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INDICADORES DE ESTRESSE OXIDATIVO E CONSUMO DE VITAMINAS ANTIOXIDANTES ASSOCIADOS À INFECÇÃO PELO HPV E CITOLOGIA CERVICAL DE UM GRUPO DE MULHERES

FRANCISCO BELTRÃO – PR
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LISTA DE ABREVIATURAS E SIGLAS

HPV – Papillomavírus Humano
IST – Infecção Sexualmente Transmissível
ASC-US – Células Escamosas Atípicas de Significado Indeterminado, possivelmente não neoplásicas
LSIL – Lesão Intraepitelial de Baixo Grau
HSIL – Lesão Intraepitelial de Alto Grau
EROS – Espécies Reativas de Oxigênio
NIC – Neoplasia Intraepitelial Cervical
EO – Estresse Oxidativo
ORFs – *Open Reading Frames*
OMS – Organização Mundial da Saúde
IARC – Agência Internacional de Pesquisa sobre o Câncer
ASC-H – Células Escamosas Atípicas de Significado Indeterminado, não podendo excluir HSIL
AGC-US – Células Glandulares Atípicas de Significado Indeterminado, possivelmente não neoplásicas
AGC-H – Células Glandulares Atípicas de Significado Indeterminado, quando não se pode excluir HSIL
AIS – Adenocarcinoma in situ
SOD – Superóxido Dismutase
GSH – Glutationas
CEONC – Centro de Oncologia
CEP – Comitê de Ética em Pesquisa
ABEP – Associação Brasileira de Empresas de Pesquisa
QFA – Questionário de Frequência Alimentar
IMC – Índice de Massa Corporal
PCR – Reação da Polimerase me Cadeia
QL – Quimioluminescência
TRAP – Capacidade Antioxidante Total

Indicadores de estresse oxidativo e consumo de vitaminas antioxidantes associados à infecção pelo HPV e citologia cervical de um grupo de mulheres

Resumo

A maioria das infecções desencadeadas pelo Papilomavírus humano (HPV) é eliminada espontaneamente pelo sistema imune. Contudo, parte delas apresentam carga viral alta e a persistência dos subtipos oncogênicos do vírus são fatores decisivos para progressão de lesões pré-cancerosas. Entre os fatores que contribuem para essa condição está a capacidade viral de intervir em processos que elevam a produção de espécies reativas de oxigênio (EROS). No organismo, a produção de EROS é equilibrada através de sistemas de enzimas antioxidantes e demais moléculas antioxidantes que auxiliam nesse balanço. O presente estudo investigou a associação entre os níveis plasmáticos de lipoperóxidos e a capacidade antioxidante total plasmática com consumo de vitaminas A, C e E em mulheres atendidas em um centro especializado de oncologia. Ao todo 113 mulheres com desfechos positivos e negativos à infecção por HPV e para o Papanicolau responderam questionários contendo dados socioeconômicos, de comportamento sexual, consumo alimentar e hábitos de vida. Também foram submetidas à coleta para o exame Papanicolau, para detecção do HPV e de sangue para determinar a lipoperoxidação e a capacidade antioxidante total. Foram utilizados os softwares R e XLSTAT® para as análises estatísticas. Além da frequência, mediana, média e desvio padrão, conforme o tipo de variável foram utilizados teste de Mann-Whitney ou Qui-quadrado com posterior regressão logística. Ainda, foram determinadas correlações entre lipoperoxidação, capacidade antioxidante plasmática total e desfechos do Papanicolau, HPV e do consumo de vitaminas, seguindo para Análise da Variância Permutacional para averiguar interações dentre elas. A prevalência do HPV foi de 8% e de alterações cervicais de 5,3%. Os resultados apontam para chance aumentada à infecção para ex-fumantes (OR: 5,07; IC95%: 0,9388 – 27,3559; p=0,0592) e às que usufruem de anticoncepcional (OR=6,6923; IC95%: 0,7643 – 58,5999; p=0,0860). Os níveis plasmáticos de lipoperoxidação foram mais elevados em mulheres sem alterações

cervicais (M_d: 462400; IIQ: 394642 – 535365; p=0,015) e naquelas com HPV (M_d: 876855; IIQ: 589295 – 1031243; p=0,077). A capacidade antioxidante total revelou correlação com o consumo de chimarrão (R^2_S = 0,196; p= 0,038) e com os níveis de lipoperóxidos ($R^2_{Pearson}$ = -0,262; p = 0,005). Entretanto, nenhuma interação foi observada entre os indicativos de EO e as vitaminas A, C e E para os desfechos da Infecção Sexualmente Transmissível (IST) e do Papanicolau. Por fim, a busca por informações sobre a relação entre estresse oxidativo, potencial antioxidante e consumo de vitaminas em indivíduos soropositivos para o HPV ou com distúrbios cervicais é escassa, portanto, sugere-se que novos estudos sejam desenvolvidos para esclarecer a relação ou interação entre eles.

Palavras-chave: IST; Displasia do colo uterino; Oxirredução; Vitaminas.

Indicators of oxidative stress and consumption of antioxidant vitamins associated with HPV infection and cervical cytology in a group of women

Abstract

Most infections triggered by Human Papillomavirus (HPV) are spontaneously eliminated by the immune system. However, part of them have a high viral load and the persistence of the virus oncogenic subtypes are decisive factors for the progression of precancerous lesions. Among the factors that contribute to this condition is the viral ability to intervene in processes that uplift the production of reactive oxygen species (ROS). In the organism, the production of ROS is balanced through the systems of antioxidant enzymes and other antioxidant molecules that assist in this balance. The current study investigated the association between the lipoperoxide plasma levels and the total antioxidant capacity with consumption of vitamins A, C, and E in women from a specialized oncology center. Altogether 113 women with positive and negative outcomes of HPV infection and Pap smear answered questionnaires with socioeconomic data, sexual behavior, food consumption and lifestyle habits. They also underwent the collection for the Pap smear, detection of HPV, and blood tests to determine the lipoperoxidation and the total antioxidant capacity. The XLSTAT-R® and R software were used for statistical analysis. In addition to the mean, median, and standard deviation frequency, according to the type of variable, the Mann-Whitney or Chi-square test were used with subsequent logistic regression. In addition, correlations among lipoperoxidation, total plasma antioxidant capacity and outcomes of Pap smear, HPV and vitamin consumption were determined, leading to the Permutational Analysis of Variance to investigate interactions among them. The prevalence of HPV was 8% and cervical changes were 5.3%. The results point to an increased chance of infection for ex-smokers (OR: 5.07; 95% CI: 0.9388 - 27.3559; p = 0.0592) and for those who use contraceptives (OR = 6.6923; CI95 %: 0.7643 - 58.5999; p = 0.0860). Plasma levels of lipoperoxidation were higher in women without cervical changes (Md: 462400; IIQ: 394642 - 535365; p = 0.015) and in those with HPV (Md: 876855; IIQ: 589295 - 1031243; p = 0.077). The total antioxidant capacity exposed a correlation with the consumption of mate (R2S = 0.196; p = 0.038) and with the

levels of lipoperoxides (R2Pearson = -0.262; p = 0.005). However, no interaction was observed between oxidative stress (OS) indicatives and vitamins A, C and E for sexually transmitted infection (STI) and Pap smear outcomes. Lastly, the search for information on the relationship between oxidative stress, antioxidant potential and the consumption of vitamins in individuals who are HPV seropositive or with cervical disorders is scarce, therefore, it is suggested that further studies develop to clarify the relationship or interaction between them.

Keywords: STI. Cervical Dysplasia. Lipoperoxides. Vitamins.

SUMÁRIO

1. INTRODUÇÃO GERAL

O Papilomavírus Humano (HPV) é um dos agentes etiológicos mais importante e valioso para o prognóstico do câncer de colo de útero no mundo (SAIT et al., 2019). É um vírus epiteliotrópico capaz de infectar a pele e mucosas de ambos os sexos (TRISTÃO et al., 2012) e é reconhecido como uma das infecções sexualmente transmissíveis (IST) mais comum (CEZAR-DOS-SANTOS et al., 2019). Dos mais de 200 subtipos de HPV, 20 são de alto risco oncogênico com destaque para os subtipos 16 e 18 (STANLEY, 2010; DOORSLAER et al., 2013; BRASIL, 2015; EGAWA et al., 2015; KRESS et al., 2015; CHOI, PARK, 2016). Contudo, 80% das infecções pelo HPV são subclínicas, transitórias e assintomáticas sendo eliminadas, na maioria das vezes, espontaneamente pelo sistema imune ou apresentam uma baixa carga viral restringindo sua identificação (NARISAWA-SAITO, 2007).

Apesar disso, a prevalência do vírus ainda é alta, em torno de 12% na população e se intensifica conforme a severidade da lesão (COLPANI et al., 2016). Guan et al. (2012) verificou a frequência do HPV em 52% das alterações citopatológicas definidas como células escamosas atípicas de significado indeterminado, possivelmente não neoplásicas (ASC-US), 74% nas lesões intraepiteliais de baixo grau (LSIL) e 88,9% nas lesões intraepiteliais de alto grau (HSIL) com chances ampliadas para progressão do câncer (STANLEY et al., 2010; DOORSLAER et al., 2013; BRASIL, 2015; CHOI, PARK, 2016). Além do vírus exercer forte influência no estabelecimento das lesões cervicais e da carcinogênese outros fatores podem ser determinantes nesses processos. Dentre eles estão o componente genético, a resposta imunológica do indivíduo com ênfase ao microambiente cervical, as coinfecções por outras IST, o comportamento sexual, o uso de tabaco, a dieta e o estresse oxidativo (EO) à nível sistêmico (BOSCH et al., 2013; RICHARD et al., 2015; BERTI et al., 2017; NAYKI et al., 2017).

Levando em consideração o componente dietético, como potenciais substâncias preventivas para o câncer cervical estão as vitaminas A, C e a E, as quais atuam como antioxidantes em sistemas biológicos (BARCHITTA et al., 2018). Os mecanismos pelos quais elas agem envolve a formação, ação e reparo de danos causados pelas espécies reativas de oxigênio e nitrogênio (ERONS) (FEDRIZZI, 2010; ROCK, MICHAEL, REYNOLDS, RUFFIN, 2004; MARTELLI,

NUNES, 2014). Em particular, as espécies reativas de oxigênio (EROS), quando exacerbadas podem caracterizar o estado de EO. As EROS são constantemente produzidas durante o metabolismo aeróbico das células, inclusive durante as infecções e inflamações (POLJSAK, SUPUT, MILISAV, 2013). Essa condição pode se instituir com a IST causada pelo HPV à nível de microambiente (GUO et al., 2015). A presença viral desencadeia uma resposta imunológica tanto pela sua entrada quanto pelas patologias decorrentes dele. Ainda, uma das condições de EO é originada pelo aumento de EROs somada a capacidade antioxidante reduzida da célula (POLJSAK, SUPUT, MILISAV, 2013). Alguns estudos afirmam que pacientes infectados com HPV de alto risco, com neoplasia intraepitelial cervical (NIC) e carcinoma uterino possuem alterações na peroxidação lípidica e comprometimento dos sistemas antioxidantes celulares, devido ao uso exacerbado de suas fontes, situação peculiar do EO (LOOI et al., 2008; KIM et al., 2010; NAYKI et al., 2017).

Segundo Myles (2014), as vitaminas antioxidantes possuem papel fundamental na modulação da resposta imune perante a presença e a persistência da infecção pelo HPV, impedindo à progressão do câncer invasivo. Maus hábitos alimentares são cofatores nutricionais que contribuem de 20% a 60% para a carcinogênese no mundo (JIA et al., 2012), assim como, o EO e a inflamação crônica (DE MARCO et al., 2012). Logo, as vitaminas antioxidantes podem atuar neutralizando a ação dos radicais livres minimiza a vulnerabilidade à infecção pelo vírus (GEORGESCU et al., 2018). Muitos trabalhos têm priorizado compreender a relação entre o EO e a infecção pelo HPV e a conexão com a carcinogênese. Porém, são escassos aqueles que abordam a relação entre o consumo alimentar, EO, alterações cervicais e a infecção do HPV. Deste modo, uma temática envolvendo uma associação entre eles pode redimensionar algumas condutas à saúde da mulher, sugerindo novas intervenções à prevenção da IST causada pelo HPV e à sua evolução patológica.

1.1 Papilomavírus Humano (HPV): genoma e aspectos epidemiológicos

O HPV é um vírus pertencente à família Papilomaviridae, o qual compreende 16 gêneros diferentes, sendo o α-Papillomavírus o responsável pela infecção nos seres humanos (FEDRIZZI, 2010). O vírus possui um genoma composto por uma

única molécula de DNA circular de aproximadamente 8kb, onde estão distribuídas pelo menos oito regiões gênicas importantes com suas respectivas fases de leitura aberta, as ORFs (*Open Reading Frames*). Duas delas denominadas de tardias (do inglês “late”) as L1 e L2 e seis regiões de síntese precoce (do inglês “early”), respectivos genes E1, E2, E4, E5, E6 e E7 (EL-ALIANI et al., 2017; WEELE et al., 2019). As ORFs de L1 e L2 codificam as proteínas estruturais que formam o capsídeo viral (STOLER, 2003; SOUTO, FALHARI, CRUZ, 2005). E aquelas da região precoce codificam proteínas envolvidas na replicação do DNA episomal ou circular (E1 e E2), na regulação da transcrição viral (E2), na maturação viral e alteração da matriz celular (E4) e as E5, E6 e E7 estão envolvidas na transformação celular. Além do que, as duas últimas constituem oncoproteínas virais fundamentais à adaptação anatômica e celular do HPV, na inativação de genes supressores de tumores do hospedeiro e consequente progressão oncogênica (MCLAUGHLIN-DRUBIN, MÜNGER, 2009).

