemographic information, such as age, years of exposure, health history, cancer history in family, lifestyle, cigarette consumption, alcohol consumption, medication, use of labor protection, duration of service (years), respiratory disease, previous exposure to X-rays, and treatment with known carcinogens. The inclusion criteria included being healthy, voluntary acceptance, and occupational exposure ≥ 3 years; exclusion criteria included be smoker, medical treatment up to 3 months prior to sampling, X-ray exposure up to one year prior to sam-pling, diagnosis of cancer, chemotherapy or radiotherapy, intake of therapeutic drugs known to be mutagenic, exposure to other occupa-tional risk factors, history of genetic or respiratory diseases, and family cancer. The individuals included in the study were non-smokers, both in the exposed and non-exposed groups. For statistical analysis, the exposed workers were matched with the non-exposed group with respect to age and socio-economic status.

The study population was informed about the details of the work and the methodologies to be used. All volunteers signed informed consent before providing samples. This study was approved by the University of Simon Bolívar Ethics Committee (CIE-USB-CE-0234-01).

2.2. Sample collection

A total of 10 mL of peripheral blood was collected by venipuncture in heparinized tubes from the welding group and the non-exposed group. Subsequently, 5 mL blood was used for the CBMN-Cyt assay and the analysis of metals by the PIXE method each. Thereafter, urine samples were obtained in collection flasks. All samples were coded and transported on ice to the laboratory for processing.

2.3. Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay

Heparinized whole blood (0.5 mL) was added to 4.5 mL RPMI 1640 medium (Sigma R8758, USA) supplemented with 2 mM L-glutamine (Sigma A5955, USA), 10% fetal bovine serum (Gibco/Invitrogen 15000-044, Brazil), 100 μL/mL antibiotic–antimycotic solution (Sigma A5955, USA), and 2% phytohemagglutinin (Sigma L8754, USA) to stimulate the lymphocytes. Two cultures were carried out per individual for the CBMN-Cyt assay. Cytochalasin B (Sigma, C6762) was added at 44 h of incubation at a final concentration of 6 μg/mL. Cells were harvested at 72 h, treated with a hypotonic solution (0.075 M KCl), centrifuged immediately, and fixed three times with methanol/acetic acid (3:1) cold. The fixed cells were placed on humidified slides. The air-dried slides were stained with 6% Giemsa for 10 min

For the analysis, the cells were scored under a light microscope (magnification 1000 x). The frequencies the micronuclei (MN), nucleoplasmic bridge (NPB), and nuclear bud (NBUD) per 1000 binucleated (BN) cells per slide were used as indicators for DNA damage and chro-mosomal instability. Cytotoxic effects were assessed using the frequency of necrotic (NECR) and apoptotic (APOP) cells in 500 randomly scored cells. All slides were scored by one reader blinded to the exposure status of the individuals. All the analyses of biomarkers in the CBMN-Cyt assay were performed according to the recommendations of Fenech (2007).

2.4. Quantitative polymerase chain reaction (qPCR) for telomere length measurement

The DNA samples were quantified using the NanoDrop 1000 spectrophotometer and diluted according to experimental requirement (5 ng/ μL). For telomere length (TL) measurement, the protocol of O'Callaghan

(2011) was followed with minor modifications by Kahl et al. (2016). A standard curve was made using serial dilutions of known amounts of a pooled DNA sample. The single copy gene 36B4, which encodes the acidic ribosomal phosphoprotein PO, was used for amplification control. All samples were analyzed in triplicate, with a negative and positive control and a standard curve, using the Step One Plus ™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). A master mix was prepared using SYBR Green PCR Master Mix (Quatro G Ltda., Porto Alegre, Brazil), 20 ng DNA, injection water, 0.2 μmol of telomere primers (forward: 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′; reverse: 5′-CCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′), and 0.2 μmol of 36B4 primers (forward: 5′-CAGCAAGTGGGAAGGTGTAATCC-3′; reverse: 5′-CCCATTCTATCATCAACGGGTACAA-3′). The qPCR was run under the following conditions (for both telomere and 36B4 amplicons): Taq polymerase activation for 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. The cycle threshold (Ct) of each sample was used to calculate total telomere in kb per human diploid genome.

2.5. Analysis of metals particle-induced X-ray emission (PIXE)

Urine samples were processed on filter paper and dried for 48 h. Fresh samples of blood were dried at 40 °C for 72 h, macerated using a mortar and pestle, and finally pressed into pellets. PIXE (Johansson et al., 1995) analysis was carried out at the Ion Implantation Laboratory (Institute of Physics at the Federal University of Rio Grande do Sul). The samples were placed inside a reaction chamber and kept under vacuum (approximately 10^{6} mbar). The 3 MV Tandetron accelerator delivered 2.0 MeV protons with an average current of 1 nA. The beam spot size on the sample was 4 mm^2. During the experiments, an electron flood gun was used to avoid charging effects in the samples (Shubeita et al., 2005).

The X-rays produced in the samples were detected by a Si (Li) de-tector (Model Sirius 80/Be/PIXE – SGX Sensortech (MA) Limited (UK)) placed at 135° with respect to the beam direction. The energy reso-lution of the detector was 150 eV at 5.9 keV. The PIXE system is cali-brated against several thin and thick targets from Micromatter TM and NIST (National Institute of Standards and Technology) (Debastiani et al., 2019). The X-ray spectra were analyzed with the GUPIXWIN software developed at the University of Guelph (Canada) (Campbell et al., 2000). This software considers all physical parameters, including X-ray pro-duction cross sections and fluorescence yields. Briefly, this software converts peak areas into elemental concentrations. The chemical ele-ments detected in the samples by the PIXE technique were: sodium (Na), magnesium (Mg), Al, silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), Ti, Cr, Mn, Fe, nickel (Ni), Cu, zinc (Zn), and rubidium (Rb). The Limit of Detection (LOD) for the elements analysis with PIXE varies from element to element and depends on the matrix. For low atomic numbers (Z), the LOD is relatively larger compared to those elements with a higher atomic number (Z > 20).

2.6. Statistical analysis

First a normality test was performed using the Kolmogorov–Smirnov test. Analysis of differences in the biomarkers was performed using the non-parametric Mann–Whitney *U* test. For the concentration of inor-ganic elements, statistical differences between the groups were analyzed using the unpaired *t*-test (Welch's correction). Spearman's correlation was used to analyze the concentration of metals in the blood and urine with the formation of micronuclei in the lymphocytes in each study group. Principal Component Analysis (PCA) bi-plot based on descriptive analysis was applied to the concentrations of metals detected in blood and urine samples and the DNA damage, chromosomal instability and cytotoxic effects to assess the relationships between variables and the relative influence of different parameters on the results general. The critical level for rejection of the null hypothesis was considered to be a two-tailed P-value of 0.05. Statistical analysis was performed with GraphPad PRISM software, version 6.01 (GraphPad Inc., San Diego, CA), with the Statistical Package for the Social Sciences version 17 (SPSS Inc., Chicago, IL) and with XLSTAT® 2020.3.1. (ADDINSOFT SARL, Paris, France).

Table 1
Main characteristics of the non-exposed and exposed groups.

Population characteristics	Non-exposed	Exposed
Number of individuals	100	98
Age (mean ± SD)	30.16 ± 10.37	40.60 ± 10.84
Time of service (mean years ± SD)	……	19.15 ± 8.39
Use of PPE[a]		
- Yes	……	(69)73.40%
- No	……	(25)26.59%
Alcohol consumption[b]		
- Yes	70.20%	71.19%
- No	29.80%	28.80%
Vitamins consumption		
- Yes	31.0%	17.0%
- No	69.0%	83.0%
Meat consumption		
- Yes	95.80%	90.50%
- No	4.20%	9.50%
Consumption of fruits and vegetables		
- Yes	85.50%	89.60%
- No	14.50%	10.40%
Family history of cancer		
- Yes	30.50%	32.60%
- No	69.50%	67.40%

SD, standard deviation.
[a] PPE, personal protective equipment.
[b] Drink more than three beers/day or in excess once a week.

3. Results

The features of the study population are shown in Table 1. The mean age of the non-exposed group was 30.16 ± 10.37 years (range, 18–63 years) and that of the exposed group was 40.60 ± 10.84 years (19–66 years). The mean duration of service in the exposed group was 19.15 ± 8.39 years (3–39 years). Table 1 also describes the use of personal protective equipment (PPE) used by 73.40% of mechanics and not used by 26.59%. Moreover, 70.20% of the non-exposed group and 71.19% of the exposed group consumed alcohol (defined as drinking > 3 beers per day or drinking in excess once a week). The data demonstrated that most individuals in the non-exposed and exposed group did not consume vi-tamins; both study groups had a high consumption rate of meat, vege-tables, and fruits. Table 1 shows the low rates of family history of cancer in the exposed and non-exposed groups (32.60% and 30.50%, respectively).

Table 2 shows the values of the CBMN-Cyt assay biomarkers for the two groups. A significant increase was seen in the frequency of MN, NPB, NBUD, and NECR in the exposed group compared to the non-exposed

Table 2
Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay biomarkers in the non-exposed and exposed groups.

Biomarkers	Non-exposed (n = 100)	Exposed (n = 98)	P
CBMN-Cyt			
MN	3.83 ± 2.90	6.07 ± 4.71	**< 0.001**
NPB	2.11 ± 2.09	4.67 ± 3.49	**< 0.001**
NBUD	2.13 ± 1.91	5.10 ± 3.80	**< 0.001**
APOP	5.82 ± 3.71	8.43 ± 7.61	0.0691
NECR	3.21 ± 3.12	7.02 ± 6.80	**< 0.001**
NDI	1.97 ± 0.05	1.99 ± 0.04	0.0698

MN, micronuclei; NPB, nucleoplasmic bridge; NBUD, nuclear bud; APOP, apoptotic cells; NECR, necrotic cells; NDI, nuclear division index. Data are expressed as the mean ± standard deviation. P, in bold significant between exposed group and non-exposed group; Mann–Whitney *U* test.

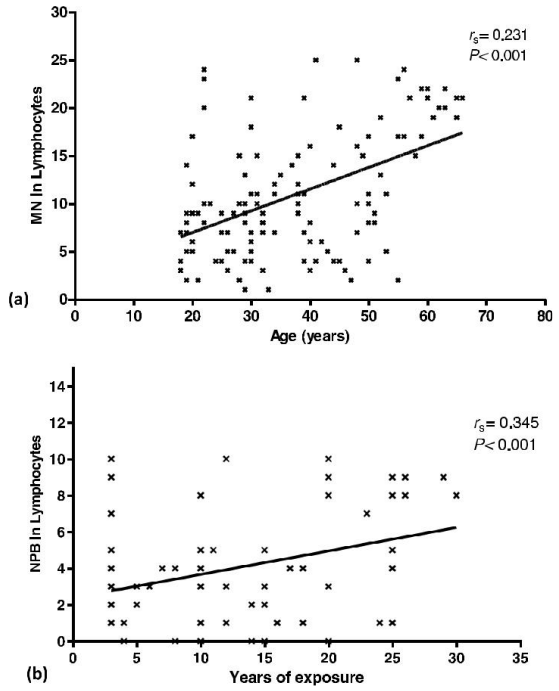

Fig. 1. Non-parametric Spearman's correlation analyses between (a) MN frequency in lymphocytes versus age (19–66 years) and (b) NPB frequency in lymphocytes versus years of exposure (3–30 years) for the exposed group.

group (Mann–Whitney *U* test, $P < 0.001$). The means obtained in the frequency of micronuclei of the individuals who used PPE (4.91 ± 3.30) vs those who did not use these (5.01 ± 4.75), were not statistically significant. In the analysis of the frequency of micronuclei of those who consume alcohol (5.89 ± 4.20) vs those who do not consume (3.02 ± 2.91) they were statistically different but this may be associated with the fact that most of the exposed individuals are alcohol consumers.

