Genetic polymorphisms in one-carbon metabolism increased the risk of persistence of pre-neoplastic cervical lesions

Nayara Nascimento Toledo Silva (nayarants@gmail.com)
Universidade Federal de Ouro Preto https://orcid.org/0000-0002-0970-291X
Ana Carolina Silva Santos
Universidade Federal de Ouro Preto
Verlândia Mendes Nogueira
Centro Estadual de Atenção Especializada de Itabirito
Cláudia Martins Carneiro
Universidade Federal de Ouro Preto
Angelica Alves Lima
Universidade Federal de Ouro Preto

Research article

Keywords: Genetic polymorphisms, Thymidylate Synthase, TS3'UTR, pre-neoplastic cervical lesions, HPV

Posted Date: December 3rd, 2019

DOI: https://doi.org/10.21203/rs.2.18058/v1

Abstract

Background: Cervical cancer is caused by high-risk Human Papillomavirus (hr-HPV) infection associated with cofactors that has been analyzed as predictors of cytological abnormalities remission or persistence. These cofactors may be classified as environmental, epigenetic or genetic. Polymorphism in genes of enzymes that act on one-carbon metabolism alter their activity and may be associated with cervical carcinogenesis because they affect DNA synthesis and repair, and gene expression. Therefore, the objective of this study was to analyze the risk of persistence of pre-neoplastic cervical lesions according to genetic polymorphisms involved in one-carbon metabolism. Sample group was divided in Remission (n=60) - presence of pre-neoplastic lesion at first meeting (T_1), and normal cytology after six months of follow-up (T_2), and Persistence (n=46) - presence of pre-neoplastic lesion at T_1 and T_2. Cervical samples were obtained for cytological analysis (T_1 and T_2), HPV detection (T_1), and evaluation of polymorphism C667T of Methylenetetrahydrofolate Reductase (MTHFR C677T), A2756G of Methionine Synthase (MS A2756G), A66G of Methionine Synthase Reductase (MTRR A66G), double or triple 28 bp tandem repeat in 5'-untranslated enhanced region of Thymidylate Synthase (TSER), and 6 bp deletion at nucleotide1494 in TS 3'-untranslated region (TS3'UTR). Genetic Risk Score (GRS) was calculated for analyze all genetic polymorphism simultaneously.

Results: No differences were observed between Remission and Persistence groups of GRS, or genotypic and allelic distribution of MTHFR C677T and MS A2756G polymorphisms. However, higher risk of persistence was observed among women presenting heterozygote genotype - ins/del [OR (IC95%): 3.22 (1.19 – 8.69), p=0.021], or polymorphic genotype – del/del [OR (IC95%): 6.50 (1.71 – 24.70), p=0.006] of TS3’UTR.

Conclusions: Presence of TS3’UTR polymorphism increased risk of persistence of cervical abnormalities. This genetic variant could be considered as potential marker of cervical carcinogenesis, assisting follow-up of women with persistent pre-neoplastic cervical lesions.

Background

Persistent high-risk Human Papillomavirus (hr-HPV) infection is the main cause of cervical cancer. However, only 10% of women with viral infection will develop pre-neoplastic lesions, and less than 1% will progress to cervical cancer. Furthermore, only approximately 30% of high-grade cervical lesions progresses to cancer in uterine cervix, and spontaneous regression occurs in 20–40% of cases (1-4). Thus, risk of cervical carcinogenesis depends on hr-HPV infection and host-dependent features (5, 6). Environmental, genetic and epigenetic cofactors of cervical carcinogenesis has been analyzed as markers for diagnosis, prognosis, and for auxiliary in treatment of pre-neoplastic cervical lesions, since they could be predictors of cytological abnormalities remission or persistence (7-10).

Several genetic alterations characterize cervical cancer and they may have a substantial impact on risk of cervical carcinogenesis, as genomic instability, chromosomal aberration, and integration of HPV DNA into host genome (4, 7). Polymorphism in genes of enzymes that act on one-carbon metabolism, such as Methylenetetrahydrofolate Reductase (MTHFR), Methionine Synthase (MS), Methionine Synthase Reductase (MTRR), and Thymidylate Synthase (TS), alter their activity and may be associated with cervical carcinogenesis (11-13).

MTHFR enzyme, whose gene is located on chromosome 1p36.3, is a flavoprotein that acts on folate metabolism, being essential for DNA integrity (14). MTHFR C677T polymorphism consists of cytosine (C) exchange for thymine (T) at nucleotide 677, and results in substitution of Alanine for Valine, leading to decrease in MTHFR activity (14, 15). This Single Nucleotide Polymorphism (SNP) may modify the susceptibility to carcinogenesis by modulating the availability of 5,10-methyleneTHF at different points in folate metabolism (16).