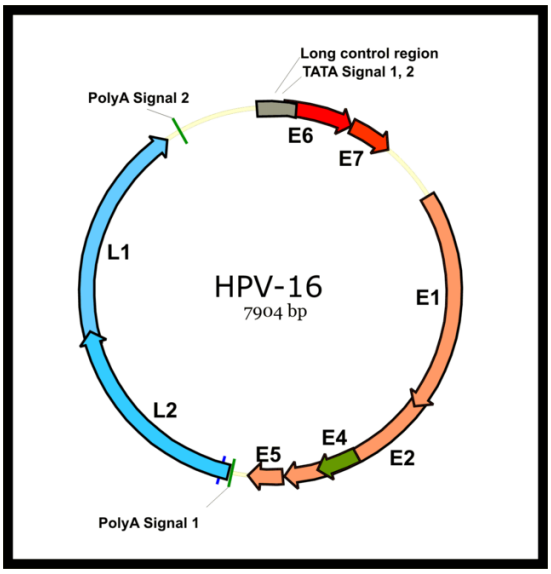

Figura 1 - Representação do genoma do Papilomavírus Humano. Fonte: MUÑOZ et al., 2006.

Apesar de possuir uma estrutura genômica conservada, a região L1 detém mais de 10% da diversidade nucleotídica do HPV, a qual é responsável pelos quase 200 genótipos virais distintos (BASTO et al., 2017). Desse total, 40 subtipos infectam o epitélio do trato ano-genital masculino e feminino (BZHALAVA, EKLUND, DILLNER, 2015). Vinte deles são considerados de alto risco oncogênico, com destaque para os 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 e 82,

fortemente associados ao surgimento de lesões mais graves como as neoplasias intraepiteliais cervicais de graus 2 e 3 (NIC2 e 3), potenciais precursoras do câncer, especialmente, de colo uterino (STANLEY et al., 2010; DOORSLAER et al., 2013; BRASIL, 2015; EGAWA et al., 2015; CHOI, PARK, 2016). Os subtipos de baixo risco são os HPVs 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, associados a condilomas benignos e lesões genitais de baixo grau com reduzida probabilidade de progressão para neoplasias (BRASIL, 2015; EGAWA, STANLEY et al., 2010).

A prevalência global feminina da infecção pelo HPV é de 12% (COLPANI et al., 2016). Segundo a Organização Mundial da Saúde (OMS), os cincos genótipos mais frequentes são os HPV de alto risco 16, 18, 31, 58 e 52 (AYRES, AZEVEDO-SILVA, 2010; OMS, 2012). E, não menos frequente em mulheres com citologia normal, cuja principal prevalência são dos respectivos subtipos, 16 com 3,2%, seguido do 18 (1,4%), 52 (0,9%), 31 (0,8%) e 58 (0,7%) (BRUNI et al., 2010; BOSCH et al., 2013). Especificamente no Brasil, a prevalência de HPV pode chegar a 54,3% na população geral e 38,4% dela se refere aos subtipos de alto risco (BRASIL, 2017).

Além disso, no Brasil destaca-se a presença do vírus mesmo em mulheres com diagnóstico de citologia normal, cuja frequência varia de 2,4% a 55,4%, superior a prevalência em escala global (AYRES, AZEVEDO-SILVA, 2010; WENDLAND et al., 2018). Na cidade de Pelotas, região sul do Brasil, foi verificado prevalência de 29,9% da infecção em mulheres, com 41,3% e 17,3% para os genótipos de alto risco oncogênico, 16 e 18, respectivamente (ENTIAUSPE et al., 2014). No nordeste brasileiro, especificamente em Alagoas, a prevalência do HPV para o sexo feminino se apresentou com 21,55%, pouco inferior à região sul, mas dentro dos subtipos os de alto risco se mantiveram como mais frequente (SANTOS-FILHO et al., 2015). Deste modo, as pesquisas de distribuição do vírus em território nacional, alertam para prevalência de HPV no país.

Os casos de lesões que regridem sem tratamento em um período de até 24 meses ainda ocorrem na maioria da população feminina, decorrente da ação da resposta imunológica do indivíduo. Contudo, ocasionalmente de 10% a 30% das infecções por HPV acabam sendo persistentes e, sem diagnóstico prévio, estão sujeitas a progredir de uma lesão de baixo grau para de alto grau em um curto período de tempo (MCCREDIE et al., 2008; KYRGIOU et al., 2016).

1.2 Infecção pelo HPV e alterações no colo uterino

A Agência Internacional de Pesquisa sobre o Câncer (IARC) estimou cerca de 570 mil novos casos de câncer cervical em todo o mundo para 2018 e mais de 776 mil em 2040. A instituição adverte que o câncer do colo de útero é o segundo tipo mais comum entre as mulheres, especialmente, em países em desenvolvimento e está ranqueado em 4º lugar em número de mortes (WHO, 2012; IARC, 2018). Geograficamente, as regiões de maior incidência dessa neoplasia são Sudeste Asiático, América Latina e África Subsaariana (MARTEL et al., 2017). Aproximadamente 99,7% de todos os cânceres do colo do útero estão associados à presença de algum subtipo de HPV, dentre eles, 70% correspondem aos genótipos 16 e 18 (MARTEL et al., 2017) e, praticamente metade deles são diagnosticados em mulheres com menos de 50 anos (WALBOOMERS et al., 1999; GARLAND et al., 2016).

Após a detecção precoce de uma lesão no colo uterino, a primeira conduta é o tratamento para subsequente eliminação, procedimento de sucesso em mais de 80% dos casos. Contudo, se houver persistência viral pode existir recorrência e progressão da lesão para estágios mais severos, atingindo ápice com o câncer cervical (MARTIN-HIRSCH et al., 2014). A evolução da infecção do HPV à neoplasia cervical é determinada, entre outros, pela expressão de proteínas virais que promovem instabilidade genômica do hospedeiro, alterações no ciclo celular e apoptose (NARISAWA-SAITO, KIYONO, 2007; KINES et al., 2009). Além disso, o vírus é capaz de modular o sistema imune ocasionando a inativação de genes supressores de tumor, tolerância imunológica do tumor e favorecimento da oncogenicidade (TINDLE, 2002; DOORBAR, 2016; KYRGIOU et al., 2016).

As principais alterações encontradas no epitélio cervical, conforme a classificação do Sistema Bethesda (2001) (SOLOMON et al., 2002; WHO, 2013; INCA, 2016) agrupam-se em escamosas e glandulares, com ênfase à citologia. As classes de alterações nas células escamosas são: ASC-US (células escamosas atípicas de significado indeterminado, possivelmente não neoplásicas); ASC-H (células escamosas atípicas de significado indeterminado não podendo excluir HSIL); LSIL (lesão intraepitelial de baixo grau) (compreende o efeito citopático pelo HPV e considera a NIC grau 1); HSIL (lesão intraepitelial de alto grau) (compreende as NIC2 e 3) e Carcinoma Epidermóide Invasor. Para as células glandulares as

categorias são AGC-US (células glandulares atípicas de significado indeterminado, possivelmente não neoplásicas), AGC-H (células glandulares atípicas de significado indeterminado quando não se pode excluir HSIL), AIS (Adenocarcinoma in situ), Adenocarcinoma Invasor (KIM et al., 2010; INCA, 2012; SCHIFFMAN et al., 2016). Uma meta-análise realizada no continente africano, verificou como subtipos mais prevalentes nas lesões do colo uterino os HPV 16, 52,18, 35, 45, 51, 66, 53, 39 e 58 para ASC-US e os HPV 16, 35, 52,18, 53, 56, 58, 51, 45 e 66 para LSIL. Os autores ainda, trazem os genótipos 16,18, 35, 52, 58, 33, 31, 53, 45 e 66 como os mais frequentes nas HSIL, inclusive, para faixa etária de 45 a 54 anos, onde a prevalência da lesão foi de 87,1% (OGEMBO et al., 2015). Estudo realizado no Brasil, por Martins e colaboradores (2016) verificou como subtipos mais comuns nas categorias LSIL e HSIL, os HPV 56 (28,8%) e HPV 16 (37,2%), respectivamente.

Uma outra forma de categorizar as alterações observados no exame Papanicolau remete a uma abordagem histológica e se baseia na classificação de Richart (1967), que estabelece o conceito de neoplasia intraepitelial cervical (NIC), subdividida em três graus 1, 2 e 3 (SELLORS, SANKARANARAYANAN, 2003; STANLEY, 2010; SCHIFFMAN et al., 2011; INCA, 2012). A NIC1 é, também, reconhecida como um diagnóstico histológico de replicação viral benigna, a qual deve ser tratada de forma conservadora, sem procedimentos invasivos (TAINIO et al., 2018). Já a NIC2 possui um curso clínico e comportamento biológico intermediário e, em alguns casos, regridem espontaneamente para NIC1 e, em outros evoluem para NIC3 (KALLIALA et al., 2005; WHO, 2014; TAINIO et al., 2018). Esta última pode ser considerada como uma lesão precursora pré-invasiva de alto potencial oncogênico, apresentando os piores prognósticos dentre as três (MASSAD et al., 2013). Ainda, o tratamento com possível excisão do colo do útero pode ser uma conduta comum tanto à NIC2 quanto a NIC3 (KALLIALA et al., 2005; WHO, 2014; TAINIO et al., 2018).

Em países desenvolvidos, a cada mil mulheres 1,5 são diagnosticadas com NIC2 ou NIC3, anualmente. Esse número amplia para 8,1 na faixa etária de 25 a 29 anos (TORRE et al., 2015). A prevalência de HPV de alto risco normalmente é elevada nas NIC2 e NIC3. Um reflexo disso, foi observado em mulheres tailandesas que receberam diagnostico de NIC2 e 3 com frequência de 64,8% e 90,1%, respectivamente, para os subtipos 16, 58 e 18 (KIETPEERAKOOL, KLEEBKAOW,

SRISOMBOON, 2015). Nesses casos ou com o câncer de colo de útero instalado, o tratamento passa a ser mais agressivo, com mortalidade chegando a 50% (LACEY et al., 2013). A imunoterapia tem apresentado resultados promissores, inclusive para NIC2, extinguindo até 90% das lesões em 24 meses (DISCACCIATI et al., 2011; SIEGEL, MILLER, JEMAL, 2019).

Em contrapartida, é importante destacar que mulheres com citologia normal não estão isentas, obrigatoriamente, da ausência do HPV. Um estudo de meta-anàlise conduzido com mulheres sul-africanas, apresentou a condição descrita com uma prevalência de 57,3% do vírus, dentre eles os subtipos mais frequentes foram o 16 e 18, respectivamente, 9,9% e 5,8% (OGEMBO et al., 2015). Outra situação, foi observada nos laudos citológicos normais de mulheres do sudeste do Brasil, com prevalência dos genótipos HPV 16 (23,2%) e 56 (21,0%) (MARTINS, 2016).

1.3 Estresse oxidativo e antioxidantes

O estresse oxidativo (EO) pode ser definido como o resultado de um desequilíbrio na formação e eliminação de radicais livres ou espécies oxidantes (AZZI, DAVIES, KELLY, 2004). No entanto, os radicais livres são produzidos de forma contínua em processos fisiológicos normais, os quais podem ser mediados pela manutenção do metabolismo ou pelos processos inflamatórios (VELLOSA et al., 2013). Quando ocorre o acúmulo destes radicais, um processo de modificações oxidativas é iniciado e como consequência o organismo é afetado pela disfunção celular (DEVASAGAYAM et al., 2004; BIRBEN, 2012). Logo, com presença de concentrações elevadas de radicais livres não neutralizados e intermediários ativos o organismo entra em estado de EO (SILVA et al., 2018). Essa condição pode alterar o metabolismo aeróbico, interferir na resposta inflamatória e propiciar a proliferação celular anormal, favorecendo a disseminação de qualquer processo infeccioso (BIRBEN et al., 2012).

Conforme mencionado, a oxidação é parte fundamental da vida aeróbica e do metabolismo celular. Existem espécies reativas intermediárias, as quais sob condições fisiológicas são produzidas para atuar na regulação metabólica, no ciclo celular e nas vias de sinalização intracelular (NATHAN, 2003; MARTELLI, NUNES, 2014). Entretanto, alguns fatores endógenos e exógenos podem interferir na produção de radicais livres (OLIVEIRA, SCHOFFEN, 2010). O citocromo P450 na

mitocôndria e os peroxissomos são responsáveis pela produção endógena de radicais livres, os quais levam à formação de espécies reativas de oxigênio (EROS) e espécies reativas de nitrogênio (ERN). Podem ser exemplos de fatores exógenos indutores da produção desses radicais a radiação, o tabagismo, a quimioterapia e a dieta restrita em nutrientes (BHATTACHARYYA et al., 2014).