A significant correlation was observed between age and formation of MN in lymphocytes ($r_s = 0.231$, $P < 0.001$) and formation of NPB with the years of exposure ($r_s = 0.345$, $Y = 1.099X + 14.41$, $P < 0.001$) (Fig. 1).

The concentration of inorganic elements in the urine and blood samples of the non-exposed and exposed group by PIXE are shown in Table 3. In this analysis, higher and significant concentrations of Cr, Fe,

Table 4
Spearman correlation analyses between MN formation and inorganic elements in peripheral blood and urine for non-exposed and exposed group.

Elements[1]		Peripheral Blood		Urine	
		Non-exposed group	Exposed group	Non-exposed group	Exposed group
Na	r_s	0.613	0.765	0.080	0.063
	P	0.195	0.060	0.108	0.052
Mg	r_s	0.107	0.316	0.286	0.683
	P	0.291	**0.002**	0.005	0.117
Al	r_s	0.064	0.046	0.961	0.252
	P	0.526	0.654	0.021	0.028
Si	r_s	0.078	0.057	0.055	0.783
	P	0.439	0.578	0.705	0.347
P	r_s	0.130	0.328	0.072	0.081
	P	0.199	0.081	0.326	0.127
S	r_s	0.132	0.246	0.081	0.212
	P	0.190	**0.004**	0.138	0.234
Cl	r_s	0.349	0.242	0.172	0.060
	P	**0.010**	0.066	0.195	0.168
K	r_s	0.324	0.309	0.052	0.098
	P	**0.001**	0.061	0.051	0.262
Ca	r_s	0.077	0.067	0.615	0.089
	P	0.235	0.072	0.015	0.178
Ti	r_s	0.197	0.039	0.879	0.079
	P	**0.049**	0.705	0.141	0.023
Cr	r_s	0.055	0.064	0.760	**0.022**
	P	0.200	0.061	0.002	0.128
Mn	r_s	0.058	0.087	0.983	0.209
	P	0.233	0.070	0.057	0.269
Fe	r_s	0.132	0.210	0.572	**0.007**
	P	0.191	**0.038**	0.027	0.226
Ni	r_s	0.087	0.071	0.057	**0.025**
	P	0.071	0.059	0.211	0.113
Cu	r_s	0.196	0.252	0.055	**0.026**
	P	0.051	**0.012**	0.118	0.136
Zn	r_s	0.746	0.848	0.242	0.183
	P	0.085	0.778	0.021	0.013
Rb	r_s	0.534	0.732	0.839	0.902
	P	0.296	0.298	0.192	0.836

[1] Spearman correlation coefficient / p-value. Bold for a statistically significant difference between non-exposed and exposed groups (for the same tissue). Bold, $P < 0.05$.

Table 3
Concentration of inorganic elements in the urine and blood samples (μg/cm^2) of the non-exposed and exposed group by PIXE method (mean ± standard deviation).

Elements	Peripheral Blood		Urine	
	Non-exposed group	Exposed group	Non-exposed group	Exposed group
Na	7028 ± 6402	**12695 ± 5601***	3251 ± 2539	3229 ± 2471
Mg	92 ± 96	**496 ± 383***	110 ± 105	135 ± 107
Al	83 ± 65	**1671 ± 1535***	106 ± 160	144 ± 132
Si	534 ± 1776	767 ± 2068	689 ± 1333	643 ± 571
P	4129 ±584	6233 ± 4512	19822 ± 21941	19685 ± 22983
S	2428 ±2580	**5977 ± 1163***	12711 ± 8377	12848 ± 10497
Cl	1621 ±9065	**9266 ± 4171***	12549 ± 6244	17859 ± 11528
K	17954 ± 13305	**10634 ± 2954***	10475 ± 7488	14970 ± 12313
Ca	8446 ±11017	**557 ± 845***	7148 ± 16892	8435 ± 10802
Ti	7912 ±10754	**29 ± 87***	460 ± 2422	496 ± 729
Cr	138 ± 132	**362 ± 273***	52 ± 49	**2779 ± 8254***
Mn	10 ± 13	**2750 ± 8269***	556 ± 462	629 ± 1627
Fe	704 ± 634	**1854 ± 979***	17 ± 43	**183 ± 177***
Ni	47 ± 30	**67 ± 59***	708 ± 699	808 ± 790
Cu	14 ± 13	16 ± 12	22 ± 19	**175 ± 558***
Zn	54 ± 37	85 ± 50	23 ± 34	23 ± 31
Rb	30 ± 55	46 ± 53	37 ± 41	38 ± 38

* Significant increase in relation to the control group at $P < 0.05$; unpaired t- test (Welch`s correction). Bold, $P < 0.05$. For LOD information see Material and Methods.

and Cu were found in the urine samples from exposed group than non-exposed group; and a significant increase in the concentration of Na, Mg, Al, S, Cl, Cr, Mn, and Fe was found in the blood samples from exposed group when compared with non-exposed group. Interestingly, we found low concentrations of Cl, K, Ca, and Ti in the blood samples when compared to urine samples from exposed group.

Table 4 shows Spearman correlation analyses between MN formation and concentration of inorganic elements in peripheral blood and urine, respectively. A significant correlation was observed between the for-mation of MN and the increase in Mg, S, Fe, and Cu concentration ($P < 0.05$) in the peripheral blood of the exposed group. In the urine of the exposed group, a significant correlation was observed between MN and the concentration of Cr, Fe, Ni, and Cu ($P < 0.05$).

In our results we find a significant increase in telomere length of the exposed group (5653.0 ± 6791.0) compared to that of the non-exposed group (2549.5 ± 1776.3) (Mann–Whitney *U* test, $P < 0.05$) (Fig. 2a). Furthermore, we found a significant correlation between the frequency of MN and NPB with telomere length in the exposed group ($P < 0.001$) (Table 5). Significant correlation between age (19–66 years) or years of exposure (3–30 years) with telomere length in the exposed group was found (Y = 0.4628X + 9.459, $P < 0.001$) (Fig. 2b, c).

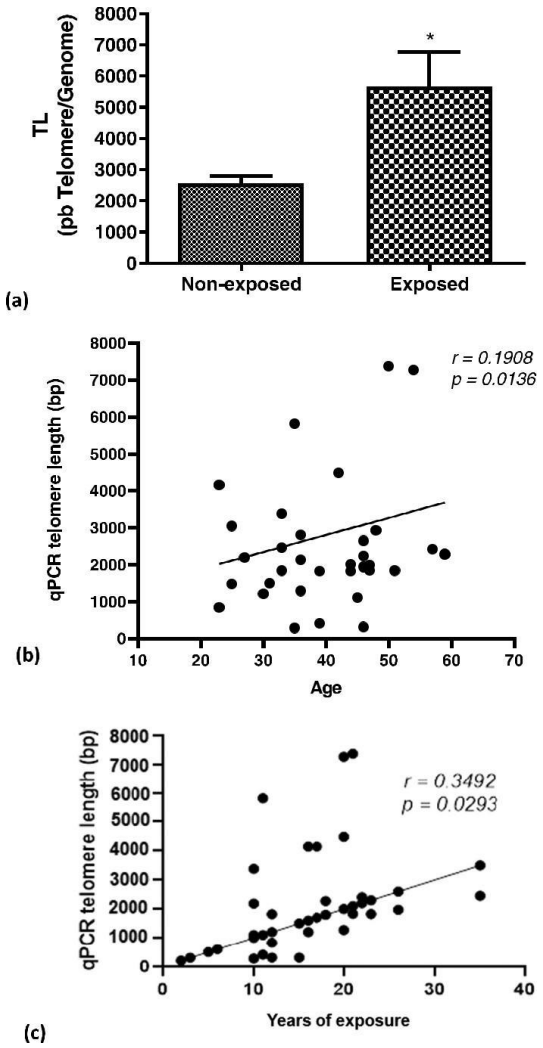

Fig. 2. (a) Mean and standard deviation of telomere length (TL) for non-exposed (control) and exposed group; *significantly different respect to non-exposed group (P < 0.001, Mann–Whitney U test).); (b) Non-parametric Spearman's correlation analyses between telomere length versus age (19–66 years) and (c) telomere length versus years of exposure (3 -30 years) for the exposed group.

Table 5
Spearman correlation analyses between cytokinesis-block micronucleus cytome (CBMN-Cyt) assay biomarkers and telomere length TL (bp) for non-exposed and exposed group.

TL (bp) Vs	Non-exposed		Exposed	
CBMN-Cyt	r_s	*P*	r_s	*P*
MN	0.1027	0.2256	0.6637	< 0.001
NPB	0.2284	0.0621	0.8646	< 0.001
NBUD	0.1894	0.0811	0.2588	0.0534
NECR	0.1084	0.2133	0.1108	0.251

TL, telomere length; MN, micronuclei; NPB, nucleoplasmic bridge; NBUD, nu-clear bud; NECR, necrotic cells.

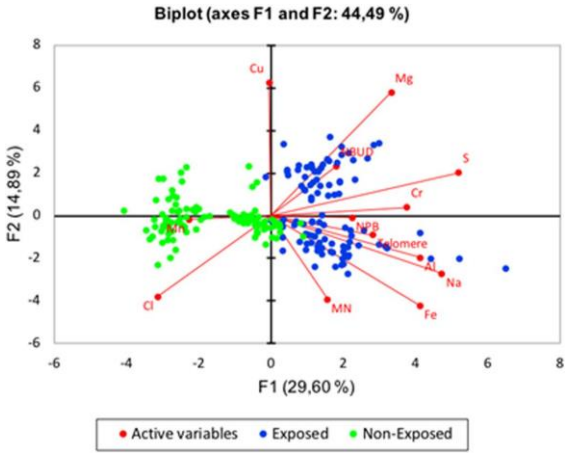

Fig. 3. Principle of Component Analysis (PCA) integrating data from inorganic elements detected in blood samples and DNA damage parameters.

Fig. 3 demonstrated the results of the PCA that shows a spatial distribution of the chemical and biological results and to evaluate the relative implication of these data in each sample. The plot of scores shows the position of each sample in the ordination plane of the two significant main components (F1 and F2), explaining, respectively, 29.60% and 14.98% of the total variation. Thus, factor analysis identified two components to be responsible for data structure explaining 44.49% of the total variance. F1 was loaded by S, Na, Al, Fe and Cr, and negatively loaded by Cl. The F1 axis discriminated the samples of Non-Exposed and Exposed groups, lower and higher values, respectively. While the component F2 was loaded by Cu and Mg and negatively loaded by MN frequencies.

4. Discussion

More than 10 million people work with welding globally (Honaryar et al., 2019). Several studies have described the toxic potential of exposure to welding fumes and hazardous components, including metals, that are released during welding (CCOHS 2016; Rana et al., 2019a). Based on numerous studies, IARC has established that there is sufficient evidence to classify welding fumes as carcinogenic to humans (Group 1) (IARC, 2018).

In this study, we proved that the group exposed to welding fumes showed a significant increase in the biomarkers of genomic instability (MN, NPB, and NBUD) and cell death (NECR) using the CBMN-Cyt assay. Several studies have shown the genotoxic risk due to chronic exposure to compounds released in welding fumes (Jara-Ettinger et al., 2015; Pesch et al., 2015; Wong et al., 2014). DNA damage can lead to responses such as cell cycle arrest and DNA repair; however, if the damage is excessive or irreparable this can lead to the activation of cell death (Jarvis et al., 2014; Leon´-Mejía et al., 2019). These changes can be attributed to a continuous exposure to the components present in welding fumes. Our results demonstrated a significant correlation between MN frequency and an increase in Mg, S, Fe, and Cu concentration in the peripheral blood and Cr, Fe, Ni, and Cu concentration in the urine of the exposed group. Many studies showed that welding fumes containing a mixture of metals such as Cr, Fe, Ni and Mn [besides Mn, beryllium (Be), Cd, Cr, Al, cobalt (Co), Cu, Fe, Pb, mercury (Hg), molybdenum (Mo), Ni, Zn, Ti, antimony (Sb), and V], and represent a harmful exposure, as well as welders are a high risk group of cancer and neurodegenerative diseases (Al-Lozi et al., 2017; Falcone et al., 2018; Yang et al., 2018; Michalek et al., 2019; Pesch et al., 2019; Rana et al., 2019a).

However, the composition and the rate of generation of welding fumes depends on the components of the metal being welded, coatings, types of electrodes or filler materials, operating conditions of the welding process (temperature and current), and the technique and skill of the welder (Balkhyour and Goknil, 2010).

These compounds enter the systemic circulation via inhalation and cause damage to organs such as the liver, kidney, and brain (NIOSH, 2013; IARC, 2018). Urinary excretion is the main route of elimination of most metals (Barbier et al., 2005; Jin et al., 2018). We analyzed the concentration of inorganic elements in the urine and blood samples. Our results indicate that a significantly higher concentration of Mg, Al, S, Cr, Mn, and Fe in the blood and Cr, Fe, and Cu in the urine of workers when compared with the non-exposed group. All these elements are present in welding fumes (Balkhyour and Goknil, 2010; Falcone et al., 2018; Michalek et al., 2019). The welding of Cr-containing steel, such as stainless steel (steel that contains between 10.5% and 27% Cr), is a primary source of exposure to hexavalent Cr (Cr VI) (Meeker et al., 2010). Fumes from mild steel welding contain mostly Fe, along with small amounts of additive metals like Mn, molybdenum (Mo), and Cu and the composition and toxicity of welding fumes may vary (Balkhyour and Goknil, 2010). Although Mn is essential at low concentrations, prolonged exposure cause Mn accumulation in the brain causing damage to the central nervous system (Chen et al., 2015). Cr comes from stainless-steel, high-alloy materials, and welding rods and it is also used as plating material. Exposure to Cr increases the risk of lung cancer; in fact, Cr VI is considered to be a group I carcinogen by the IARC (IARC, 2018). Iron oxide and iron-containing particles can cause siderosis—a benign form of lung disease caused by particles deposited in the lungs. Acute symptoms include irritation of the nose and lungs (CCOHS, 2016).

Prolonged exposure to metals, such as Ni, Al, Cr, and Cu, found in our samples and their correlation with DNA damage is associated with genotoxicity and carcinogenesis (Magaye et al., 2012; Willhite et al., 2014; IARC, 2018; El Safty et al., 2018; Guo et al., 2019). Metals can directly interact with DNA and alter replication and cause DNA damage, such as base modifications, depurination, inter and intra-molecular crosslinks between DNA and proteins, chromosomal rearrangements and induction of single strand breaks. Other alterations associated with the interaction of metals with DNA are activation of proto-oncogenes and inactivation of tumor suppressor genes, epigenetic alterations, sequence alteration, point mutations, and chromosomal aberrations in cells; this damage is produced mainly by the induction of alterations and oxidative modifications (Angel´e-Martínez et al., 2014; Morales et al., 2016). In contrast, the inflammation induced by the particles in the lungs is shown to cause the release of reactive oxygen species (ROS) by macrophages. The transition metals on the surface of the particles can also generate ROS through the Fenton reaction and lead to oxidative damage in the DNA (Vidrio et al., 2008).

Particles released during welding have different diameters, but the majority are less than 2.5 μm, including nanoparticles, which can reach the alveoli and deposit there, generating interstitial fibrosis and eventually, respiratory diseases such as bronchitis, airway irritation, lung function changes (Antonini, 2003), siderosis (Patel et al., 2009), asthma (Storaas et al., 2015), COPD (Ithnin et al., 2019) and lung cancer (Honaryar et al., 2019; Wong et al., 2017). Studies have described that these nanoparticles are rich in different metals (McCarrick et al., 2019; Shoeb et al., 2017). The mechanism of induction of these pathologies may be related to oxidative damage and inflammation caused by the metals contained in the welding fumes (Krishnaraj et al., 2017a; Leonard et al., 2010).

Notably, although 73% of the welder population reported using PPE, the levels of DNA damage were highly significant, which indicates that either PPE or clothing used during welding are not adequate (CCOHS, 2017) or that exposure to genotoxins is prolonged and cannot be repaired efficiently (García-Leston´ et al., 2012, Leon´-Mejía et al., 2019). Other variables that can influence levels of damage are those related to lifestyle. In both study groups, we found high consumption of alcohol, meat, fruits, and vegetables; however, more than 60% of the individuals in the exposed group did not consume vitamin supplements. Vitamins and minerals are important enzyme cofactors or are a part of metal-loenzymes, associated with DNA synthesis and repair, prevention of oxidative DNA damage, and maintenance of DNA methylation (Fenech and Bonassi, 2011).

Another important variable is the association between age and DNA damage. In our findings, a significant correlation of age was found with the formation of MN in lymphocytes. Several studies have indicated that aging leads to a decrease in DNA repair capacity (Fenech and Bonassi, 2011; Leon´-Mejía et al., 2019). In contrast, we found a direct association between the years of exposure and the formation of NPB in lymphocytes. These results coincide with other human biomonitoring studies where genetic instability and exposure to genotoxic agents have been analyzed (Singh et al., 2011; Barron´ Cuenca et al., 2019; Leon´-Mejía et al., 2019). This indicates that there is a cumulative effect of DNA damage or poor DNA repair due to continuous exposure to welding fumes, which can lead to lung disorders and cancer in the long term(Krishnaraj et al. 2017; Mehrifar et al. 2019; Vallieres` et al., 2012) NPBs can be seen during telophase and are a useful biomarker for loss of telomere integrity and fusion of chromosomal ends (Fenech, 2007; Zeljezic et al., 2015).

We found a significant increase in telomere (Krishnaraj et al., 2017) length in the exposed group compared to the non-exposed group. In-crease in telomere length has been described in different types of tu-mors, including gliomas, which have the highest rate of longer telomeres, demonstrating high telomerase activity (Barthel et al., 2017). Similarly, we found a significant correlation between the frequency of MN and telomere length. Different studies have associated changes in telomeres with the effects of welding fumes (Wong et al., 2014; Li et al., 2015; Shoeb et al., 2020, 2017). In fact, we found a significant corre-lation of age and years of exposure with telomere length. Telomeric length and DNA damage processes are related to the progression of aging. Alterations in telomere length and genetic instability can be impacted by the accumulation of DNA damage and oxidative stress (Kahl et al., 2018; Ko et al., 2017). Shoeb et al. (2020) showed that when rats were exposed to welding fumes, Trf1 and Trf2 were down-regulated and ATM, shelterin complex proteins, and telomere elongation were activated, corroborating our data. Trf1 is a telomerase inhibitor and Trf2 is involved in telomere length regulation. These proteins are a part of the chromosome protection network against nucleolytic degradation, end-to-end fusion, and breakage-fusion-bridge-cycle (Kahl et al., 2016). Trf2 is a key component of the shelterin complex, and any change that affects this component results in the loss of function of other proteins, triggering chromosomal instability (Baird et al., 2018).

PCA was conducted to obtain an overview of the spatial distribution of the chemical and biological data and to evaluate the relative

implication of these data in differences among samples. The principal component analysis indicated associations between S, Na, Al, Fe and Cr concentrations with exposed group. On the other hand, the non-exposed group was associated to Cl, as well Mn concentrations. Is possible to observe an association between NBUD, that is a result of gene amplifi-cation (Fenech, 2007), a reparation disbalance, and Mg, S concentra-tions, that acts as enzymatic co-factors. Still, was observed an association between telomere and NBUD with Cr and Al concentrations, elements associated with genotoxicity and carcinogenesis (Magaye et al., 2012; Willhite et al., 2014; IARC, 2018; El Safty et al., 2018; Guo et al., 2019).

In conclusion, the results obtained using different biomarkers of CBMN-Cyt show a significant increase in DNA damage and cell death in the welder group compared to the non-exposed group. High concen-trations of toxic metals such as Cr, Fe, and Cu in the urine and Mg, Al, S, Cr, Mn, and Fe in the blood were detected. Thus, there may be an as-sociation between oxidative damage and the components of the welding fumes, such as metals, toxic gases, and organic vapors. All these com-ponents constitute a complex mixture and due to their synergistic effect, it is difficult to relate the genotoxic and cytotoxic effects found to the actions of a single compound. Moreover, increased telomere length of the exposed group, compared to the non-exposed group, may have a close relationship with genetic instability found. The significant corre-lation of telomere length with the frequency of micronuclei, and its correlation with age and years of exposure represent very important results in the risk assessment of exposed populations and constitute very relevant and pioneering data in Colombian populations.

CRediT author statement

All authors declare that they have participated in the review and improvement of the manuscript and that this presentation is suitable for delivery as a revised version for the EES journal.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was supported by COLCIENCIAS Grant (657/125365741083/2014) and Universidad Simon´ Bolívar, Barranquilla, Colombia.

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CAPÍTULO II

4 CAPÍTULO II - *Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay analysis and its relationship with genetic polymorphisms in welders*

APRESENTAÇÃO

O segundo capítulo corresponde a uma publicação que foi submetida à revista “Mutation Research- Genetic Toxicology and Environmental Mutagenesis”, na qual é abordada a influência dos polimorfismos genéticos *OGG1 Ser326Cys, XRCC1 Arg280His, XRCC1 Arg194Thr, XRCC1 Arg399Gln, XRCC3 Thr241Met, GSTM1* e *GSTT1* nos danos ao DNA em uma população de pessoas profissionalmente expostas aos fumos de solda, em comparação com outra de pessoas não expostas.