A homeostase redox diz respeito ao equilíbrio do fluxo de elétrons em reações de redução-oxidação, a qual é estritamente controlada, a não ser que a célula esteja exposta a situações de oxidações extremas. Nestes casos, os antioxidantes ou moléculas de proteção são acionadas para equilibrar a formação e a remoção dos radicais livres (EROS e ERN) (SIES, 1986; HALLIWELL, GUTTERIDGE, 2015). Basicamente, os antioxidantes podem ser definidos como moléculas capazes de retardar ou inibir a oxidação de outro substrato. Os sistemas antioxidantes celulares são divididos em dois grupos, os enzimáticos e os não enzimáticos (VALKO et al., 2007). O primeiro é composto por enzimas produzidas no organismo, sendo as principais, a catalase (CAT), a superóxido dismutase (SOD) e as glutationas (glutationa peroxidase e glutationa-S-transferase). O componente não enzimático é constituído por moléculas normalmente obtidas através da alimentação, como as vitaminas C e E, o ácido lipóico, carotenoides, flavonoides, além de outras substâncias como tripeptídeo endógeno denominado glutationa reduzida (GSH) (SCHMIDT, LISA, 2015).

De modo geral, os antioxidantes podem atuar de forma direta neutralizando a ação dos radicais livres, ou indiretamente, quando participam dos sistemas enzimáticos (GALLAGHER et al., 2009). Ainda, é válido ressaltar que a eficiência dos antioxidantes não enzimáticos in vivo depende tanto da ingestão e biodisponibilidade dos mesmos em condições fisiológicas quanto dos tipos de radicais livres gerados no processo oxidativo (MARAKALA, MALATHI, SHIYASHANKARA, 2012; SILVA et al., 2018).

1.4 HPV e estresse oxidativo

A infecção pelo HPV, seu ciclo de vida e a progressão para neoplasia cervical dependem de vários fatores como subtipo viral, o estado imunológico, a suscetibilidade genética, fatores ambientais e o EO (FOPPOLI, DE MARCO, PERLUIGI, 2015). Quanto ao EO, estudos apontam para um papel importante dele

em diversas patologias, dentre elas à carcinogênese (GOODMAN et al., 2011; DE MARCO, 2013; BREITENBACH, ECKL, 2015). Nesse contexto, o desenvolvimento de lesões cervicais, especialmente, as percursoras do câncer de colo do útero assim como a persistência do HPV podem estar associadas as condições de EO (RIBAS, SUEN, 2013).

Para esclarecer o mecanismo de ação do EO na progressão de neoplasias, é necessário compreender os processos que favorecem a produção de EROS, os quais estão relacionados ao metabolismo celular em casos de inflamações, infecções, estresse mecânico, químico ou câncer (DARR, FRIDOVICH, 1994; SILVA, JASIULIONIS, 2014). A partir da sinalização, proliferação e diferenciação celular, a síntese fisiológica das EROS se inicia e sua produção fica exacerbada, no momento, em que essas moléculas superam a ação dos antioxidantes, caracterizando o estado de EO (VALKO et al., 2007). Quando essa condição persiste, surgem danos oxidativos em lipídios, proteínas e ácidos nucléicos e, como resposta celular geram processos de apoptose, necrose e câncer (CADENAS, 1997; MENDONÇA, CARIOCA, MAIA, 2014).

Alguns estudos sugerem possível cooperação entre o EO e a IST causada pelo HPV, com subsequente evolução para as lesões intraepiteliais e o câncer (VASCONCELOS et al., 2007; PINTO, FUZII, QUARESMA, 2011). Para De Marco (2013) a atividade genotóxica proporcionada pelo EO pode favorecer a instabilidade genômica, que naturalmente, é induzida por alguns subtipos de HPV (16 e 18) durante o ciclo viral, aumentando a probabilidade da integração entre os genomas, progredindo para neoplasia.

Além do EO ter sido proposto como um fator crítico no desencadeamento do câncer do colo do útero (DE MARCO, 2013) é fundamental ressaltar o processo mediado pela expressão das proteínas virais. As oncoproteínas, E6 e E7, se ligam na membrana das células do hospedeiro e modulam algumas proteínas celulares, levando a transformação e imortalização das células. A E6 se associa e inativa as proteínas do domínio p53, as quais detectam alterações no DNA, reparam ou promovem a morte celular (THOMAS, PIN, BANCOS, 1999; HEBNER, LAIMINS, 2006; GHITTONI et al., 2010). A oncoproteína E7 é capaz de inativar a proteína retinoblastoma (pRb), reguladora universal do ciclo celular (BOYER, WAZER, BAND, 1996; BURK, CHEN, DOORSLAER, 2009; GARCIA-TAMAYO, MOLINA, BLASCO-OLAETXEA, 2010).

O controle da transcrição dos genes E6 e E7 ocorre durante o ciclo de vida viral tendo como base a regulação negativa da proteína viral E2, que atua de forma combinada com a proteína E1. A E1 é uma helicase viral dependente de ATP, que se associa à origem da replicação para desenrolar o DNA viral. A proteína E2 tem funções antiproliferativas, promove a inibição do crescimento celular e induz a apoptose, além de suprimir a transcrição dos genes E6 e E7 (DESAINTES et al., 1997; BERGVALL, MELENDY, ARCHAMBAULT, 2013). Ainda, o DNA do HPV é episssomal, mas pode estar integrado ao do hospedeiro (VINOKUROVA et al., 2008; MCBRIDE, 2013). Se assim o for, essa integração irá interferir diretamente na expressão do gene E2, comprometendo sua transcrição, condição que favorece a superexpressão das oncoproteínas E6 e E7 (GARCIA-TAMAYO, MOLINA, BLASCO-OLAETXEA, 2010).

A partir da compreensão do mecanismo de atuação do ciclo viral, alguns estudos passaram a investigar qual seria a sua provável relação com o EO (LAI et al., 2013; WILLIAMS et al., 2014; MARULLO et al., 2015). Estudo realizado por Cruz-Gregorio e colaboradores (2018) descreveu como as proteínas expressas precocemente pelos subtipos de HPV de alto risco podem modular diferencialmente o estado redox. Conforme a pesquisa, nas células infectadas pelo HPV, E1 e E2 causam a diminuição da síntese do antioxidante enzimático SOD e da CAT, por conseguinte, eleva-se a concentração de EROS, que depleciona as glutationas e ocasiona danos ao DNA. Os autores também verificaram que após a integração genômica a E6 aumenta os níveis de EROS e induz danos ao DNA, reduzindo as concentrações de GSH e CAT. Entretanto, enquanto os danos celulares não estão concretizados, observou-se que a ação de E7 diminui juntamente com os níveis de EROS, devido ao aumento da CAT e GSH como forma de proteger o material genético (CRUZ-GREGORIO et al., 2018).

Prioritariamente, os mecanismos que correlacionam o HPV, com o EO e a progressão do câncer do colo do útero, são investigados em infecções persistentes, incluindo os subtipos mais prevalentes nas biópsias neoplásicas, HPV 16 e 18 (BURD, 2003; SYRJÄNEN, 2012). No mais, a compreensão dos mecanismos moleculares relacionados às proteínas do HPV pode orientar a busca de marcadores tumorais confiáveis, permitindo caracterizar melhor a patologia, além de possibilitar a identificação de novos alvos terapêuticos (LIMA, SILVA, RABENHORST, 2013).

1.5 Nutrição, antioxidantes e HPV

O diagnóstico positivo para HPV não significa, necessariamente, que a mulher apresente lesões intraepiteliais cervicais ou neoplásicas (MACHADO et al., 2017). Outros fatores podem interferir no avanço da infecção como, o tabagismo, alta paridade, uso de anticoncepcionais, fatores socioeconômicos e nutricionais (SAMPAIO; ALMEIDA, 2009; GADDUCCI et al., 2011; SCHETTINO, MAININI, AMMATURO, 2018). O fator nutricional potencialmente relacionado à neoplasia cervical envolve o sistema de antioxidantes, cujas vitaminas e minerais, apresentam um papel muito importante na defesa contra as EROS, sintetizadas nos processos enzimáticos, levando a inibição dos mecanismos neoplásicos (LEE et al., 2005; CHIH et al., 2013). Estudos têm sugerido que na história natural do câncer há possibilidade de prevenção através do consumo suficiente de nutrientes que estimulem modificações do sistema imunológico do organismo (HAUSEN, 2002; CHIH et al., 2013; SENAPATI, SENAPATI, DWIBEDI, 2016), inclusive alguns reiteram que as fontes de antioxidantes nutricionais são agentes fundamentais na prevenção da carcinogênese (GOODMAN et al., 2011, DE MARCO, 2013; BREITENBACH, ECKL, 2015).

Além disso, outros trabalhos já verificaram que o consumo de frutas e vegetais, fontes de nutrientes antioxidantes (vitamina C e E, carotenoides, folatos e minerais), tem sido associado a um risco reduzido de infecção pelo HPV e consequente progressão da ação viral (PIYATHILAKE et al., 2004; KIM et al., 2010; SIEGEL et al., 2010; TOMITA et al., 2010; ZHANG et al., 2012; GUO et al., 2015; CAO et al., 2016; ZHOU, MENG, 2016). Estes achados passaram a dar suporte a hipótese do papel das vitaminas como nutrientes protetores contra o câncer cervical, inibindo a proliferação de células cancerígenas, estabilizando a p53, prevenindo danos no material genético celular e revertendo a imunossupressão (GARCIA-CLOSAS et al., 2005). Ainda, as estruturas moleculares de alguns antioxidantes podem interagir com os receptores e substratos celulares e regular a atividade das EROS, contribuindo para inibição do fator de transcrição das oncoproteínas E6 e E7 (CASTLE, GIULIANO, 2003). Com a interrupção do mecanismo de ação do vírus, o organismo preservará o estado imunológico e a proteína p53 se manterá estável, prevenindo assim, danos ao DNA (AMES, WAKIMOTO, 2002; REDDY, ODHAV, BHOOLA, 2003). Deste modo, a estratégia

mediada por nutrientes segue o princípio de que um determinado constituinte dietético, neste caso os antioxidantes, podem interagir com enzimas metabolizadoras e proteínas, regulando a metilação do DNA, síntese e organização da cromatina, evitando consequentemente, danos ao material genético (GARCIA-CLOSAS et al., 2005; CHIH et al., 2013).

Entre os nutrientes antioxidantes mais citados que atuam na linha de defesa da progressão da neoplasia cervical, estão a vitamina A (grupo de carotenoides), vitamina C e vitamina E (grupo de tocoferóis) (BORUTINSKAITE, NAVAKAUSKIENE, MAGNUSSON, 2006; BARCHITTA et al., 2018). É importante compreender que o termo, vitamina A, identifica o retinol e todos os carotenoides dietéticos que apresentam atividade biológica de transretinol. As formas metabolicamente ativas desta vitamina são o retinol e o ácido retinóico (LEMOS-JÚNIOR, LEMOS, 2010). A vitamina A pode ser encontrada em alimentos de origem animal (fígado, leite e ovos) e de origem vegetal (vegetais folhosos e legumes verde-escuros, frutas amarelas e/ou alaranjadas, e óleo de buriti, pupunha, dendê e pequi) (SHERWIN et al., 2012). Ainda, este nutriente se destaca por ser um potente modulador do crescimento e da diferenciação celular. Neste caso, ele limita tanto o crescimento de células malignas no epitélio escamoso do colo uterino, como também o desenvolvimento do vírus e, por conseguinte, a evolução das lesões displásicas (PORTANTIOLO, 2014). Uma meta-análise com estudos em humanos fortaleceu a relação da vitamina A com o câncer cervical por identificar que o aumento do seu consumo está associado a um risco reduzido de câncer do colo do útero. Além do que, níveis séricos totais dentro dos valores de referência 0,4 a 1,5 mg/dL desta vitamina têm apresentado uma associação significativa e inversa ao risco de câncer do colo do útero, e o efeito provém principalmente do caroteno (ZHANG et al., 2012).

A vitamina C é comumente encontrada no organismo humano na forma de ascorbato e suas principais fontes são frutas cítricas (laranja, abacaxi, limão), não cítricas (acerola, caju, morango, goiaba, kiwi, mamão papaia) e hortaliças (brócolis, couve-flor, repolho, tomate). De modo geral, esta vitamina participa da manutenção e regeneração do tecido epitelial (TOMITA, 2007; VANNUCCHI, ROCHA, 2012). Ela pode evitar a formação de carcinógenos a partir de compostos precursores, alterando sua estrutura a fim de inibir ou dificultar o seu acesso ao tecido-alvo (GONZÁLEZ et al., 2011; PORTANTIOLO, 2014). Os resultados apresentados por

Cao e colaboradores (2016), inferem que o aumento da ingestão de vitamina C (50 mg/dia) apresentou uma correlação significativa com a redução do risco do desenvolvimento de neoplasia cervical, reforçando assim, a importância do seu consumo diário.

Por fim, a vitamina E que compreende o grupo dos tocoferóis e é composta por oito formas naturais, nomeadas de alfa (α), beta (β), gama (γ) e delta (δ) (NIKI, TRABER, 2011). As fontes desta vitamina podem ser encontradas em uma variedade de alimentos como óleo de soja e de coco, azeite de oliva, banana, couve manteiga, nozes, amendoim, gergelim, linhaça e carnes (COHEN, SILVA, VANNUCCHI, 2014). A vitamina E é reconhecida pela sua ação antioxidante, a qual inibe a peroxidação lipídica, protegendo a célula de danos oxidativos no DNA. Ela também é capaz de impedir que células tumorais continuem o seu ciclo celular estagnando-as na fase G1 promovendo a apoptose (DIAO et al., 2016; ZHENG et al., 2016). Um estudo recente de revisão sistemática e meta-análise, avaliou diversos estudos e sugeriu que tanto a ingestão quanto os níveis circulantes adequados de vitamina E podem reduzir o risco de neoplasia cervical, substanciando a importância da suplementação em casos de deficiência na ingestão desse antioxidante (HU et al., 2017).