CYTOKINESIS-BLOCK MICRONUCLEUS CYTOME (CBMN-CYT) ASSAY ANALYSIS AND ITS RELATIONSHIP WITH GENETIC POLYMORPHISMS IN WELDERS

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Abstract

The fumes generated in the welding process is composed of micrometer and nanometric particles that form when metal fumes condense. International Agency for Research on Cancer established that many of the compounds derived from the welding process are carcinogenic to humans, but there are few studies related to the role of genetic polymorphisms. The aim of this work was analyzing the influence of *OGG1 Ser326Cys*, *XRCC1 Arg280His*, *XRCC1 Arg194Thr, XRCC1 Arg399Gln, XRCC3 Thr241Met, GSTM1*, and *GSTT1* genes polymorphisms on DNA damage of 98 subjects occupationally exposed to welding fumes and 100 non-exposed individuals. In results were found that individuals exposed to welding fumes with the *XRCC3 Thr241Thr, XRCC3 Thr241Met* and *GSTM1 null* genotype demonstrated a significantly higher frequency of micronucleus in binucleated lymphocytes. On the other hand, individuals with *XRCC1 Arg399Gln* and *XRCC1 Gln399Gln* genotype had significant levels of nucleoplasmic bridges. *OGG1 326 Ser/Cys, OGG1 326 Cys/Cys, XRCC1 194Arg/Thr, XRCC1 194Thr/Thr* and *GSTT1 null* genotype exhibited significantly higher apoptotic values. Also, *XRCC1 194Arg/Trp, XRCC1 194Thr/Thr* and *GSTM1 null* genotype carriers had higher necrotic levels compared to *XRCC1 194Arg/Arg* and *GSTM1 non null* carriers. Compositional analysis revealed the presence of iron, manganese, silicon, and the presence of particles smaller than 2 µm; that adhere to each other and form agglomerates. These results may be associated with the mixture of components such as gases of nitrogen dioxide, carbon monoxide and metallic fumes leads to significant DNA damage and cell death processes. These findings demonstrate the importance and association between individual susceptibility and levels of DNA damage due to occupational exposure to welding fumes and constitute the first results for Colombia on the effects of this exposure on workers.

Key words: Occupational exposure, welding fumes, micronucleus test, polymorphisms, particles.

1*. Introduction*

The job of welding is a very widespread activity worldwide (Anand et al., 2014). During welding processes, and also in operations related to these processes, such as cutting or melting a material, a large number of genotoxic agents are generated to which the welder may be exposed such as aluminum (Al) oxides, cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), titanium (Ti), vanadium (V), among others. In addition to gases such as ozone, nitrogen dioxide and carbon monoxide (Anand et al., 2014; Pesch et al., 2019). The type of compound that is generated depends on the type of welding, the filler material (electrode, rod) and its coating. Also, micrometric particles are generated, which have different diameters and can be easily inhaled and absorbed by the body. Many of the generated agents can cause damage to the respiratory tract, lungs and nervous system and even cause cancer (Heltoft et al., 2017; Kazi et al., 2021; Pesch et al., 2019).

One way to quantify exposure to xenobiotics and its possible impact on the body is the use of biological monitoring procedures through biomarkers. Currently, the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay has a promising approach for the measurement of DNA damage, cytostatic effects, and cytotoxicity (Fenech, 2007; Fenech et al., 2011). Quintana-Sosa et al. (2021) using different biomarkers of CBMN-Cyt show a significant increase in DNA damage and cell death in the welder group compared to the non-exposed group. Despite significant correlations between micronuclei (MN) frequency and the increase in the concentration of magnesium (Mg), sulfur (S), Fe and Cu in blood samples of the exposed group, and between MN and Cr, Fe, Ni and Cu in urine were demonstrated a high variability among individuals was observed (Quintana-Sosa et al., 2021).

Importantly, DNA damage can be influenced by genetic polymorphisms, causing variability among individuals exposed to different pollutants (Wright, 2005). Besides, polymorphisms in DNA repair genes may represent susceptibility factors that affect DNA integrity, increasing the risk of some types of cancer in humans (Dylawerska et al., 2017). DNA repair is essential in the protection of the cellular genome from environmental aggressions, in fact it has been shown that a reduced capacity in DNA

repair is associated with an increased risk of lung cancer (Li et al., 2019; Minina et al., 2019).

The aim of this study was analyzing the cytotoxic and genotoxic effects of welding fume and the possible influence of variability between individuals, specifically the influence of two Base excision repair (BER) gene polymorphisms (*OGG1 Ser326Cys* and *XRCC1 Arg280His, XRCC1 Arg194Thr, XRCC1 Arg399Gln*), one homologous recombinational repair (HRR) gene (*XRCC3 Thr241Met*) and two metabolic detoxification genes (*GSTM1* and *GSTT1*) on genetic damage in welders.

2. Materials and methods

2.1. Individuals and sampling

This study was approved by the University of Simón Bolívar's Ethics Committee (CIE-USB-CE0233-00), and informed written consent was obtained from everyone before the start of the study. The study population comprised 198 male individuals (in total) from Monteria, on the north coast of Colombia. Within the study population, 98 were welders (mean age: 40.60 ± 10.84 years; exposed for 19.15 ± 8.39) and 100 were controls (mean age: 30.16 ± 10.37). This sample size was calculated according to the total number of welders in the sampling areas. Confounding and exclusionary factors were collected from all participants through an interviewer-administered questionnaire. Data included age, health status, cancer history, other chronic diseases, lifestyle, nutrition, smoking habits, medication intake, frequency of alcohol consumption (total number of drinks and the most common alcoholic beverage consumed), specific occupation and time of service, protective measures and previous exposure to medical X-rays or treatments with known carcinogens. Individuals were selected according to the following inclusion criteria: voluntary participation and time of occupational service ≥ 3 years. Exclusion criteria were smoking (current or past), exposure to other genotoxic factors, medical treatment for up to 3 months or receipt of X-ray examinations up to 1 year before the start of this study, previous or current cancer diagnosis and current or previous chemotherapy,

radiotherapy or intake of therapeutic drugs known to be mutagenic. All data were organized and recorded in databases. There were no major differences in socioeconomic status or dietary habits among the final study population. We obtained consent from all study participants after a detailed explanation of study aims, benefits, risks and methodology. All identifying information was maintained at the Unidad de Genética y Biología Molecular of the University Simón Bolívar in Barranquilla, Atlántico Department, and this was the only institution with identifiable information on the study participants. Detailed demographic characteristics of the studied population can be found in a previous study in Quintana-Sosa et al. (2021).

2.2. Sample collection

Peripheral blood samples were collected by venipuncture into heparinized vacutainer tubes (Becton Dickenson) for the MN test and into EDTA tubes (5 mL) for polymorphisms analysis. All sample tubes were coded and kept upright on ice during transportation to the laboratory. Samples were processed immediately upon arrival.

2.3 Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay

CBMN-Cyt assay was carried out according to previous methodology described in Quintana-Sosa et al. (2021). Briefly, heparinized whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 medium (Sigma R8758, USA) supplemented with 2 mM L-glutamine (Sigma A5955, USA), 10% fetal bovine serum (Gibco/Invitrogen 15000-044, Brazil), 100 µL/mL antibiotic–antimycotic (Sigma A5955, USA) and 2% phytohemagglutinin (Sigma L8754, USA) to stimulate lymphocytes. Duplicate cultures were set up for each sample. Cultures were incubated at 37◦C for 44 h, under 5% CO_2. Cytocalasin B (Sigma, C6762; 6 µg/mL) was added at the 44 h of incubation. The cells were harvested after 72 h, treated with hypotonic solution (0.075 M KCl), immediately centrifuged and fixed three times with methanol/acetic acid (3:1). The fixed cells were dripped onto humidified slides, air-dried and treated with 1% Giemsa stain for 10 min. For cytome analysis, were analyzed MN, nucleoplasmic bridges (NPB) and nuclear buds (NBUD) frequency per 1000

binucleated (BN) cells per slide and the frequency of necrotic (NECR) and apoptotic (APOP) cells in 500 randomly scored cells. The scoring criteria followed those proposed by Fenech et al. (2007).

2.4 DNA isolation

Genomic DNA was isolated from peripheral blood lymphocytes using the DNeasy Blood and Tissue extraction kit (Qiagen, USA) following the manufacturer's instructions and stored at − 20 °C until further analysis.

2.5 Polymorphisms in xenobiotic metabolism and DNA repair genes

2.5.1 Determination of BER pathway genotypes

The *OGG1 Ser326Cys* genotype was determined using the primers pairs and conditions described by De Ruyck et al. (2005). For this analysis, PCR product was used as template and was digested using the *SatI* restriction enzyme. The *XRCC1 Arg280His* genotype was determined by PCR/RFLP using the primers and conditions described by Tuimala et al. (2002). PCR products were digested using the *RsaI* enzyme. The *XRCC1 Arg194Thr* genotype was carried out as described by Lunn et al. (1999). After obtain the PCR product, this was digested with *PvuII* enzyme. *XRCC1 Arg399Gln* genotype was carried out as described by Ryu et al. (2011) and the PCR product were digested using *Msp I* restriction enzyme (Ryu et al., 2011). All genotypes were assessed using 3% ultrapure agarose gels (Invitrogen-Brazil) stained with Syber Safe and visualization by Gel Doc XR+ (BioRad).

2.5.2 Determination of the HRR pathway genotype

The *XRCC3 Thr241Met* genotype was determined by PCR/RFLP using the primers and conditions described by Tuimala et al. (2002). The PCR product was digested using the NlaIII

enzyme, and the genotype was assessed using 3% ultrapure agarose gels (Invitrogen-Brazil) stained with Syber Safe and visualization by Gel Doc XR+ (BioRad).

2.5.3 Determination of GSTM1 and GSTT1 genotypes

For the analysis of GSTM1 and GSTT1 metabolism genotypes, multiplex PCR was performed using the CYP1A1 (exon 7) gene 312-bp product as an internal control (Iarmarcovai et al., 2006). When the internal control was present, the absence of GSTM1 (215 bp) and GSTT1 (480 bp) products indicated the *GSTM1 null* and *GSTT1 null* genotypes, respectively.

2.6 Particulate Matter (PM) Sampling

The samples were collected in the work area of a welding shop using a Haz-Dust EPAM-5000 system (Environmental Devices Corporation, Plaistow, NH, USA) and polytetrafluoroethylene filters for $PM_{2.5}$. The samples were collected continuously for 24 h including the working hours of all subjects.

2.6.1. SEM-EDS analysis

Particles were analyzed on porous silicon using a field emission scanning electron microscope (JEOL JSM 6490 LV, JEOL, Tokyo, Japan). Briefly, samples were fixed on adhesive graphite tape and coated with a thin gold (Au) coating (DENTON VACUUM Desk IV).

2.7 Statistical analysis

The agreement of genotypic frequencies with Hardy–Weinberg equilibrium (HWE) expectations for *OGG1 Ser326Cys*, *XRCC1 Arg280His, XRCC1 Arg194Thr, XRCC1 Arg399Gln*, *XRCC3 Thr241Met* and *GSTM1null*, *GSTT1null* was performed using a χ^2 test with 1° of freedom. The influence of genotype on Cytokinesis-block micronucleus cytome (CBMN-Cyt) biomarkers was determined using Poisson regression analysis. For continuous variables a Gaussian link function was used.

For all tests, a two-tailed p <0.05 was considered statistically significant. Statistical analyses were carried out using software R version 3.6.0.

3. Results

The peripheral blood lymphocytes of 98 welders chronically exposed to welding fumes and 100 non- exposed individuals were assessed for DNA damage and genotyped to detect polymorphisms in genes mediating DNA repair and xenobiotic metabolism.

PM collected in the workplace was analyzed by SEM-EDS (Figure 1). Compositional analysis revealed the presence of Fe, Mn, P, Si, K and revealed the presence of particles smaller than $PM_{2.5}$, specifically, particles smaller than 2 µm and nanoparticles. These particles have the ability to form agglomerates.

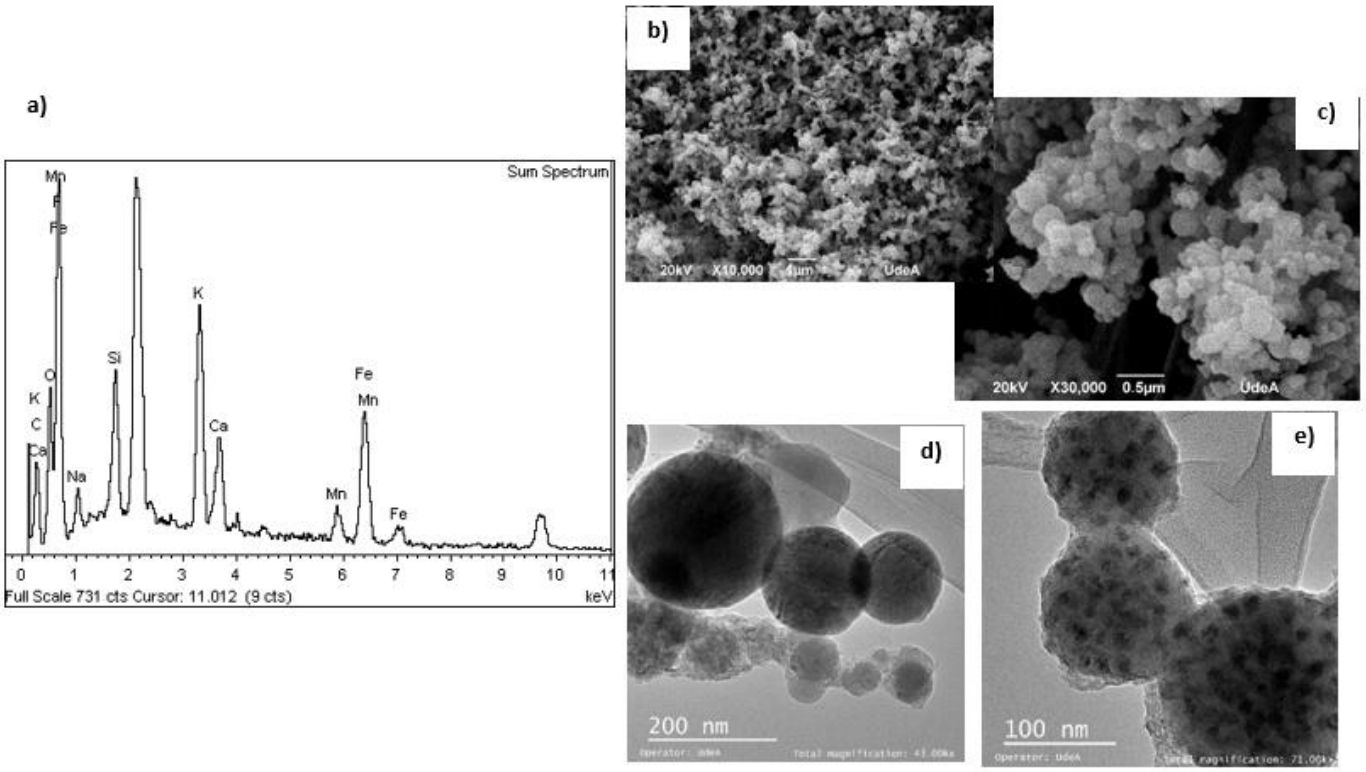

Figure 1. SEM analysis. **a)** SEM-EDS spectra of the welding fume particles. Peaks without identification correspond to the standards of the analyzes **b)** General view of the filter with particulate matter. **c), d)** and **e)** Agglomerate formation.

Table 1 shows the genotypic distribution and allelic variant frequencies of the metabolic, BER and HRR genes studied in welders-exposed and non-exposed individuals. The genotype frequency of *OGG1 Ser326Cys, XRCC3 Thr241Met, XRCC1 Arg280His* complied with HWE, whereas the *XRCC1 Arg194Thr, XRCC1 Arg399Gln, GSTM1* and *GSTT1* polymorphisms deviated from HWE. This deviation from HWE may be due to a moderate effect of genetic drift previously described for sampled population (Espitia-Pérez et al., 2016; Rojas et al., 2013) or incomplete panmixia, strongly associated to assortative mating aspects of indigenous populations that constitutes the main racial component in this region from northern Colombia. All variant alleles agreed with literature values for Caucasian populations (Duarte et al., 2005; Hoyos-Giraldo et al., 2009).

Table 2 and Table 3 summarize the effects of genotype on different damage biomarkers (MN, NPB, NBUD, APOP and NECR). In the non-exposed group is observed that individuals carrying the *XRCC1 280 Arg/His* and *XRCC1 280 His/His* genotype demonstrated significantly higher MN frequency in lymphocytes than those with the *XRCC1 280 Arg/Arg* genotype ($p < 0.05$) (FR: 0.77, P = 0.003). Individuals with the *GSTM1 null* and *GSTT1 null* genotype exhibited significantly higher NPB than individuals with the *GSTM1 non-null* and *GSTT1 non-null* genotype respectively

(p < 0.05) (FR: 0.74, P = 0.046; FR:0.63, P = 0.001, respectively). Also was observed that subjects with *GSTT1 null* genotype had higher levels of NBUD (FR: 0.6, P = 0) and APOP (FR: 0.74, P = 0) compared to *GSTT1 non-null.* Besides, individuals with *GSTM1 null* genotype showed significant levels of apoptosis compared to *GSTM1 non-null* (FR: 0.75, P = 0.001). Regarding the levels of NECR in the non-exposed group, subjects with the *XRCC3 Thr241Thr* and *XRCC3 Thr241Met* genotype had significant levels compared to the *XRCC3 Met241Met* individuals (FR: 2.45, P = 0.044).

Regarding the results of the effect of the genotype on CBMN-Cyt biomarkers in the exposed group (Table 2 and 3), was observed that subjects with the *XRCC3 Thr241Thr, XRCC3 Thr241Met* and *GSTM1 null* genotype demonstrated a significantly higher frequency of MN than *XRCC3 Met241Met* (FR: 0.68, P = 0) and *GSTM1 non-null* respectively (*p* < 0.05) (FR: 0.84, P = 0.012). Considering the frequency of NPB, it was found that individuals with *XRCC1 Arg399Gln* and *XRCC1 Gln399Gln* genotype had significant levels of this DNA damage biomarker compared to *XRCC1 Arg399Arg* individuals carriers (*p* < 0.05) (FR: 0.82, P = 0.036). Also, *OGG1 326 Ser/Cys, OGG1 326 Cys/Cys, XRCC1 194Arg/Trp, XRCC1 194Trp/Trp* and *GSTT1 null* genotype exhibited significantly higher APOP values than *OGG1 326 Ser/Ser, XRCC1 194Arg/Arg* and *GSTT1 non null* carriers respectively (*p* < 0.05) (FR: 1,71, P = 0.040; FR:1.17, P = 0.041, FR:0.8, P = 0.004, respectively). In relation to genotype and NECR levels, it was found that *XRCC1 194Arg/Trp, XRCC1 194Trp/Trp* and *GSTM1 null* genotype carriers had higher NECR levels compared to *XRCC1 194Arg/Arg* and *GSTM1 non null* carriers (*p* < 0.05) (FR: 0.73, P = 0; FR:1.44, P = 0, respectively).

Table 1. Genotypic distribution and allelic variant frequencies in welders-exposed and non-exposed individuals.

Gene	Genotype	Non-exposed group	Exposed group	p*	Total frequency observed	Equilibrium frequency	p**	Allele/Genotype Frequency
		N (%)	N (%)		N (%)	N (%)		
	Ser/Ser	67(67)	3(3.1)		70(35.4)	55(27.8)		*Ser:*0.53
	Ser/Cys	20(20)	48(49)	<0.010	68(34.3)	99(50)	**<0.010**	
OGG1 326	*Cys/Cys*	13(13)	47(48)		60(30.3)	44(22.2)		*Cys:*0.47
	Thr/Thr	76(76)	43(43.9)		119(60.1)	111(56.1)		*Thr:*0.75
	Thr/Met	21(21)	38(38.8)	<0.010	59(29.8)	74(37.3)	**<0.010**	
XRCC3 241	*Met/Met*	3(3)	17(17.3)		20(10.1)	13(65.6)		*Met:*0.25
	Arg/Arg	34(34)	18(18.4)		52(26.3)	38(19.2)		*Arg:*0.44
	Arg/His	13(13)	58(59.2)	<0.010	71(35.9)	98(49.5)	**<0.010**	
XRCC1 280	*His/His*	53(53)	22(22.4)		75(37.9)	62(31.3)		*His:*0.56
	Arg/Arg	51(51)	30(30.6)		81(40.9)	86(43.4)		*Arg:*0.66
	Arg/Trp	43(43)	55(56.1)	<0.010	98(49.5)	89(44.9)	0.171	
XRCC1 194	*Trp/Trp*	6(6)	13(13.3)		19(9.6)	23(11.6)		*Trp:*0.34
	Arg/Arg	50(50)	56(57.1)		106(53.5)	100(50.5)		*Arg:*0.71
	Arg/Gln	42(42)	28(28.6)	0.093	70(35.4)	81(40.9)	0.052	
XRCC1 399	*Gln/Gln*	8(8)	14(14.3)		22(11.1)	17(8.6)		*Gln:*0.29
GSTM1	*Positive*	63(63)	33(33.7)	<0.010	96(48.5)	96(48.5)	0.800	*Non-Null:* 0.45
	Null	37(37)	65(66.3)		102(51.5)	102(51.5)		*Null*:0.55
GSTT1	*Positive*	25(25)	24(24.5)	0.934	49(24.7)	49(24.7)	0.400	*Non-Null:* 0.33
	Null	75(75)	74(75.5)		149(75.3)	149(75.3)		Null:0.67

*Statistical significance of genotypic differences between exposed and non-exposed groups determined by 2 test; **Statistical significance of genotypic differences between expected and observed values.

Table 2. Effects of genotype on Cytokinesis-block micronucleus cytome (CBMN-Cyt) biomarkers (mean ± S.D) in non-exposed and exposed group.