Para Gonçalves (2015) e Koshiyama (2019) as vitaminas antioxidantes A, C e E apresentam diferentes habilidades biológicas e podem intervir na história natural da infecção pelo HPV. Logo, recomendam que a alimentação saudável, com ingestão de frutas, verduras e legumes, pode ser uma medida efetiva na prevenção e controle da carcinogênese mediada pelo vírus em nível sistêmico. Em controvérsia, alguns trabalhos apresentam ausência de associação entre algumas vitaminas antioxidantes plasmáticas com a presença da IST (SEDJO et al., 2002; GUO et al., 2015). Além disso, em alguns casos a associação entre as vitaminas passa a ser comparada apenas com a neoplasia cervical e não, necessariamente, com a presença do vírus ou com alterações citopatológicas. Contudo, são necessárias mais pesquisas para elucidar o papel que os nutrientes antioxidantes e os alimentos desempenham na redução do risco de câncer do colo do útero. É necessário levar em consideração o consumo alimentar e suas respectivas concentrações plasmáticas, de forma a confiabilizar a interpretação da ação das vitaminas sobre a ação do vírus.

2. OBJETIVOS

2.1 Geral

Determinar a associação entre o consumo de vitaminas antioxidantes e indicadores de estresse oxidativo plasmático em um grupo de mulheres diagnosticadas com HPV e com alterações no exame Papanicolau.

2.2 Específicos

- Caracterizar a população a partir das variáveis socioeconômicas, sexuais, ginecológicas e hábitos de vida;
- Determinar a prevalência do Papilomavírus Humano na população em estudo;
- Estabelecer a prevalência das alterações citopatológicas do colo uterino na população;
- Determinar o índice de massa corporal e o consumo alimentar de vitaminas antioxidantes A, C e E da população em estudo;
- Estimar tanto os níveis de biomarcadores de EO, da população em estudo;
- Associar o consumo alimentar de vitaminas antioxidantes com os níveis de biomarcadores de EO;
- Verificar se há relação entre consumo de vitaminas antioxidantes e os biomarcadores de EO com a infecção pelo HPV.
- Verificar se há relação entre consumo de vitaminas antioxidantes e os biomarcadores de EO com o resultado do Papanicolau.

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4. Oxidative stress and consumption of antioxidant vitamins associated with HPV infection and cervical cytology in a group of women

Abstract

The production of ROS is balanced through the systems of antioxidant enzymes and small molecules that assist in this control. This study investigated the association between the plasma lipoperoxide levels and the total plasma antioxidant capacity with consumption of A, C and E vitamins in women with positive and negative outcomes of HPV infection and Pap smear. 113 women answered questionnaires and also underwent the collection for the Pap smear, detection of HPV, and blood tests to determine the lipoperoxidation and the total antioxidant capacity. According to the type of variable, the Mann-Whitney or Chi-square test were used with subsequent logistic regression. In addition, correlations among then were determined leading to the Permutational Analysis of Variance. The prevalence of HPV was 8% and cervical changes were 5.3%. The results point to an increased chance of infection for ex-smokers (OR: 5.07) and for those who use contraceptives (OR = 6.6923). Plasma levels of lipoperoxidation were higher in women without cervical changes and in those with HPV. The total antioxidant capacity exposed a positive correlation with the consumption of mate and negative with the levels of lipoperoxides. However, no interaction was observed between oxidative stress parameters and antioxidant vitamins consumption for sexually transmitted infection and Pap smear outcomes.

Keywords: STI. Cervical Dysplasia. Lipoperoxidation. TRAP.

1. Introduction

The global prevalence of Human Papillomavirus (HPV) infection is 12% [1]. Specifically, in Brazil, it can reach 54.3% in the general population [2] and even be present in women diagnosed with normal cervical cytology, with frequency varying from 2.3% to 55.4% [1,3,4]. The main agent involved in the origin of cervical cancer in the world is HPV [5,6,7]. According to the National Cancer Institute in Brazil (INCA), the estimates for 2018 and 2019 were more than 16,000 new cases of cervical cancer for every 100,000 women [8]. The progression of the viral infection is correlated to more than 200 HPV subtypes, with 16 and 18 being the most virulent oncogenic genotypes, associated with 70% of all invasive cervical cancer cases in the world [9,10].

Most HPV infections are spontaneously eliminated by the immune system [11,12]. However, most of them have a high viral load and the persistence of oncogenic subtypes are decisive factors for the progression of precancerous lesions [13]. Among the factors that contribute to this condition is the ability of the virus to intervene in processes that increase the production of reactive oxygen species (ROS) in cells [14]. This increase in the production of ROS induces transformations in the cervical cells and favors the integration of the viral genome with that of the host, in addition to causing damage to RNA molecules, proteins and lipids [15].

In the body, the production of ROS is balanced through systems of antioxidant enzymes and small molecules that assist control these reactive species [16]. The imbalance between ROS synthesis and the antioxidant capacity results in intracellular damage – a condition known as oxidative stress (OS) [17]. In this context, Marulo et al. [18] listed OS as an important cofactor in promoting HPV-induced carcinogenesis.

Based on the investigation of the antioxidants function, they can be described in actions involving the synthesis, repair and methylation of the genetic material, both at the systemic level, as well as in loco, to contain the carcinogenic progression [19]. Studies have also shown that HPV infection is associated with an imbalance between the production of ROS and the organism's antioxidant response [20].

The intake of antioxidant nutrients can modulate the immune response and decrease viral replication [21]. Among the main non-enzymatic antioxidants with these characteristics are vitamins A, C, and E, which express lines of defense in

preventing DNA damage and improve the performance of immunological functions [22,23]. Henceforth, studies have investigated the association between antioxidant vitamins and the prevention of cervical cancer, and some sought the relationship with HPV infection [23,24,25,26,27,28]. A few studies have evaluated the association between OS condition, antioxidant vitamins consumption, and the presence of HPV. According to Nirmala et al. [29], virus infection is an OS inducing factor in cervical cells, mainly when serum concentrations of vitamins C and E are reduced. Within this context, the current study investigated the association of the OS parameters, plasma lipoperoxides levels and total plasma antioxidant capacity, with the estimate of the consumption of antioxidant vitamins A, C and E in women with positive and negative outcomes for HPV infection and for the Pap smear.

2. Materials and methods

The current study is a retrospective and cross-sectional study carried out between April and December 2019, with women who attended an Oncology Center (CEONC) in southwestern Paraná state, Brazil. The main inclusion criterion to compose the target population was women referred to CEONC due to previous alterations in the Pap smear, or who were going through medical monitoring after the diagnosis and treatment of cervical cancer, and who had already had sexual intercourse. The sample size was obtained for convenience, since it is an annually fluctuating population. The research was approved by the Ethics Committee in Research with Human Beings of the Western Paraná State University (CEP/UNIOESTE), approval number 3,254,342, and the signatures of the participants in an Informed Consent Form were requested.

2.1 Instruments for collecting general and nutritional data

Individual interviews were conducted through a questionnaire, elaborated with socioeconomic nature questions about sexual/gynecological/reproductive behavior, and lifestyle habits. Afterwards, a specific, validated Food Frequency Questionnaire (FFQ) [30] was used to determine the daily intake of antioxidant vitamins A, C and E. A specific question also seeks to investigate or consume yerba mate by the population.

The assessment of nutritional status was defined by the Body Mass Index (BMI), which is the ratio between weight in kilograms (kg) and height in meters-squared (m^2). For this calculation, the women were weighed on a digital scale, from Omron® brand, with a capacity of up to 150 kg, and the height was measured by a portable stadiometer from Filizola brand. The participants' BMI was classified according to the recommendations of the World Health Organization [31].

2.2 Collection and diagnosis of the gynecological examination Pap test

The collection for the gynecological exam, Pap test, was conducted by a hospital nurse, which followed the recommendations of the Ministry of Health (2013) [32]. An Ayre's spatula was used to sample the endocervix cells, after the preparation of the cytological lamina, the spatula was preserved in a falcon tube with 2mL of TE buffer (Tris-HCl and EDTA), and stored at -20ºC for later detection of HPV [33,34]. After this procedure, the laminas were destined for analysis in a laboratory accredited to CEONC, and the results followed the classification of the Bethesda System (2001) [35,36].

2.3 HPV detection

For the isolation of the viral genome, a 200µL aliquot was taken from each original sample (cervical cells retained in the endocervical brush) and processed, using the Biological Fluid/Blood Genomic DNA extraction kit – "Purelink® Genomic DNA Mini Kit" (Invitrogen by Thermo Fisher Scientific) – according to the manufacturer's protocol, and stored in a freezer at -20ºC.

For the detection of HPV via PCR (DNA Polymerase Chain Reaction), the MY09 (5′-CGTCCMAARGGAWACTGATC-3′) and MY11 (5′-GCMCAGGGWCATAAYAATGG-3′) primer pair were used, which amplifies a conserved region of approximately 450pb of the L1 gene of the viral genome [37,38]. The amplification conditions for each reaction was 190 nM of dNTPs, 500 nM of each primer, 2 mM $MgCl_2$, Buffer (200 mM Tris-HCL, 500 mM KCl), 1.25 U of DNA polymerase (Ludwig ™), about 80 ng of DNA subjected to a temperature of 55ºC for the annealing of the primers. Simultaneously, a 268 bp segment of the human β-globin gene was synthesized for each sample from the following primers GH20 (5′-

GAAGAGCCAAGGACAGGTAC-3′) and PC04 (5′-CAACTTCATCCACGTTCACC-3′), in order to verify the quality of the total DNA extraction process. In addition, a negative control was included, PCR without DNA to verify the absence of contamination and, a positive control, PCR with DNA from cells of the Hela Lineage (HPV 18), whose viral DNA is integrated into the human genome [38].

The amplicons were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide, under a difference in electrical potential of 150 volts for one hour, and subsequently, viewed under ultra-violet (UV) light, and photodocumented.

2.4. *Blood collection, determination of lipoperoxide levels and total plasma antioxidant capacity (TRAP)*

At the research site, the participants had 5mL of blood collected – using disposable syringes and needles – and packed in a tube with EDTA anticoagulant. Thereafter, the biological sample was centrifuged for 24 minutes, at 4000 g, to separate the cell sediment from the plasma, which was aliquoted in 2mL microtubes and kept in a freezer at -20ºC.

For each plasma sample, the total plasma non-enzymatic antioxidant capacity (TRAP) [39,40] and lipoperoxidation [40,41] was determined. To estimate the TRAP, plasma samples were diluted in glycine buffer (0.1 M, pH 8.6) at 37 ° C, in proportions ranging from 1:20 to 1:500. Then, the ABAP solution (54.24 mg of 2.20'-azobis dissolved in 1 ml of ultrapure distilled water) was added. To this was added the luminol solution as a reaction amplifier (3.98 mg in 250 ml of KOH 1 M added to 10 ml of glycine buffer and diluted 1:10 at the time of the reaction). The soluble vitamin E (Trolox) was the reference antioxidant (2.5 mg in 5 ml of glycine buffer [0.1 M, pH 8.6] at 37 ° C). The final solution was processed in the luminometer apparatus, GloMax (TD 20/20, Turner Designs), where the sample readings provided curves in which the results were expressed in nM of Trolox and tabulated in the Excel program spreadsheets.

To determine the levels of lipoperoxidation [40], 865 µL of phosphate buffer was added to each 125 µL aliquot of plasma. The lipoperoxide estimation was performed by chemiluminescence reaction initiated by the addition of 10µL of tert-butyl (3 mM) for processing and reading in a GloMax luminometer (TD 20/20 Turner

Designers), for 40 minutes. The results were expressed in relative light units (RLU) and the generated curve was used as a qualitative indicator of lipoperoxidation. Quantitative data were obtained by the integral of the area under the curve, using OriginLab software, 7.5 version. The final values were tabulated in Excel spreadsheets, as well as all variables obtained in the research.

2.5. Statistical analyses

The descriptive and inferential analyses were processed using the R software (R Development Core Team, 2019) and XLSTAT® software (Addinsoft, 2017), with the results presented in different tables and graphs. The descriptive components were the frequency, the mean and the standard deviation to determine, respectively, the prevalence of HPV infection and the Pap smear result. The quantitative variables were analyzed for statistical assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) to define the choice of tests. Once these assumptions were accepted, parametric tests were used and, otherwise, the respective non-parametric tests.

For the qualitative variables, the association with the results of the Pap test and the diagnosis of HPV, through the Chi-square test for independence, were verified and, in situations of injury of the assumption of minimum expected frequency equal to 1, the Chi-square test for independence with the Monte Carlo permutational method was applied. The variables that presented significance of p-value less than 0.20, followed the construction of the binary logistic regression model, in order to identify the factors effectively associated with the outcomes. For the selection of the predictor variables, the criterion of $p < 0.10$ was used, if the odds ratio (OR = Odds Ratio) was statistically equal to 1, subsequently, of $p < 0.05$ for the adjusted odds ratio. In the regression analysis, all possible multiple interactions were tested using the maximization of the Wald function, with adjustment of the model by the Hosmer & Lemeshow statistics. A ROC (Receiver Operating Characteristic) curve was constructed to assess the model's ability to represent reality, with sensitivity and specificity of the adjusted model. These analyzes were performed using the XLSTAT® software (Addinsoft, 2017).

To compare the plasma levels of lipoperoxides, the total plasma antioxidant capacity and the ratio between both with Pap test and HPV test outcomes, Mann-

Whitney-U non-parametric tests (α = 0.05) were performed, and the results presented in graphics through boxplots with median and interquartile range. Pearson's (continuous variables) and Spermann's (categorical variables) correlation analysis was also performed to determine the relationship strength between lipoperoxidation and between the total plasma antioxidant capacity (TRAP) with the aforementioned outcomes, and the consumption of vitamins A, C and E and mate, considering $p < 0.05$. The results of this analysis were expressed in scatter plots. Finally, the variables of lipoperoxidation, TRAP and their ratio were also evaluated in relation to the daily consumption of antioxidant vitamins (A, C and E), and the results of the Pap smear and HPV to investigate possible interactions through the Permutational Variance Analysis (α = 0.05) (non-parametric test), also called PERMANOVA. The results were presented in graphs.

3. Results

During the study period, 172 women visited the Cancer Hospital and 114 women agreed to participate in the research. However, one of the samples was excluded due to problems in the processing of blood tissue, totaling a group of 113 participants. The prevalence of HPV infection was 8% and cervical changes were 5.3%. Most women with the virus had normal cytology for gynecological examination, with a prevalence of 7.1%. The main alterations found were the atypical squamous cells of undetermined significance (ASC-US) and high-grade intraepithelial lesion (HSIL), both with a frequency of 1.8%.

There was no significant difference between the average age of the infected population (49.33 ± 13.25) and not infected by HPV (50.24 ± 13.39), as well as between women with (57.83 ± 9.15) and without cytological changes (49.74 ± 13.42). The general description of socioeconomic aspects, lifestyle, immunization, and BMI; as well as the sexual, reproductive, and gynecological behavior of women with and without the virus, and who showed cervical changes or not, are reported in tables 1 and 2, respectively.

Table 1 - Association between HPV infection outcomes and cervical changes (Pap test), and socioeconomic variables, lifestyle and BMI of a group of women assisted at CEONC, in the town of Francisco Beltrão, in 2019.

Variables	**HPV Diagnosis**		***p*†**	**OR_b (IC95%)**	***p*††**	**Pap Test**		***p*†**
	Negative	Positive				Normal	Changed	
	(n*= 104)	(n*=9)				(n*= 107)	(n*=6)	
Marital status	N (%)	N (%)				N (%)	N (%)	
Married/stable union	76 (73.1%)	5 (55.6%)	< 0.2			78 (72.9%)	3 (50.0%)	< 0.2
Single and others	28 (26.9%)	4 (44.4%)				29 (27.1%)	3 (50.0%)	
Education								
≤ 9 years of study	60 (57.7%)	6 (66.7%)	NS*			78 (72.9%)	3 (50.0%)	NS*
> 9 years of study	44 (42.3%)	3 (33.3%)				29 (27.1%)	3 (50.0%)	
Monthly household income								
≤ R$ 2.063,78	71 (91.0%)	7 (77.8%)	NS*			62 (57.79%)	4 (66.7%)	NS*
> R$ 2.063,78	33 (94.3%)	2 (22.2%)				45 (42.1%)	2 (33.3%)	
Tobacco use								
Never smoked	71 (68.3%)	5 (55.6%)	< 0,2	1		72 (67.3%)	4 (66.7%)	NS*
Smoker	21 (20.2%)	1 (11.1%)		0.762 (0.079-7,347)	0.814	20 (18.7%)	2 (33.3%)	
Table sequence								

Former smoker	12 (11.5%)	3 (33.3%)		5,067 (0.939-27,356)	**0.0592**	15 (14.0%)	0 (0.0%)	
Alcoholic drink								
No	67 (64.4%)	6 (66.7%)	NS*			67 (62.6%)	6 (100.0%)	< 0.2
Yes (up to twice a week)	37 (35.6%)	3 (33.3%)				40 (37.4%)	0 (0.0%)	
BMI (Kg/m^2)‡								
≤ 24,9	28 (26.9%)	5 (55.6%)	< 0,2			32 (29.2%)	1 (16.7%)	< 0.2
≥ 25,0	76 (73.1%)	4 (44.4%)				75 (70.1%)	5 (83.3%)	
Use of condom								
Don't use/sometimes	91 (87.5%)	7 (77.8%)	NS*			93 (86.9%)	5 (83.3%)	NS*
Yes	13 (12.5%)	2 (13.3%)				14 (13.1%)	1 (16.7%)	
Contraceptive								
No/never used	47 (45.2%)	1 (11.1%)	< 0,2	1		46 (43.0%)	2 (33.3%)	NS*
Yes/not anymore	57 (54.8%)	8 (88.9%)		6,692 (0.764-58,599)	**0.0860**	61 (57.0%)	4 (66.7%)	
HPV§ vaccine								
No/one dose	103 (99.0%)	9 (100.0%)	NS*			106 (99.1%)	6 (100.0%)	NS*
Yes	1 (1.0%)	0 (0.0%)				1 (0.9%)	0 (0.0%)	

NS * = statistically non-significant values with p> 0.20 for the Chi-square test; OR = Odds Ratio and CI = 95% Confidence Interval (Logistic Regression); §HPV = Human Papilloma Virus.

Table 2 - Association between HPV infection outcomes and cervical changes (Pap test) and sexual, reproductive and gynecological variables of a group of women assisted at CEONC, in the town of Francisco Beltrão, in 2019.

Variables	HPV Outcome		*p*†	Pap test Outcome		*p*†	Variables	HPV Outcome		*p*†	Pap test Outcome		*p*†
	Negative	Positive		Normal	Changed			Negative	Positive		Normal	Changed	
	(n*= 104)	(n*=9)		(n*= 107)	(n*=6)			(n*= 104)	(n*=9)		(n*= 107)	(n*=6)	
Age of the 1st sexual intercourse	N (%)	N (%)		N (%)	N (%)		*Previous Pap test*	N (%)	N (%)		N (%)	N (%)	
≤ 18 years	73 (70.2%)	7 (77.8%)	NS*	76 (71.0%)	4 (66.7%)	NS*	Normal	83 (79.8%)	7 (77.8%)	NS*	86 (80.4%)	4 (66.7%)	NS*
> 18 years	31 (29.8%)	2 (22.2%)		31 (29.0%)	2 (33.3%)		Changed	21 (20.2%)	2 (22.2%)		21 (19.6%)	2 (33.3%)	
No. of partners							*Current Pap test*						
Up to one	46 (44.2%)	1 (11.1%)	< 0,2	43 (40.2%)	4 (66.7%)	NS*	Normal	99 (95.2%)	8 (88.9%)	NS*	99 (92.5%)	5 (83.3%)	NS*
≥ two	58 (55.8%)	8 (88.9%)		64 (59.8%)	2 (33.3%)		Changes	5 (4.8%)	1 (11.1%)		8 (7.5%)	1 (16.7%)	
No. of new partners/year							*Pap test Interval*						
None	91 (87.5%)	7 (77.8%)	NS*	93 (86.9%)	5 (83.3%)	NS*	≤ 1 year	79 (76.0%)	8 (88.9%)	NS*	84 (78.5%)	3 (50.0%)	NS*
≥ 01	13 (12.5%)	2 (22.2%)		14 (13.1%)	1 (16.7%)		≥ 2 years	25 (24.0%)	1 (11.1%)		23 (21.5%)	3 (50.0%)	
No.of children							*Presence of candidiasis*						
≤ one child	24 (23.1%)	3 (33.3%)	NS*	26 (24.3%)	1 (16.7%)	NS*	No	97 (93.3%)	9 (100.0%)	NS*	101 (94.4%)	5 (83.3%)	NS*

Table sequence

≥ two kids	80 (76.9%)	6 (66.7%)		81 (75.7%)	5 (83.3%)		Yes	7 (6.7%)	0 (0%)		6 (5.6%)	1 (16.7%)	
Oral sexual Intercourse							*Presence of G. vaginalis*						
No	86 (82.7%)	8 (89.9%)	NS*	88 (82.2%)	6 (100.0%)	NS*	No	86 (82.7%)	5 (55.6%)	< 0,2	87 (81.3%)	4 (66.7%)	NS*
Yes/sometimes	18 (17.3%)	1 (11.1%)		19 (17.8%)	0 (0.0%)		Yes	18 (17.3%)	4 (44.4%)		20 (18.7%)	2 (33.3%)	
Anal intercourse							*Inflammatory Response*						
No	91 (87.5%)	9 (100.0%)	NS*	95 (88.8%)	5 (83.3%)	< 0,2	No	35 (33.7%)	2 (22.2%)	NS*	37 (34.6%)	0 (0.0%)	< 0.2
Yes	13 (12.5%)	0 (0%)		12 (11.2%)	1 (16.7%)		Yes	69 (66.3%)	7 (77.8%)		70 (66.4%)	6 (100.0%)	
STIs‡ History							*Performed hysterectomy*						
No	90 (91.8%)	8 (88.9%)	NS*	92 (86.0%)	6 (100.0%)	NS*	No	69 (66.3%)	2 (22.2%)	NS*	86 (80.4%)	4 (66.7%)	NS*
Yes	14 (13.5%)	1 (11.1%)		15 (14.0%)	0 (0.0%)		Yes	35 (33.7%)	7 (77.8%)		34 (31.8%)	3 (50.0%)	
HPV§ History							*Vaginal infection*						
No	99 (95.2%)	9 (100.0%)	NS*	102 (95.3%)	6 (100.0%)	NS*	No/don't know	68 (65.4%)	5 (55.6%)	< 0.2	71 (66.4%)	2 (33.3%)	< 0.2
Yes	5 (4.8%)	0 (0%)		5 (4.7%)	0 (0%)		Yes	36 (34.6%)	4 (44.4%)		36 (33.6%)	4 (66.7%)	
Uterus Disease													
No	92 (88.5%)	9 (100.0%)	NS*	95 (88.8%)	6 (100.0%)	NS*							
Yes	12 (11.5%)	0 (0%)		12 (11.2%)	0 (0%)								

NS * = statistically non-significant values with p> 0.20 for the Chi-square test; OR = Odds Ratio and CI = 95% Confidence Interval (Logistic Regression); STIs ‡ = Sexually transmitted infection; §HPV = Human Papilloma Virus.

For the HPV infection outcome, some variables showed associations, which were the marital status, use of tobacco, use of oral contraceptives and BMI (Table 1), number of sexual partners in life, number of sexual partners in the last year, presence of *Gardnerella vaginalis* and recent vaginal infection (Table 2). Thereafter, regarding the logistic regression method, the variables that remained significant were the use of tobacco and contraceptives. The model presented an adequate adjustment according to the Hosmer & Lemeshow statistic (X2 = 7.95; GL = 8; p = 0.439) and suggests that former smokers were 5.07 times more likely to have a positive viral diagnosis (OR = 5 , 0677; 95% CI: 0.9388 - 27.3559; p = 0.0592), even if the significance was bordering. Similar information was observed among women who use the contraceptive, with almost seven times more chance of being infected (OR = 6.6923; 95% CI: 0.7643 - 58.5999; p = 0.0860) (Table 1). The probabilities were estimated, ordered and plotted on a graph, providing the ROC curve (Figure 1). The area under the ROC curve shows that the model can predict approximately 73.81% of the factors associated with the HPV infection outcome.

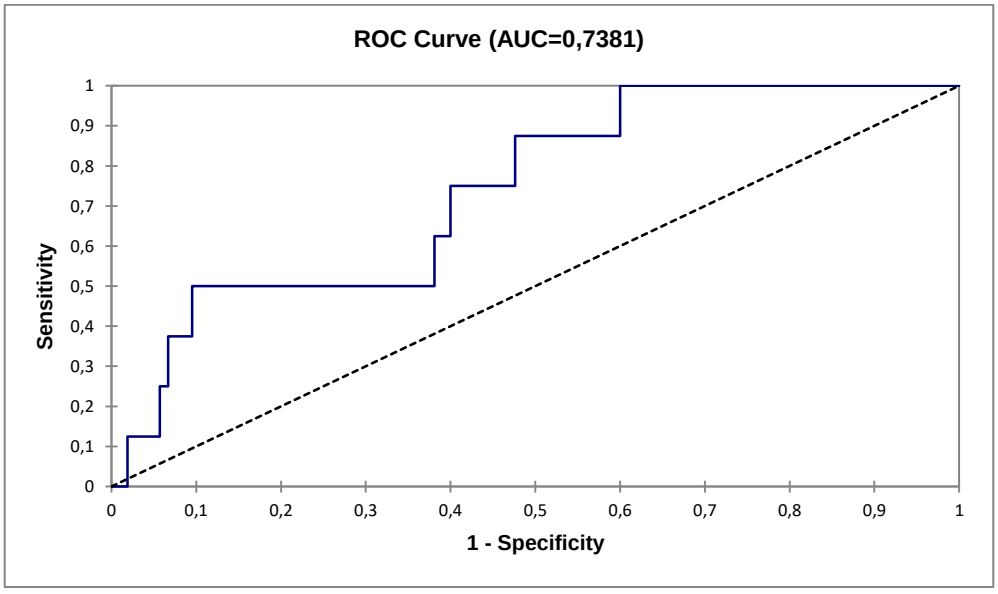

Figure 2 - ROC curve for the binary logistic regression model with factors predictive of the HPV test result. The sensitivity of the adjusted model was equal to 0%, while the specificity was equal to 100%.

Regarding the Pap test, the associated variables were the marital status, alcohol consumption and BMI (Table 1), practice of anal sex, recent occurrence of

vaginal infection, and inflammatory process (Table 2). However, it was not possible to build a logistic regression model with adequate adjustment according to the Hosmer & Lemeshow statistic, as it was for the HPV outcome.