Groups / Genotype	N	DNA Damage[a]			Cell Death[a]	
		MN	NPB	NBUD	APOP	NECR
Non-Exposed						
OGG1 326 Ser/Ser	67	3.01 ± 2.32	2.11 ± 1.21	2.13 ± 1.90	5.21 ± 2.32	3.25 ± 2.01
OGG1 326 Ser/Cys	20	4.52 ± 2.45	1.89± 0.46	3.92 ± 2.23	3.26 ± 2.22	2.61 ± 1.02
OGG1 326 Cys/Cys	13	4.90±3.35	2.68±1.66	3.66±2.23	4.44±2.46	3.55±1.46
XRCC3 241 Thr/Thr	70	3.11 ± 2.76	1.89 ± 1.55	1.16 ± 1.44	4.31 ± 2.12	**5.45 ± 2.67[b]**
XRCC3 241 Thr/Met	21	3.45 ± 2.21	2.77 ± 1.21	2.40 ± 1.33	3.45± 2.10	**6.01 ± 4.55[b]**
XRCC3 241 Met/Met	9	4.45±2.45	1.14±1.55	1.12±1.66	4.87±2.54	3.67±2.98
XRCC1 280 Arg/Arg	34	3.04 ± 5.45	2.77 ± 4.66	3.54 ± 1.55	5.11 ± 3.34	1.21 ± 1.11
XRCC1 280 Arg/His	13	**5.28 ± 3.11[c]**	3.01±5.34	2.35 ± 1.90	3.92 ± 1.45	2.23 ± 1.01
XRCC1 280 His/His	53	**6.77 ± 5.51[c]**	2.20 ± 5.91	1.66 ± 1.78	4.21 ± 2.34	2.33 ± 1.58
XRCC1 194 Arg/Arg	51	2.23 ± 1.64	1.11 ± 0.40	2.33 ± 1.41	3.32 ± 1.77	2.45 ± 2.03
XRCC1 194 Arg/Trp	43	3.34± 2.46	3.97±0.84	1.55 ±1.21	3.45 ± 1.76	1.12 ± 1.04
XRCC1 194 Trp/Trp	6	3.40 ± 2.77	1.88 ±0.28	1.29 ± 0.59	4.56 ± 2.99	0.30 ± 0.11
XRCC1 399 Arg/Arg	50	2.23 ± 1.02	1.65 ± 1.55	3.65 ± 1.51	3.34 ± 2.56	1.43 ± 1.02
XRCC1 399 Arg/Gln	42	3.54 ± 1.45	2.23 ± 0.23	0.22 ± 0.11	3.33 ± 2.34	3.74 ± 2.11
XRCC1 399 Gln/Gln	8	3.67 ± 2.89	2.54 ± 1.67	2.32 ± 1.30	4.11 ± 3.24	2.66 ± 1.23
GSTM1 non-null	37	3.22±1.68	2.83±3.04	2.87±1.49	5.48±4.04	3.96±2.20
GSTM1 null	63	4.88±3.50	**4.80±3.73[f]**	3.90±1.20	**8.5±7.39[f]**	4.04±3.84
GSTT1 non-null	75	4.38±2.17	3.52±3.51	3.02±3.84	5.06±6.95	5.98±2.48

GSTT1 null	25	3.58±2.74	**5.08±3.46[g]**	**5.87±3.57[g]**	**8.12±9.40[g]**	4.75±2.51
Exposed						
OGG1 326 Ser/Ser	3	9.53 ± 8.23	4.11 ± 4.02	4.23 ± 3.61	7.16 ± 6.10	8.65 ± 2.01
OGG1 326 Ser/Cys	48	8.52 ± 6.39	5.23 ±4.03	6.15 ± 5.12	**9.69 ± 5.32[a]**	6.61 ± 1.02
OGG1 326 Cys/Cys	47	8.82± 5.06	3.53± 2.61	3.56± 2.81	**10.06±.7.45[a]**	7.56± 0.91
XRCC3 241 Thr/Thr	43	**10.56 ± 3.43[b]**	4.39 ± 3.94	3.45 ± 3.01	5.94 ± 3.04	3.04 ± 3.42
XRCC3 241 Thr/Met	38	**9.33 ± 6.65[b]**	4.79 ± 3.89	4.06 ± 3.11	7.47 ± 5.48	4.89 ± 1.31
XRCC3 241 Met/Met	17	7.06±5.45	5.04±3.04	4.01±3.99	6.20±4.34	4.09±2.11
XRCC1 280 Arg/Arg	18	7.24 ± 6.23	4.07 ± 2.96	4.45 ± 2.92	9.11 ± 8.42	8.13 ± 1.76
XRCC1 280 Arg/His	58	8.18 ± 3.30	3.25 ± 2.20	3.78 ± 2.68	9.65 ± 8.42	7.76 ± 1.12
XRCC1 280 His/His	22	6.97 ± 5.03	3.34 ± 1.61	5.10 ± 4.03	8.37 ± 7.14	6.67 ± 0.44
XRCC1 194 Arg/Arg	30	5.88 ± 4.44	5.46± 2.40	6.75 ± 2.94	7.11 ±6.79	4.34 ± 1.16
XRCC1 194 Arg/Trp	55	5.56 ± 4.55	5.69 ± 3.64	6.58 ± 4.76	**12.62 ± 9.20[d]**	**8.98 ± 1.22[d]**
XRCC1 194 Trp/Trp	13	6.95 ± 2.60	6.80 ± 4.05	7.33 ± 3.90	**11.23± 7.65[d]**	**7.99 ± 0.11[d]**
XRCC1 399 Arg/Arg	56	9.01 ± 5.45	5.25 ± 2.27	5.43 ± 3.11	10.93 ± 8.73	6.33 ± 0.32
XRCC1 399 Arg/Gln	28	8.89 ± 7.14	**8.66 ± 2.99[e]**	6.84 ± 3.56	10.91 ± 7.85	7.98 ± 2.11
XRCC1 399 Gln/Gln	14	9.90 ± 4.87	**9.96 ± 4.56[e]**	5.71 ± 3.88	11.57 ± 9.44	6.43 ± 2.94
GSTM1 non-null	52	5.33±2.31	5.31±3.20	3.54±2.45	9.45±4.76	5.90±5.44
GSTM1 null	46	**7.88±5.33[f]**	4.34±5.56	4.67±3.98	10.78±5.66	**8.94±2.45[f]**
GSTT1 non-null	74	4.56±3.67	5.67±4.90	4.76±3.22	7.90±6.70	6.04±3.21
GSTT1 null	24	5.41±5.01	4.78±4.55	5.88±4.56	**11.10±9.90[g]**	7.03±3.96

*Bold: For statistically significant level. $p<0.05$; a Data significant in relation to genotype *OGG1 Ser/Ser* individuals; b Data significant in relation to genotype *XRCC3 241 Met/Met* individuals; c Data significant in relation to genotype *XRCC1 280 Arg/Arg* individuals; d Data significant in relation to genotype *XRCC1 194 Arg/Arg* individuals; e Data significant in relation to genotype *XRCC1 399 Arg/Arg* individuals; f Data significant in relation to genotype *GSTM1 (+)* individuals; g Data significant in relation to genotype *GSTT1 (+)* individuals. [x] Values of the biomarker (DNA damage or cell death) of the entire group (exposed or nonexposed) before subset for each genotype from da Quintana-Sosa et al. (2021).

Table 3. Cytokinesis-block micronucleus cytome (CBMN-Cyt) biomarkers Poisson regression analysis of the total, non-exposed and exposed populations considering genetic polymorphisms.

Biomarkers / Genotype	Total Population (n=198)			Non-exposed (n = 100)			Exposed (n = 98)		
	FR	CI (95%)	P	FR	CI (95%)	P	FR	CI (95%)	P
MN									
OGG1Ser/Cys;Cys/Cys a	**1.67**	**1.48-1.88**	**0**	1.17	0.97-1.40	0.088	1.13	0.77-1.95	0.547
XRCC3 241Thr/Met;Thr/Thr b	**0.61**	**0.53-0.71**	**0**	1.91	1.02-4.20	0.070	**0.68**	**0.59-0.80**	**0**
XRCC1 280 Arg/His;His/His c	1.06	0.94-1.20	0.340	**0.77**	**0.64-0.92**	**0.003**	1.13	0.95-1.35	0.177
XRCC1 194 Arg/Trp;Trp/Trp d	1.06	0.95-1.18	0.310	1.01	0.85-1.21	0.891	0.89	0.77-1.02	0.092
XRCC1 399 Arg/Gln;Gln/Gln e	0.81	0.81-1.00	0.053	0.85	0.71-1.00	0.055	0.99	0.87-1.14	0.985
GSTM1 Null f	1.02	0.92-1.14	0.630	0.88	0.73-1.06	0.193	**0.84**	**0.74-0.96**	**0.012**
GSTT1 Null g	1.06	0.94-1.21	0.305	1	0.83-1.24	0.938	1.09	0.94-1.28	0.250
NPB									
OGG1Ser/Cys;Cys/Cys a	**1.74**	**1.06-2.09**	**0**	0.86	0.64-1.15	0.332	1.17	0.69-2.21	0.584
XRCC3 241Thr/Met;Thr/Thr b	0.80	0.64-1.02	0.065	0.56	0.32-1.10	0.063	1.14	0.89-1.48	0.297
XRCC1 280 Arg/His;His/His c	1.09	0.92-1.31	0.304	0.97	0.74-1.30	0.855	0.91	0.72-1.15	0.407
XRCC1 194 Arg/Trp;Trp/Trp d	1.13	0.97-1.32	0.122	0.92	0.70-1.21	0.546	0.98	0.81-1.20	0.856
XRCC1 399 Arg/Gln;Gln/Gln e	**0.83**	**0.71-0.97**	**0.017**	1.01	0.77-1.32	0.945	**0.82**	**0.67-0.99**	**0.036**
GSTM1 Null f	**1.28**	**1.10-1.50**	**0.001**	**0.74**	**0.55-0.99**	**0.046**	1.20	0.99-1.47	0.072
GSTT1 Null g	**0.82**	**0.69-0.97**	**0.018**	**0.63**	**0.48-0.84**	**0.001**	0.92	0.75-1.14	0.458
NBUD									
OGG1Ser/Cys;Cys/Cys a	**1.86**	**1.56-2.22**	**0**	0.83	0.62-1.11	0.228	1.4	0.81-2.72	0.266
XRCC3 241 Thr/Met;Thr/Thr b	1.03	0.81-1.33	0.802	1.07	0.52-2.71	0.876	1.43	1.09-1.88	0.008
XRCC1 280 Arg/His;His/His c	**1.27**	**1.07-1.52**	**0.008**	0.97	0.73-1.29	0.819	1.15	0.91-1.46	0.256
XRCC1 194 Arg/Trp;Trp/Trp d	**1.22**	**1.05-1.42**	**0.010**	0.82	0.63-1.08	0.156	1.15	0.94-1.40	0.173
XRCC1 399 Arg/Gln;Gln/Gln e	1.08	0.93-1.25	0.303	0.95	0.73-1.25	0.732	1.24	1.04-1.48	0.016
GSTM1 Null f	**1.20**	**1.03-1.39**	**0.018**	0.91	0.68-1.20	0.495	0.94	0.79-1.14	0.530
GSTT1 Null g	0.96	0.81-1.14	0.630	**0.60**	**0.45-0.80**	**0**	1.21	0.98-1.50	0.088
APOP									
OGG1Ser/Cys;Cys/Cys a	**1.45**	**1.30-1.64**	**0**	1.16	0.98-1.38	0.079	**1.71**	**1.07-2.98**	**0.040**
XRCC3 241Thr/Met;Thr/Thr b	0.97	0.82-1.16	0.745	1.47	0.87-2.76	0.187	1.09	0.91-1.31	0.385

XRCC1 280 Arg/His:His/His c	1.10	0.97-1.24	0.130	0.91	0.77-1.08	0.289	1.15	0.96-1.39	0.129
XRCC1 194 Arg/Trp:Trp/Trp d	**1.13**	**1.02-1.26**	**0.025**	0.92	0.78-1.08	0.313	**1.17**	**1.01-1.37**	**0.041**
XRCC1 399 Arg/Gln:Gln/Gln e	0.97	0.87-1.08	0.568	1.03	0.87-1.21	0.740	0.97	0.85-1.12	0.703
GSTM1 Null f	**1.08**	0.97-1.20	0.148	**0.75**	**0.63-0.89**	**0.001**	1.16	1.00-1.34	0.052
GSTT1 Null g	**0.78**	**0.69-0.87**	**0**	**0.74**	**0.62-0.88**	**0**	**0.80**	**0.69-0.93**	**0.004**
NECR									
OGG1Ser/Cys:Cys/Cys a	**1.92**	**1.66-2.23**	**0**	1.2	0.95-1.50	0.121	0.96	0.64-1.51	0.835
XRCC3 241 Thr/Met:Thr/Thr b	**0.74**	**0.62-0.89**	**0.001**	**2.45**	**1.05-7.93**	**0.044**	0.91	0.76-1.11	0.331
XRCC1 280 Arg/His:His/His c	**1.28**	**1.10-1.49**	**0.001**	1.09	0.86-1.38	0.470	1.10	0.90-1.34	0.357
XRCC1 194 Arg/Trp:Trp/Trp d	0.97	0.85-1.10	0.598	1.01	0.81-1.26	0.937	**0.73**	**0.63-0.86**	**0**
XRCC1 399 Arg/Gln:Gln/Gln e	1.07	0.95-1.21	0.279	1.15	0.93-1.44	0.200	1.12	0.96-1.30	0.140
GSTM1 Null f	**1.62**	**1.43-1.84**	**0**	1.14	0.91-1.43	0.237	**1.44**	**1.22-1.72**	**0**
GSTT1 Null g	1.08	0.94-1.26	0.284	0.93	0.73-1.19	0.540	1.16	0.97-1.40	0.101

CI: Confident intervals; *Bold: For statistically significant level. $p<0.05$; a Data significant in relation to genotype *OGG1 Ser/Ser* individuals; b Data significant in relation to genotype *XRCC3 241 Met/Met* individuals; c Data significant in relation to genotype *XRCC1 280 Arg/Arg* individuals; d Data significant in relation to genotype *XRCC1 194 Arg/Arg* individuals; e Data significant in relation to genotype *XRCC1 399 Arg/Arg* individuals; f Data significant in relation to genotype *GSTM1 (+)* individuals; g Data significant in relation to genotype *GSTT1 (+)* individuals.