When evaluating the OS parameters, the plasma levels of lipid peroxidation, it was possible to verify that the positivity of the results of the cytopathological exams and the HPV promoted, respectively, lower values (Md: 462400; IIQ: 394642 - 535365; p = 0.015) and higher (Md: 876855; IIQ: 589295 - 1031243; p = 0.077) of lipoperoxides when compared to patients with negative results (Figure 2). In contrast, the estimate of total plasma antioxidant capacity (TRAP) when compared to patients with negative and positive Pap smear and HPV reports did not show statistical differences (p> 0.05). It should be stressed that women with cervical disorders have an average age of 57.83 years, the majority, potentially, in post-menopausal status.

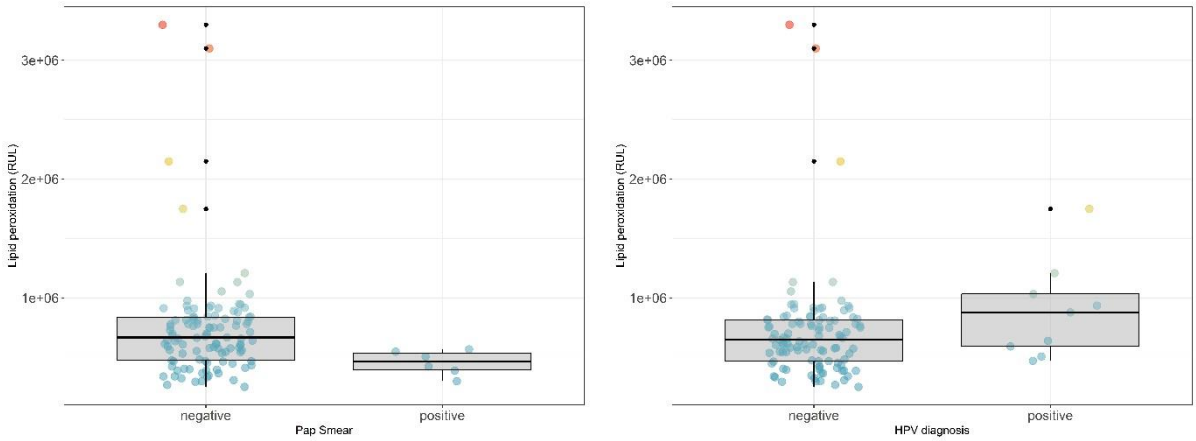

Figure 3 - Boxplot (median, interquartile range, inter-percentile range) of plasma lipoperoxidation values versus results of the Pap test (to the left) and HPV diagnosis (to the right). Mann-Whitney test with p <0.05.

Still, the results showed an inverse relationship between lipoperoxidation and TRAP, suggesting the consumption of non-enzymatic antioxidants through pro-oxidants in the systemic scope (R2Pearson = -0.262; p = 0.005) (Figure 3). An inverse correlation was also observed of lipoperoxidation exclusively with vitamin E, estimated by food intake (R2Spearman = - 0.193; p = 0.04).

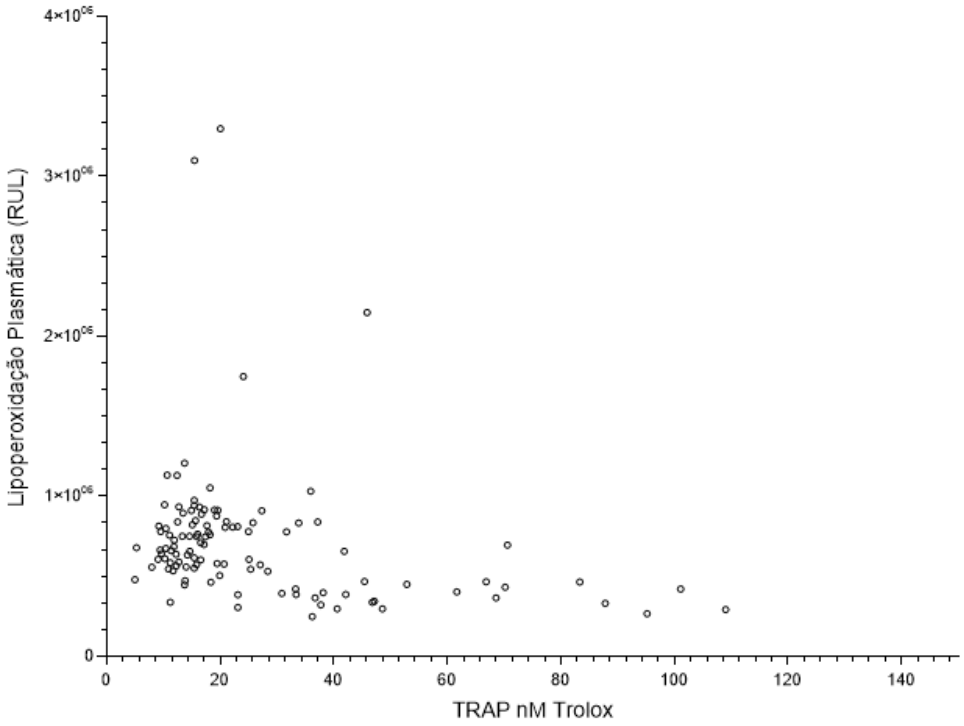

Figure 4 - Correlation between the plasma levels of lipoperoxides and the total plasma antioxidant capacity (TRAP) of the women investigated at CEONC. * p = statistically significant values <0.05, referring to Pearson's correlation coefficient.

Although TRAP did not reveal any correlation with the estimated intake of vitamins A, C and E, it showed a direct relationship with yerba mate consumption (R2Spearman = 0.196; p = 0.038). The average consumption of mate in quantity of gourds was 9.83 units/day for the group with cytopathological changes, and 8.93 units/day for the group without changes. However, just as lipoperoxidation showed an inverse relationship with TRAP, vitamin E also showed the same relationship. These data may suggest that a large part of the source of vitamin E is being suppressed through the consumption of mate and not through the intake of source foods, since there was no relationship between this vitamin and TRAP, as well as other antioxidant vitamins. Likewise, the consumption of mate in both groups is very high, which may be supporting the levels of antioxidants analyzed.

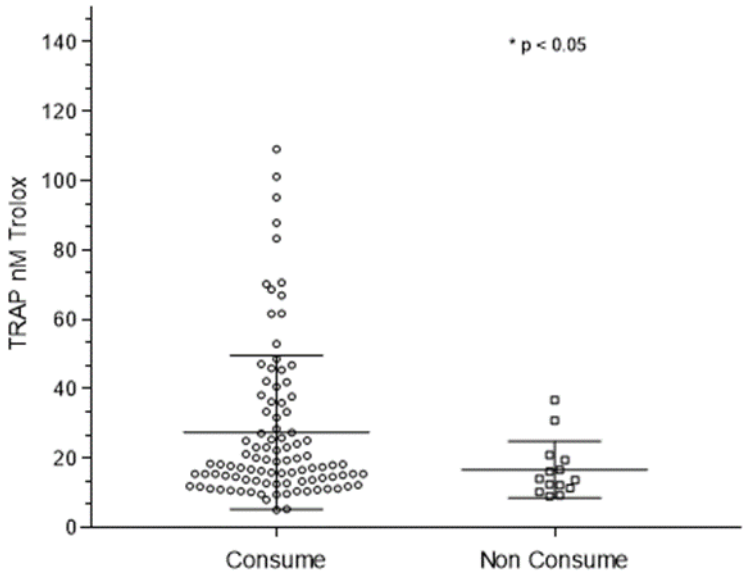

Figure 5 - Plasma levels of total antioxidant capacity (TRAP) and the consumption of yerba mate from the female population investigated at CEONC. * p = statically significant values, referring to the Mann-Whitney Test.

No significant association was between HPV infection and the changes diagnosed on the Pap test with the estimated consumption of antioxidant vitamins A, C and E. When assessing levels of pro-oxidants, the total plasma antioxidant capacity and the ratio between both, with the interaction of the HPV outcome and daily consumption of antioxidant vitamins A, C and E, there was no found relationship noted ($p < 0.05$; Figure 5). Likewise, the result was repeated for the outcome of cytopathological changes ($p < 0.05$; Figure 6). However, it is worth mentioning that the lack of significance may be due to the low number of observations in groups with sufficiency and insufficiency of vitamins, which had a positive outcome for the virus and for cervical changes.

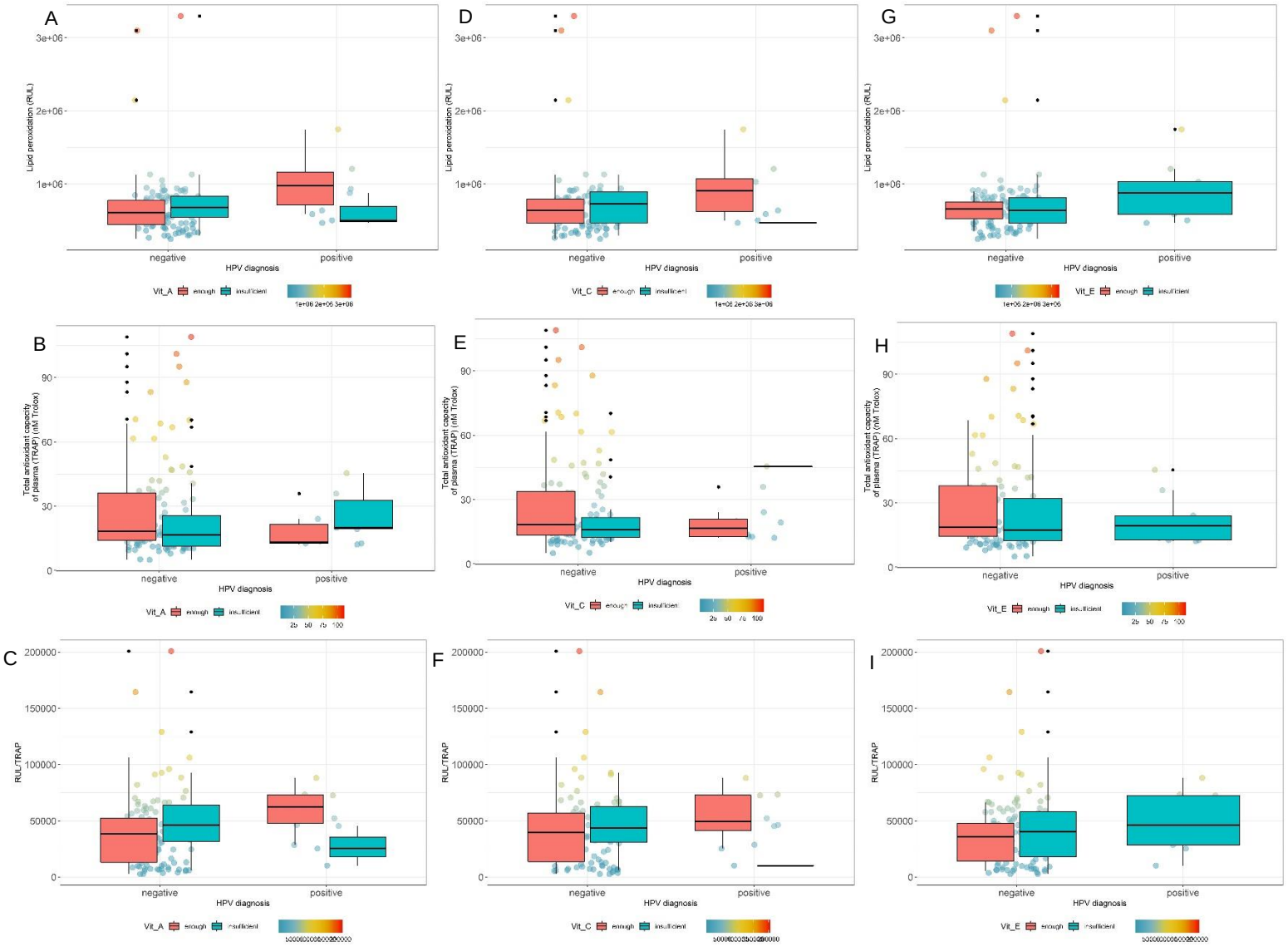

Figure 6 - Boxplot (medians, interquartile range and inter-percentile range) of lipoperoxidation plasma values, total antioxidant capacity (TRAP) and their ratio according to the results of the HPV diagnosis and daily consumption of vitamin A (graphs A, B and C) vitamin C (graphs D, E and F) and vitamin E (graphs G, H and I). All comparisons are not significant (p> 0.05).