4. Discussion

Exposure to welding fume can cause a great number of illnesses and health complications (Anand et al., 2014; Pesch et al., 2019), in fact the International Agency for Research on Cancer (IARC) has classified welding fumes as Group 1 carcinogens (IARC, 2017). It has been described that welding fumes are a varied mixture of airborne gases and fine, ultrafine and nanoparticles (Riccelli et al., 2020). Compositional analysis revealed Fe, Mn, and Si, associated with particles smaller than 2 μm; these particles have the ability to adhere to each other and form agglomerates. It is known that the particles containing these metals can generate oxidative DNA damage, cytotoxic effects, cell death and inflammatory processes in the lung (León-Mejía et al., 2020; Valavanidis et al., 2013). These nanoparticles constitute the greatest health hazard for welders due to their ability to penetrate deep into the lungs and are not easily removed by the cilia of the respiratory tract (Geiser and Kreyling, 2010; Valavanidis et al., 2013). The composition of the mix depends on the welding method and the products being welded (Kazi et al., 2021), and multiple components that are generated during process are considered carcinogenic to humans (IARC, 2017).

In this sense, genetic polymorphisms can be very useful to establish the relationship between individual susceptibility, genotoxic risk and the development of diseases such as cancer (Dylawerska et al., 2017; Wright, 2005). The XRCC1 gene is located on chromosome 19q13.2 and encodes a protein considered a central factor in the BER pathway (Campalans et al., 2005). In our study demonstrated be related with DNA damage (MN, NPB) and cell death (APOP, NECR). The XRCC1 protein has an important function in the repair of single strand breaks in mammalian cells, due to its ability to interact with multiple enzymatic components in repair reactions. Within the BER pathway, it is proposed that it interacts with PARP and DNA ligase III in the recognition and binding of single strand breaks and that in the ternary complex of DNA-XRCC1-Polβ it acts as an "anchor protein" (Campalans et al., 2005; Hanssen-Bauer et al., 2012). There are three variants of the XRCC1 gene (point mutants) *Arg194Trp*, *Arg280His* and *Arg399Gln*, which correspond to amino acid substitutions

(Huang et al., 2009). *Arg194Trp* and *Arg280His* lie between the binding domains of Polβ and PARP, while *Arg399Gln* is located in proximity to the binding domain of PARP (Hanssen-Bauer et al., 2012). These substitutions can alter the function of the XRCC1 protein and obviously affect DNA repair capacity (Huang et al., 2009); in fact, it has been associated with the development of cancer of the lung (Feng et al., 2014; Pachouri et al., 2007), bladder (Wang et al., 2010), breast (Isakova et al., 2017), esophagus (Li et al., 2013) and skin (Chiyomaru et al., 2012). Our study demonstrated *XRCC1 399 Gln/-* as a risk genotype for welders in the formation of NPB, and *XRCC1 194 Trp/-* in cell death. Iarmacovai et al. (2005) similarly demonstrated that *XRCC1* variant allele coding *Gln* amino acid at position 399 associated with a higher number of DNA breaks. Analysis of NPB formation can be a very important biomarker of the effects of occupational exposure, since NPB are formed by poor repair following a DSB, or may be chromosome end fusion when telomeres become shortened and/or dysfunctional (Fenech, 2007; Fenech et al., 2011). Significant NPB formation has been also associated with cancer risk (León-Mejía et al., 2019; Gashi et al., 2018). The study of these polymorphisms and their relationship with occupational exposure to metals has been of great relevance for understanding the carcinogenic effects in exposed welders (Iarmarcovai et al., 2005; Ko et al., 2017).

XRCC3 DNA repair protein is a protein that in humans is encoded by the XRCC3 gene. The XRCC3 gene is located on chromosome 19q13.3. The XRCC3 protein is involved in the DSB (double-strand breaks), HRR pathway and cross-link repair in mammalian cells and interacts directly with RAD51, stabilizing it (Pierce et al., 1999). In this study, individuals exposed to welding fumes with the *XRCC3 241 Thr/-* demonstrated increased frequency of MN, showing be a risk genotype for this biomarker. A single nucleotide polymorphism leads to change from C to T and to change from the amino acid Thr to Met at codon 241 (Hsiao et al., 2018; Nissar et al., 2014). This single nucleotide polymorphism can affect the function of the enzyme and/or its interaction with other enzymes involved in DNA damage and repair (Nissar et al., 2014). Several studies show a positive association of the *XRCC3 Thr241Met* polymorphism with lung (Bei et al., 2015), prostate (Xuan et al., 2015) and colorectal

cancer (Krupa et al., 2011). Interestingly, some studies demonstrate the association of these polymorphisms and the effects of metal-containing particles (León-Mejía et al., 2020; Mateuca et al., 2007).

When comparing the genotypes of the unexposed and the exposed and their association with the levels of damage and cell death, it is notable to observe that the exposed show significant differences. DNA oxidative damage is processed through the BER pathway, including XRCC1, XRCC3 and 8-oxoguanine DNA glycosylase 1 (OGG1). XRCC1 and XRCC3, which play an important part in the BER pathway of DNA and in the double strand break repair pathway, respectively, but is OGG1 the essential protein involved in oxidative stress-induced DNA demethylation. First, the damaged base is recognized and excised by OGG1, leaving an apurinic site (AP-site), afterwards, these AP sites are eliminated by the action of an AP-lyase and end processing through flap-endonuclease 1 (FEN1) and later the base is replaced (Wang et al., 2018). If this type of process does not occur properly, these AP sites can generate genomic instability and compromise transcription, DNA replication, and telomere maintenance (Simonelli, 2005). Or even, the damage to the DNA can be maintained and the damage can be so aggressive that the cell death process is triggered (León-Mejía et al., 2019). In concordance with these findings, our results, when considered the entire population, demonstrated that the *OGG1 Cys/-* may be a risk genotype for DNA damage and death cell.

Much of the existing information on the effects of Glutathione S-transferase (GST) polymorphisms on individual susceptibility to chemical carcinogens is related to the isoenzymes GSTM1 (Glutathione S-transferases M1) and GSTT1 (Glutathione S-transferases T1) (Buzio, 2003; Safarinejad et al., 2013). In this study, individuals exposed to welding fumes with the *GSTM1 null* genotype demonstrated a significantly higher frequency of MN and NECR than non-null; and *GSTT1 null* genotype demonstrated a significantly higher frequency of APOP than non-null. In addition, in this study related to non-exposed individuals both null genotypes demonstrated be a risk factor for NPB and APOP. These polymorphic enzymes participate in the metabolism of a wide range of carcinogens, are responsible for glutathione conjugation, and can detoxify various reactive oxygen species (Buzio,

2003; Safarinejad et al., 2013). The *GSTM1* and *GSTT1* genes show high variability in humans. Due to a homozygous deletion, about 50% of the Caucasian population have a null genotype (no enzyme activity) for the *GSTM1* gene, while 13-28% of Caucasians lack the *GSTT1* gene (null for the GSTT1 gene) (Piacentini et al., 2011). Some authors show that the *GSTM1 null* genotype and *GSTT1 null* is associated with an increased risk of lung (Piao et al., 2013), skin (Rinck-Junior et al., 2019), bladder (Berber et al., 2013) and colon cancer (Klusek et al., 2018). The association of these genotypes and the genotoxic effects of exposure to metals, such as those found in our study, has also been described (Coelho et al., 2013; Iarmarcovai et al., 2005; León-Mejía et al., 2020). Similarly, *GSTM1* and *GSTT1 null* genotypes have been associated with increased inflammatory processes derived from exposure to particulate matter, demonstrating the risk of cardiovascular and pulmonary diseases (Chahine et al., 2007; Ritambhara et al., 2019; Schwartz et al., 2005).

In conclusion, the mixture of components that are generated during welding constitute for gases such as ozone, nitrogen dioxide, carbon monoxide and metallic fumes including Fe, Mn, Si, leads to significant DNA damage and cell death processes which can be influenced by inter-individual variations in DNA repair processes and can increase the risk of some types of cancer in exposed workers. Therefore, an effective biological surveillance of these workers is very important, taking into consideration the understanding of the mechanisms involved in the development of occupational pathologies typical of these individuals.

Acknowledgment

We cordially thank the welders from Monteria city who participated in this study. This study was supported by COLCIENCIAS Grant (657/125365741083/2014) and Universidad Simón Bolívar, Barranquilla, Colombia.

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CONSIDERAÇÕES FINAIS

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A população mundial estimada de soldadores profissionais é de 11 milhões, e é provável que cerca de 110 milhões de trabalhadores adicionais incorram em exposições relacionadas à soldagem (Guha et al., 2017). A capacidade tóxica dos fumos de solda tem sido objeto de estudos devido a seus componentes perigosos, incluindo metais e seus óxidos, liberados durante a prática deste ofício (CCOHS 2016; Rana et al., 2019a). Com base em numerosos estudos e experimentos, o IARC determinou que há evidências suficientes para classificar alguns dos compostos de fumos de solda como carcinogênicos para humanos (Grupo 1) (IARC, 2018). Gustavsson (2020) prevê uma possível epidemia silenciosa na União Europeia (EU) se os limites de exposição ocupacional não protegerem suficientemente a saúde individual dos trabalhadores. Os limites atuais de exposição da UE são de 0,1 mg/m^3, entretanto, a Administração de Segurança e Saúde Ocupacional dos EUA (OSHA) chegou a uma conclusão diferente e decidiu que 0,05 mg/m^3 como o limite mais apropriado para a proteção individual.

De acordo com o que foi exposto no parágrafo anterior, o grupo de trabalhadores dos países do terceiro mundo é, claro, e o grupo que faz parte deste estudo, integrará inevitavelmente as estatísticas como vítimas da epidemia silenciosa, enquanto políticas de segurança para este tema na medicina do trabalho não forem concebidas pelas autoridades competentes.