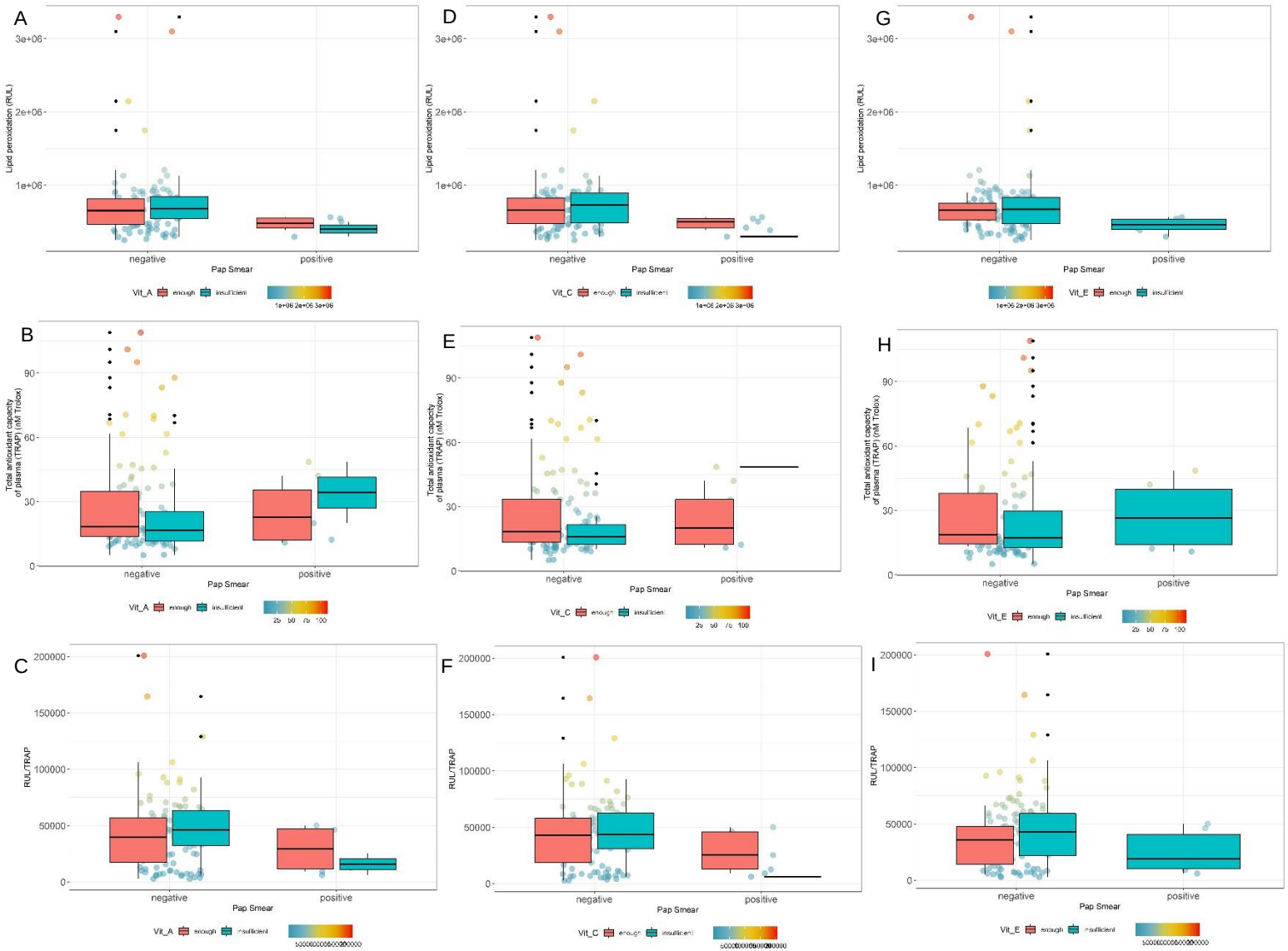

Figure 7 - Boxplot (medians, interquartile range and inter-percentile range) of lipoperoxidation plasma values, total antioxidant capacity (TRAP) and their ratio according to the results of the Pap test and daily consumption of vitamin A (graphs A, B and C) vitamin C (graphs D, E and F) and vitaminE (graphs G, H and I). All comparisons are not significant (p> 0.05).

4. Discussion

It is known, based on the literature, that HPV infection presents, in most cases, asymptomatic and with subclinical lesions that are not apparent [45]. The main alterations found were the ASC-US and HSIL, that values are consistent with some studies that have a prevalence from 0.5% to 7.9% for ASC-US and 0.4% up to 1.6% for HSIL [34,42,43]. Although the results of the present study revealed that the majority of women with HPV did not present cytopathological changes, representing a prevalence of 7.1%, within the range from 2.4% to 55.4%, for the prevalence of the virus in women without cervical changes in Brazil (POP) [4]. This reality is reproduced in other studies with a positive diagnosis for the virus in more than half of the cytopathological tests without changes [46]. The findings can also be compared with a study carried out in Paraná, whose HPV prevalence was 6.7% in women with normal Pap test [47]. Recently, Guthrie [48] found the antigen in 59% of women without cervical changes and, in 69% of them, infections were due to high-risk subtypes. The close relationship of them with the development of cervical cancer brings a warning and reinforces the importance of inserting the viral diagnosis in addition to the traditional Pap test, since it does not aim to identify one of the main etiological agents of cervical neoplasia.

In the present study, the exposure to tobacco and the use of oral contraceptives can increase up to 5 and 7 times, respectively, the chances for the occurrence of HPV infection in the participants. The relationship between tobacco and viral infection contributed to the evasion of the virus before the immune system, since it is linked to a decrease in natural killer cells, IgG and IgA and, especially, Langerhans cells (LC). The interference on the LC leads to a decreased response of T cells, favoring the persistence of the viral infection [49,50]. The LC are presenter of skin and mucous antigens and decrease the population both in cases of HPV infection and in cervical intraepithelial neoplasms [51,52,53]. Some researchers also report that smoking history is positively associated with HPV, especially for high-risk subtypes [50]. Some studies describe that tobacco can be a possible cytokine inhibitor, therefore, it reduces apoptosis and, as a consequence, favors tumor development [54]. With regard to the use of oral hormonal contraceptives, Sadate-Ngatchou and collaborators [55] found that both women who use it and

those who do not use it anymore are, approximately, twice as likely to be diagnosed with HPV. Studies refer to contraceptives as direct mediators of the suspension of the immune system [56]. The chronic estrogen exposure, normally present in oral contraceptive pills (OCP), can modulate carcinogenesis, through genetic polymorphisms that can act synergistically with contraceptives and viral infections [57]. The OCPs, both combined with three-phase and low-dose, are associated with increased viral transcription, suspension of the immunovigilance, and a bigget exposure of the glandular epithelium to infectious agents [57,58]. Moreover, when they have been used for more than five years, the risk of infection with oncogenic subtypes and of developing high-grade cervical injury is increased [59,60].

In addition, one of the main objectives of the present study concerns HPV and possible changes in OS at the systemic level. The results, at first, point to the absence of a significant association between the levels of lipoperoxides with a positive viral diagnosis. However, the values found for the levels of lipoperoxidation in the group of women with HPV were higher than those without the virus and, almost double of those with cervical changes. Studies that measured lipoperoxide levels to estimate the OS, found higher values in women with STI when compared to those with a negative diagnosis [61]. In addition, Marco et al. [62] tracked the OS indices in carcinogenesis promoted by HPV and detected an increase in ROS in women with dysplastic or cervical neoplastic lesions. Although the work has proven the presence of OS, it is important to note that the analyzes concern the cervical microenvironment, especially because the microenvironment of malignant cells is characterized by high levels of OS biomarkers [63]. It may be questionable that the current study was limited to the assessment of OS in a systemic way, however, it is based on the simultaneous relationship with the measured consumption of vitamins, which acts in a generalized way in the individual. In addition, the systemic manifestation of inflammatory processes originated by antigens can establish the state of OS. Normally, HPV causes a mild chronic induction on the host's immune system, without arousing a systemic response. After a while of infecting the keratinocytes, a viral replication and a synthesis of E6 and E7 proteins occur, which interfere with the cell cycle preventing an apoptosis. Additionally, a scarcity or simply the no release of pro-inflammatory cytokines is observed, ensuring the virus the evasion of the host's immune system, without necessarily triggering an oxidative stress at the systemic level [64]. Gonçalves et al. [65] describe that the level of

plasma lipoperoxidation increases 2 to 3 times in patients with carcinomas, when compared to controls, but the analysis is limited and does not report an association with the HPV virus.

Analyzing the associations between lipoperoxide levels and cervical changes, the results showed higher values for the group with no changes when compared to some cytopathological changes. More recent studies, such as those by Jelic et al. [66], also verified the correlation between these two variables, however, with an increase in lipid peroxidation only in cases with high-grade lesion, that is, in situations of progression to carcinogenesis. In the current study, it should be noted that the number of women who had HSIL was limited. Nevertheless, a Brazilian study approached the findings and found lower OS conditions in women with low-grade squamous intraepithelial lesion H when compared to those with normal cervical cytology. In addition, the average age of the participants with cervical changes and low indicative of OS constitutes a group in the postmenopausal period. Victorino et al. [67] found higher values of lipoperoxides in women in the perimenopausal period compared to the postmenopausal ones. Women in the climacteric period are subject to physiological and hormonal changes such as increased estrogen [68]. This hormone can have antioxidant properties, due to its chemical structure and, in this context, it can minimize the impact of OS, when it binds to the estrogen receptor-α [69].

In the research findings, it was also possible to observe an inverse correlation between the pro-oxidant profile and the degradation of antioxidants. Among the stages of lipid peroxidation, the initiation is one of the main indicators of OS, where it acts directly on lipoproteins. The availability of antioxidants in this stage is able to delay the initiation process due to the intense consumption of lipid hydroperoxides and, therefore, it reduces the status of OS, a situation observed in this work, which characterizes the search for redox balance [67]. No interaction was observed between the consumption of vitamins A, C and E, lipoperoxides and the total antioxidant capacity in the participants according to positive and negative diagnoses for HPV and Pap tests. Guo and collaborators [70] did not evaluate this interaction, however, they show the association between high plasma concentrations of α-carotene, β-carotene, vitamin C and E with a reduced risk of cervical cancer, suggesting a protective effect on the consumption of these micronutrients. Some systematic review works also strengthen the correlations between the consumption

of antioxidant vitamins as a protective factor for cervical neoplasms [21,71]. Nonetheless, it is necessary to clarify that the studies that make this correlation, investigated people with cervical cancer, which differs from the reality of the population in question. Results similar to the current research were observed in the study of Kim et al. [72]. The researchers identified mean consumption, tendentially, of higher retinol, vitamin E and C in the diet of women in the control group, when compared to the group with cervical changes, even without significant differences. However, the correlation between dietary consumption of foods that are sources of antioxidants and the delay in cervical neoplasms is not yet fully understood. The action of the antioxidant vitamins can modulate the formation of ROS, the effective immune system response to HPV clearance or squamous maturation mediated by retinol sources [73,74]. In addition, it appears that women who frequently consume foods rich in vitamins A, C and E may have other healthy habits, reflecting positively on health compared to those who do not.

In contrast to the vitamins, the total antioxidant capacity was positively associated with the consumption of mate. The yerba mate, Ilex paraguariensis, the main constituent of the typical drink in the study region, has several antioxidant components, in greater abundance of flavonoids and tocopherols, followed by B vitamins, vitamins A, C and E, in addition to tannins and chlorogenic acids [44]. In this context, a series of biological functions have already clarified the antioxidant, anti-inflammatory, immunomodulatory and anticancer action of yerba mate [75]. It is worth noting that, possibly, mate is influencing the antioxidant action due to its abundant source of total phenolics and flavonoids, and even the response to OS may be supplied by yerba mate instead of the estimated vitamins [44]. According to Boaventura et al. [76], individuals with a compromised endogenous antioxidant defense system can be recovered through widely consumed non-alcoholic beverages, including mate. It is estimated that the women investigated ingested an average of one liter of mate a day. In the long run, this consumption can contribute to the increase of the antioxidant potential, as well as the concentration of glutathione (GSH) and to the reduction of serum lipid hydroperoxides [77]. It is also widely accepted that the containment of the harmful effects of OS can be attributed to the phenolic compounds found in yerba mate, especially chlorogenic acids and flavonoids [78,79]. Thus, it is suggested, still speculatively, that the consumption of

mate may be indirectly influencing the systemic redox system, when compared to the estimated consumption of vitamins.

5. Conclusion

The HPV infection remains a crucial public health problem, which affects a considerable number of individuals, asymptomatically and it is co-responsible for benign and malignant cervical lesions. Both tobacco exposure and the use of oral contraceptives are associated with positive STIs. Although recent researches show the association between OS and the presence and persistence of HPV infection, these findings were not significantly corroborated in the present study. Such fact can be justified by the systemic dimension of OS, and not in the cervical microenvironment or by the limited number of participants. The estimated consumption of antioxidant vitamins also showed no association with the presence of the virus and/or cervical changes, however, the consumption of yerba mate was related to the total plasma antioxidant capacity between the groups evaluated – a result that may outline new studies aiming to deepen the knowledge of the action of the herb and its antioxidant potential. Lastly, the search for information on the relationship between OS, antioxidant potential and the consumption of vitamins in individuals who are HPV seropositive or with cervical disorders is scarce, therefore, it is suggested that further studies develop to clarify the relationship or interaction between them.

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5. ANEXOS

Anexo 1 – Questionário de comportamento sexual, ginecológico, da saúde da mulher e de avaliação antropométrica.

UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ – UNIOESTE/FRANCISCO BELTRÃO - PR

HISTÓRICO SOCIOECONÔMICO, REPRODUTIVO, GINECOLÓGICO

Nome Completo: _____

Número da Requisição:_____ Número da Amostra:_____

1) Nascimento: ___/___/___ **Idade** _____

2) Raça: (1) Branca; (2) Negra; (3) Parda; (4) Indígena; (5) Amarela.