No capítulo I da presente investigação, mostramos as evidências de genotoxicidade e citotoxicidade no grupo de soldadores, causadas pela exposição às complexas misturas de fumos de solda. A expressão evidente dos biomarcadores de genotoxicidade, citotoxicidade e morte celular, detectada pela aplicação do ensaio CBMN-Cyt, foi inequívoca, revelando o aumento de todos os biomarcadores, incluindo aumento no tamanho telomérico. Também observamos a presença no sangue e na urina de 17 elementos inorgânicos, entre eles os metais pesados Cr, Cu, Ni, Zn.

A exposição prolongada a metais, tais como Ni, Al, Cr e Cu, também encontrada em nossas amostras e sua correlação com danos ao DNA, está associada à genotoxicidade e carcinogênese (Magaye et al., 2012; Willhite et al., 2014; IARC, 2018; El Safty et al., 2018; Guo et al., 2019). Os metais podem interagir diretamente com o DNA, alterar a replicação e causar danos, modificações na base do tipo purificação, reticulação inter e intramolecular entre DNA e proteínas, rearranjos cromossômicos e indução de quebras de uma única fita. Outras alterações associadas à interação de metais com o DNA são a ativação de proto-oncogenes, inativação de genes supressores de tumores, alterações epigenéticas, alteração de sequência, mutações pontuais e aberrações cromossômicas nas células. As misturas complexas representam uma exposição prejudicial. Assim, os soldadores representam um grupo de alto risco para o câncer e doenças neurodegenerativas (Al-Lozi et al., 2017; Falcone et al., 2018; Yang et al., 2018; Michael et al., 2019; Pesch et al., 2019; Rana et al., 2019a).

Nossos resultados mostraram ainda uma correlação significativa entre a frequência do MN e o aumento da concentração de Mg, S, Fe e Cu no sangue periférico, e Cr, Fe, Ni e Cu na urina, o que evidencia uma exposição prejudicial, e representa para os soldadores um alto risco de câncer e doenças neurodegenerativas (Al-Lozi et al., 2017; Falcone et al., 2018; Yang et al., 2018; Michalek et al., 2019; Pesch et al., 2019; Rana et al., 2019a). Outras alterações, associadas à interação de metais com o DNA, são a ativação de proto-oncogenes, inativação de genes supressores de tumores, alterações epigenéticas, alteração de sequência, mutações pontuais e aberrações cromossômicas nas células; o dano ocorre principalmente, pela indução de alterações e modificações oxidativas (Angel'e-Martinez et al., 2014; Morales et al., 2016). Em contraste, a inflamação induzida por partículas nos pulmões demonstrou desencadear a liberação de espécies reativas de oxigênio (ERO) por macrófagos. Os metais de transição na superfície das partículas também podem gerar ERO através da reação de Fenton e levar a danos oxidativos ao DNA (Vidrio et al., 2008).

Também encontramos um aumento significativo do comprimento do telômero no grupo exposto em comparação com o grupo não exposto. O aumento do comprimento dos telômeros foi descrito em diferentes tipos de tumores, incluindo gliomas, que têm a maior taxa de telômeros mais longos, demonstrando uma elevada atividade telomérica (Barthel et al., 2017). Do mesmo modo, encontramos uma correlação significativa entre a frequência de MN e o comprimento dos telômeros. Diferentes estudos têm associado alterações nos telômeros com os efeitos dos fumos de solda (Wong et al., 2014; Li et al., 2015; Shoeb et al., 2020, 2017). De fato, encontramos uma correlação significativa da idade e dos anos de exposição com o comprimento dos telômeros. O comprimento telomérico e os processos de dano ao DNA estão relacionados com a progressão do envelhecimento. Alterações no comprimento do telômero e na instabilidade genética podem ser afetadas pela acumulação de danos no DNA e stresse oxidativo (Kahl et al., 2018; Ko et al., 2017).

É importante considerar alguns aspectos que caracterizam a natureza dos fumos, tais como sua composição, taxa de emissão componentes do metal utilizado para soldagem, revestimentos, tipos de eletrodos, entre outros, que também fazem parte dos impactos (Balkhyour e Goknil, 2010). Neste sentido, deve-se considerar que a natureza das misturas complexas é determinada pelo tipo de elementos que a compõem; como é conhecido, estes variam de acordo com o tipo de procedimento a ser utilizado e o material a ser consumido. Nos processos de soldagem, são produzidas micropartículas e nanopartículas, algumas capazes de alcançar os alvéolos e depositar neles, favorecendo o aparecimento de patologias graves como fibrose intersticial e, eventualmente, doenças respiratórias como bronquite, irritação das vias aéreas, alterações na função pulmonar (Antonini, 2003), siderose (Patel et al, 2009), asma (Storaas et al, 2015), COPD (Ithnin et al., 2019) e câncer de pulmão (Honaryar et al., 2019; Wong et al., 2017). O resultado da análise feita por SEM-EDS revelou a presença de Fe, Mn, P, Si, K, e partículas menores que $PM_{2.5}$, ou seja, partículas menores que 2 µm e nanopartículas, formando aglomerados que têm profundas conotações biológicas, devido à capacidade das nanopartículas de atravessar a barreira hematoencefálica e penetrar no cérebro. A exposição a

pequenas partículas, incluindo fumos de solda, também é geralmente associada a infarto agudo do miocárdio e outras doenças isquêmicas do coração (Wiebert et al, 2012; Mocevic et al., 2015; Sjögren et al., 2002). Além disso, a poluição do ar, incluindo a matéria particulada que pode alcançar o cérebro, tem sido ligada a doenças do sistema nervoso central, como a doença de Parkinson e os acidentes vascular cerebral (Block 2009). Os fumos de soldagem são compostos de gases e partículas potencialmente perigosas, que muitas vezes são do tamanho de nanômetros (Floros, 2018).

A exposição de longo prazo ao PM está ligada ao aumento da mortalidade e da incidência de câncer de pulmão (Pope et al., 2002; Badyda et al., 2016; Chen et al., 2016a; Consonni et al., 2018; Turner et al., 2020), e com base em evidências científicas, a Agência Internacional de Pesquisa do Câncer (IARC) declarou a poluição do ar, incluindo o PM, como carcinógeno humano (Loomis et al., 2014). Até hoje, sabe-se que o PM induz especificamente efeitos biológicos, incluindo processos inflamatórios, estresse oxidativo e genotoxicidade (Sanchez-Perez et al., 2009; Li et al., 2018; Shao et al., 2018), que podem começar com danos diretos ou indiretos ao DNA, e este processo é considerado um evento inicial no curso da carcinogênese, levando a mudanças na expressão gênica, mas também a mutações se o DNA não for reparado corretamente (Hoeijmakers, 2001). Assim, avaliar os polimorfismos nos genes de reparo de DNA poderiam auxiliar na previsão de risco em indivíduos expostos a PM.

Além disso, na análise da influência dos polimorfismos dos genes OGG1 Ser326Cys, XRCC1 Arg280His, XRCC1 Arg194Thr, XRCC1 Arg399Gln, XRCC3 Thr241Met, GSTM1e GSTT1 sobre os danos ao DNA de 98 indivíduos profissionalmente a fumos de solda e 100 indivíduos não expostos. Todas essas variantes alélicas estavam de acordo com os valores da literatura para as populações colombianas [Hoyos-Giraldo et al., 2009], brasileiras e caucasianas (Garte, 2001). Nos resultados foram encontrados que indivíduos expostos a fumos de solda com o genótipo nulo *XRCC3 Thr241Thr, XRCC3 Thr241Met* e *GSTM1* demonstrou uma frequência significativamente mais elevada de micronúcleos em

linfócitos binucleados. Por outro lado, os indivíduos com genótipo *XRCC1 Arg399Gln* e *XRCC1 Gln399Gln* apresentaram níveis significativos de pontes nucleoplasmáticas. O genótipo nulo *OGG1 326 Ser/Cys, OGG1 326 Cys/Cys, XRCC1 194Arg/Thr, XRCC1 194Thr/Thr* e GSTT1 exibia valores apoptóticos significativamente mais elevados. Também, portadores de genótipo nulo XRCC1 194Arg/Trp, XRCC1 194Thr/Thr e GSTM1 tinham níveis necróticos mais elevados em comparação com portadores não-nulos XRCC1 194Arg/Arg e GSTM1.

Neste estudo, os indivíduos com o genótipo nulo da GSTM1 demostraram uma frequência significativamente mais alta de MN e NECR do que os não-nulos; e o genótipo nulo da GSTT1 demonstrou uma frequência significativamente mais alta de APOP do que os não-nulos. Além disso, neste estudo relacionado aos indivíduos não-nulos, ambos os genótipos nulos demonstraram ter um fator de risco para NPB e APOP. É importante notar que os genes GSTT1 (codificando o glutationa S-transferase T1 isozymes) e GSTM1 (codificando o glutationa S-transferase M1 isozymes) estão envolvidos no metabolismo de numerosos carcinógenos e ROS com efeitos prejudiciais às células de alguns órgãos diretamente envolvidos no metabolismo (Rohr et al., 2011), mas sua expressão varia substancialmente entre indivíduos. O genótipo nulo indica que as respectivas enzimas não são produzidas em indivíduos homozigotos (Halosava et al., 2012). Portanto, é mais provável que sofram danos oxidativos muito relacionados às doenças pulmonares (Qu et al., 2005. Iarmarcovai et al., 200). De fato, o genótipo nulo do GSTT1 tem sido encontrado com mais frequência em pacientes com linfoma (Yang et al., 2014), câncer de bexiga (Safarinejad et al., 2013), câncer de pulmão (Minina et al., 2017) e câncer colorretal (Djansugurova et al., 2015).

A partir dos nossos resultados, foi possível concluir que a população de soldadores deste estudo está em risco iminente de saúde ocupacional, como resultado da exposição a misturas complexas sob a forma de aerossóis constituídos por micro e nanopartículas que, devido às suas propriedades conhecidas, são capazes de atravessar a barreira hematoencefálica, atingindo e impactando os órgãos mais importantes do corpo humano e causando danos. A circulação de

metais pesados como Mn, Cr e Cd no ambiente de trabalho dos soldadores, e sua posterior detecção, em altas concentrações, no sangue e na urina dos expostos, demonstra de forma certa o risco permanente à saúde desse grupo de pessoas. Esta pesquisa demonstra conclusivamente a validade e eficácia da CBMNC-Cyt como ferramenta eficaz para detectar e avaliar danos ao DNA, e também danos celulares como APOP, NECR, NBS, NBUD, CC, BN alongamento de telômeros, provavelmente causadas pela exposição a misturas complexas contida no aerossol originado pela atividade da solda. E estes efeitos podem ser influenciados por variações genotípicas interindividuais e exercer influência nos processos de reparo do DNA, o que também favoreceria processos carcinogênicos em trabalhadores expostos. Por estes motivos, é aconselhável empreender políticas de biossegurança em trabalhadores expostos.

Os resultados deste trabalho aumentam o conhecimento atual sobre os efeitos desse tipo de exposição, e sugerem o lançamento de mais estudos que enfatizem o impacto das micro e nanopartículas associadas à exposição aos metais em humanos. Estes resultados serão úteis para sua difusão entre os gerentes das oficinas, departamentos de saúde da cidade, para seu conhecimento e socialização entre trabalhadores e cidadãos em geral com o objetivo de sensibilizá-los sobre o uso e a importância das medidas de biossegurança. É, portanto, imperativo que as políticas estatais sejam estabelecidas, com programas de monitoramento abrangente para estes trabalhadores, comuma vigilância eficaz e incluindo a biomonitorização em intervalos de tempo acordados, dependendo de anos de serviço do trabalhador.

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