3) Estado Civil: (1) Solteira; (2) Casada; (3) Divorciada/Separada; (4) Viúva; (5) União estável/Amasiada

4) Paridade (QUANTOS FILHO?): Nº _____

(0) Nenhum; (1) Um; (2) Dois; (3) Três; (4) Quatro filhos ou mais; (5) Teve aborto. Quantos?_____

5) Escolaridade: _____ **/**_____**anos de estudos**

(0) Não sabe; (1) Nenhuma; (2) Ensino Fundamental incompleto; (3) Ensino Fundamental Completo; (4) Ensino Médio incompleto; (5) Ensino Médio Completo; (6) Ensino Superior incompleto; (7) Ensino Superior completo.

6) Profissão/Ocupação: _____

7) Qual é sua renda mensal? ( ) Não tem; ( ) só sua e valor_____**; ( ) familiar e valor**_____

8) Com quantas pessoas reside? Nº_____ (1) Um; (2) Dois; (3) Três; (4) Quatro ou mais.

Quem são? _____

9) Faz uso de alguma droga lícita? Tabaco. Você fuma? (0) Nunca fumou; (1) Sim. Há quantos anos? _____

(2) Ex. fumante. Quantos anos fumou?_____ Há quanto tempo parou?_____

Álcool. Você consome bebida alcóolica? (0) Nunca; (1) 1-2 vezes na semana; (2) 3-4 vezes na semana (3) 5 ou mais vezes na semana.

10) Possui alguma doença atualmente? (1) Sim; (2) Não. Se sim,

Qual/Quais?_____

Medicamentos em

uso:_____

11) Idade da Primeira Relação Sexual: _____ **anos**

(1) ≤14 anos; (2) 15-16 anos; (3) 17-18anos; (4) 19-21 anos; (5) 22-25 anos; (6) ≥ 26 anos.

12) Número de Parceiros Sexuais na Vida: Nº _____(0) Nenhum; (1) Um; (2) Dois; (3) Três; (4) Quatro ou mais.

13) Número de Parceiros Sexuais Novos no Último Ano: Nº _____(0) Nenhum; (1) Um; (2) Mais de um.

14) Durante a Relação Sexual Realiza a Prática do Sexo Oral: (0) Não; (1) Sim; (2) as vezes.

15) Durante a Relação Sexual Realiza a Prática do Sexo Anal: (0) Não; (1) Sim; (2) as vezes.

16) Usa Anticoncepcional?

(0) Não; (1) Sim; (2) Nunca usou; (3) Não usa mais; (4) ou outro método contraceptivo.

Se usa algum? ( ) Uso oral, nome_____; ( ) Uso injetável, nome_____; ( ) ou outro método contraceptivo, nome_____.

17) Tempo de Uso: _____anos. (0) Zero; (1) < 1 ano; (2) 1-5 anos; (3) > 5 anos.

18) Usa Preservativo (camisinha) na Relação Sexual: (0) Não; (1) Sim; (3) Ás vezes.

19) História de DST (já teve IST): (0) Não; (1) Sim; (2) Não sabe.

20) Caso A Resposta Anterior for sim. Qual?_____

( ) HPV; **( )** SÍFILIS; **( )** TRICHOMONAS VAGINALIS; **( )** HERPES GENITAL; **( )** GONORREIA; **( )** HEPATITE B; **( )** CLAMIDIA.

21) Sabe se teve Ocorrência Recente de Infecção Vaginal:

(0) Não; (1) Sim; (2) Não sei.

22) Quando fez o Último Exame Ginecológico (Exame Do Papanicolau): Ano_____

(1) ≤ 1 ano; (2) ≥ 2 anos; (3) Nunca fez.

23) Resultado do Último Exame Ginecológico (Exame Do Papanicolau): (1) Normal; (2) Alterado; (3) Não sei.

24) Realizou Vacinação Para HPV:

(0) Não; Quantas doses? Nº _____ (1) 1 Dose; (2) 2 Doses; (3) 3 Doses. Com quantos anos? Nº ____

AVALIAÇÃO ANTROPOMÉTRICA

1) Peso atual:_____ Kg. **2) Estatura**:_____ m.**3) IMC (Kg/m²):** _____

Classificação: (1) Baixo peso; (2) Eutrofia; (3) Sobrepeso; (4) Obesidade de grau I; (5) Obesidade de grau II; (6) Obesidade de grau III.

5) Porcentagem de Massa Magra: _____Classificação: (1) Baixo; (2) Normal; (3) Alta; (4) Muito alta.

6) Porcentagem de Gordura Corporal: _____Classificação: (1) Baixo; (2) Normal; (3) Alta; (4) Muito alta.

Anexo 2 – Questionário ABEP 2016.

Itens de conforto	Não possui	1	2	3	4 ou +
Quantidade de automóveis de passeio exclusivamente para uso particular					
Quantidade de empregadas mensalistas, considerando apenas os que trabalham pelo menos cinco dias por semana					
Quantidade de máquinas de lavar roupa, excluindo tanquinho					
Quantidade de banheiros					
DVD, incluindo qualquer dispositivo que leia DVD e desconsiderando DVD de automóvel					
Quantidade de geladeiras					
Quantidade de *freezers* independentes ou parte da geladeira duplex					
Quantidade de microcomputadores, considerando computadores de mesa, laptops, notebooks e netbooks e desconsiderando tablets, palms ou smartphones					
Quantidade de lavadouras de louça					
Quantidades de fornos de micro-ondas					
Quantidade de máquinas secadoras de roupas, considerando lava e seca					

A água utilizada no domicílio é proveniente de?

1 ( ) Rede geral de distribuição

2 ( ) Poço ou nascente

3 ( ) Outro meio

Qual o grau de instrução do chefe de família? Considere como chefe de família a pessoa que contribui com a maior parte da renda do domicílio.

( ) Analfabeto/ Primário incompleto
( ) Primário completo/ Ginásio incompleto
( ) Fundamental completo/ Médio incompleto
( ) Médio completo/ Superior incompleto
( ) Superior Completo

Anexo 3 – Questionário de frequência alimentar para avaliar o consumo de vitaminas antioxidantes.

Alimento	Frequência	Porção Média	Sua Porção	Época
Laranja/ Suco de Laranja	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Unidade Média 180g 1 Copo 200 ml	P M G E O O O O	O
Mamão Papaia	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Fatia Média 170g	P M G E O O O O	O
Morango/ Suco de Morango	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	6 Unid Médias 72g 1 Copo 200 ml	P M G E O O O O	O
Kiwi	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1Unid Média 76g	P M G E O O O O	O
Melão	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	2 Fatias Médias 180g	P M G E O O O O	O
Manga	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Fatia Média 140g	P M G E O O O O	O
Goiaba	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1Unid Média 170g	P M G E O O O O	O
Uva	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Cacho Médio 350g	P M G E O O O O	O
Melancia	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Fatia Média 200g	P M G E O O O O	O

Suco de Limão	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	4Colh Chá 8ml	P M G E O O O O	O
Abacaxi/ Suco de Abacaxi	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 Fatia Média 75g 1 Copo 200ml	P M G E O O O O	O
Bergamota	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid Média 135g	P M G E O O O O	O
Banana	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid Média 40g	P M G E O O O O	O
Caqui	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid Média 110g	P M G E O O O O	O
Abacate	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid Pequena 370g	P M G E O O O O	O
Pêssego	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid Média 60g	P M G E O O O O	O
Polpa de Acerola	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Unid 100g	P M G E O O O O	O
Ameixa Seca	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Unid 15g	P M G E O O O O	O
Oleaginosas: Castanha do Pará, avelã, amêndoa, noz pecã, nozes, semente de girassol, amendoim, pistache.	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Unid	P M G E O O O O	O
Alface	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 Folhas Méd ou 1 Pegador 20g	P M G E O O O O	O
Tomate Fresco	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 Rodelas Méd ou 3 Peq ou 1 Grande 30g	P M G E O O O O	O

Molho de Tomate	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1Colh de Servir 45g	P M G E O O O O	O
Pimentão Amarelo ou Vermelho	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 Fatias Méd 12g	P M G E O O O O	O
Cenoura Crua	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa 24g	P M G E O O O O	O
Cenoura Cozida	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia Picada 50g	P M G E O O O O	O
Ervilha Verde	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia 54g	P M G E O O O O	O
Brócolis	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa 20g	P M G E O O O O	O
Couve-Flor	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 Pedaços 60g	P M G E O O O O	O
Couve Cozida	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia Picadas 40g	P M G E O O O O	O
Repolho Cozido	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia Picadas 40g	P M G E O O O O	O
Couve de Bruxelas (repolhinho)	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia Picadas 40g	P M G E O O O O	O
Espinafre Cozido	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia 50g	P M G E O O O O	O
Abóbora	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2Colh de Sopa Cheia Picadas 72g	P M G E O O O O	O

Batata Doce	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1Unid Média 140g	P M G E O O O O	O
Beterraba Cozida	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	2Colh de Sopa Cheia Picadas 40g	P M G E O O O O	O
Acelga	N 1 2 3 4 5 6 7 8 9 10 D S M A O O O O O O O O O O O O O O O	1 Folha Média 10g	P M G E O O O O	O

Fonte: (BEMVENUTI, 2013).

Qual o tipo de óleo que utiliza? _____

Utiliza azeite de oliva? ( ) Sim ( )Não Quantidade:_____

Frequência: _____

Faz uso de algum suplemento de vitaminas? ( )Sim ( )Não

Qual? _____ Quantidade/dose: _____

Há quanto tempo: _____ Frequência: _____

Consome chimarrão? ( ) Não. ( ) Sim. Quantas cuias por dia? Nº _____

ANEXO I

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - TCLE

Título do Projeto: DETECÇÃO DO HPV, AVALIAÇÃO NUTRICIONAL, CONSUMO ALIMENTAR, CONCENTRAÇÃO PLASMÁTICA DE ANTIOXIDANTES E ANALISE IMUNOCITOQUÍMICA EM MULHERES DO SUDOESTE DO PARANÁ DIAGNOSTICADA COM OU SEM ALTERAÇÕES CERVICAIS NO COLO UTERINO.

Pesquisador Responsável: Angela Khetly Lazarotto; Telefone: (46) 99934-5844
Pesquisador Colaborador: Léia Carolina Lucio; telefone (46) 99933-2938

Convidamos você a participar de nossa pesquisa que tem o objetivo de avaliar o estado nutricional de mulheres diagnosticadas ou não com HPV. Também buscamos avaliar possível estresse oxidativo no plasma humano das mulheres participantes. Em conjunto será analisado o consumo alimentar de antioxidantes dos participantes, para verificar a adequação do consumo de alimentos fontes de carotenoides, vitamina C e vitamina E, além de questionario socio-economico, e caracteristicas ginecológicas e comportamentais. Para isso será realizado um tratamento a sua pessoa, que consiste em aferir dados antropométricos (peso, estatura e circunferências), coletar exame de Papanicolau, realizar uma punção venosa para coletar uma pequena amostra de sangue e preencher questionários com perguntas referentes à temática da pesquisa. Durante a execução do projeto você poderá sentir algum desconforto durante o exame de Papanicolau e a coleta sanguínea, além de constrangimento, timidez ou perturbação durante a coleta de medidas antropométricas ou mesmo durante a aplicação dos diversos questionários. Após as analises, as mulheres que apresentarem o HPV serão chamadas, até a UBS onde foram convidadas a

participar do projeto, para uma consulta com médido ginecologista com intuito de informá-las sobre a presença do vírus, os cuidados que precisa ter a partir de então, seguido de acompamento ginecológico para evitar possiveis lesões do colo uterino e outras patologias. Sempre que entender necessário obter informações ou esclarecimentos sobre o projeto de pesquisa e sua participação no mesmo, você pode entrar em contato com os pesquisadores responsáveis, os quais estão identificados acima ou então pode consultar o Comitê de Ética em Pesquisa da UNIOESTE através do telefone (46) 3220-3092. Na ocorrência de qualquer imprevisto, você terá total atendimento por parte dos pesquisadores, aonde os primeiros socorros serão prestados e o acionamento e encaminhamento ao atendimento médico será imediato, conforme a necessidade. Ao final da pesquisa, você terá acesso aos resultados dos exames realizados como parte dos métodos da pesquisa sem custo algum.

Declaro estar ciente do exposto e desejo participar do projeto. Nome do sujeito de pesquisa ou responsável:

Assinatura:

Eu, **Angela Khetly Lazarotto**, declaro que forneci todas as informações do projeto ao participante e/ou responsável.

Francisco Beltrão,_____de_____de 2019.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO NUTRICIONAL, CONSUMO ALIMENTAR E CONCENTRAÇÃO PLASMÁTICA DE VITAMINAS ANTIOXIDANTES EM MULHERES QUILOMBOLAS DIAGNOSTICADAS COM PAPILOMA VÍRUS HUMANO (HPV)

Pesquisador: Angela Khetly Lazarotto

Área Temática:

Versão: 2

CAAE: 08314418.4.0000.0107

Instituição Proponente: UNIVERSIDADE ESTADUAL DO OESTE DO PARANA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.254.342

Apresentação do Projeto:

Documento informando a retificação de pendências.

Objetivo da Pesquisa:

Documento informando a retificação de pendências.

Avaliação dos Riscos e Benefícios:

Documento informando a retificação de pendências.

Comentários e Considerações sobre a Pesquisa:

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Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1273090.pdf	20/03/2019 10:50:17		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_de_Consentimento_Livre_e_Esclarecido_Modificado.pdf	20/03/2019 10:49:17	Angela Khetly Lazarotto	Aceito
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Aprovado

Necessita Apreciação da CONEP:
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[2] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
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[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
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[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
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[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
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[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/xwj98nb39r.1.

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