MS is a vitamin B12-dependent enzyme, essential to intracellular folate levels maintenance, and catalyzes the methylation of homocysteine to methionine (17, 18). MS A2756G polymorphism is caused by an exchange of adenine (A) for guanine (G) at nucleotide 2756, resulting in the substitution of Aspartic Acid for Glycine, close to the binding domain of vitamin B12 (19, 20). Van Der Put *et al.* (1997) suggested that this SNP affects the secondary structure of MS, and has functional consequences. An association between the polymorphic allele (G) of MS and reduction of the number of hypermethylated CpG islands in tumor suppressor genes has been demonstrated (21). Thus, it is possible that the presence of MS A2756G alters the activity of tumor suppressor genes, explaining its association with the development of several types of tumor (22).

MTRR catalyzes the methylation of vitamin B12, that is a cofactor of MS enzyme (23). A66G polymorphism of MTRR enzyme (MTRR A66G) leads to exchange of A by G in nucleotide 66, and to substitution of Isoleucine by Methionine, which results in decrease of MTRR affinity by MS (24). Thus, polymorphic genotype was negatively associated with homocisteinemia, which alters DNA methylation and, consequently, gene expression (23, 25).

TS enzyme catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), the only *de novo* source of thymidine for DNA synthesis and repair. TS binds to RNA for repression of translation of its own messenger RNA (mRNA) or other proteins, and can regulate cell cycle progression (26-28). Moreover, TS expression is an index of cell proliferation and biological malignancy of cancer (29). The polymorphisms most frequently studied are double or triple 28 bp tandem repeat in 5'-untranslated enhanced region (TSER), and 6 bp deletion/insertion at nucleotide 1494 in TS 3'-untranslated region (TS3'UTR). These genetic variations may influence the TS gene expression and the stability of its mRNA, respectively (28).

When the one-carbon metabolism is alter, the integrity of genetic material is compromised due to changes in nucleotide pool and uracil incorporation, leading to DNA instability. In addition, global hypomethylation

and site-specific hypermethylation are observed, which lead to activation of proto-oncogenes and silencing of tumor suppressor genes (10, 30, 31).

Therefore, this study evaluated the risk of persistence of pre-neoplastic cervical lesions according to genetic polymorphisms involved in one-carbon metabolism.

Results

Mean age of participants was 39.7±11.4 years, ranging from 19 to 71, and 32.1% (n=34) were between 35 and 44 years old. Most of women resided in urban area (n=97, 91.5%), had family financial income <US$250/month (n=70, 74.5%), high school education (n=46, 47.9%), was not smoker (n=81, 84.4%), and ingested alcoholic beverages (n=57, 59.4%). Moreover, a higher percentage of participants were married or had a fixed partner (n=77, 80.2%), reported having had the first sexual intercourse with at least 18 years old (n=53, 56.4%), and three or more sexual partners (n=55, 57.3%). In addition, most of participants was not using hormonal contraceptives (n=61, 63.5%), and have had pregnancies (n=81, 84.4%) (Table 1).

Similar frequencies of these characteristics and no significant association ($p<0.05$) were observed between Remission and Persistence groups (Table 1).

In relation to HPV, 50.9% (n=54) presented viral infection and significate higher frequency were observed among women from Persistence group (n=31, 67.4%) if compared with Remission (n=23, 38.3%) (p=0.003). Similar results were obtained analyzing only HPV-AR infection (p=0.000) (data not showed).

MTRR A66G genotypic frequencies were 10.4% (n=11), 76.4% (n=81), and 13.2% (n=14) of AA, AG, and GG, respectively. Genotypic frequency of TSER was 35.8% (n=38) of 2R/2R, 31.1% (n=33) of 2R/3R, and 33.0% (n=35) of 3R/3R. However, distributions of genotypes of MTRR A66G and TSER of Remission group were not found under Hardy-Weinberg equilibrium (p=0.000). Thus, these polymorphisms were excluded from further analyses of this study.

MTHFR C677T genotypic frequencies were 50.0% (n=53) of CC, 45.3% (n=48) of CT, and 4.7% (n=5) of TT, and T allelic frequency was 27.4%. MS A2756G polymorphic genotype was detected in 3.8% (n=4) of samples, and G allele in 19.8%. On the other hand, higher frequency of women presented polymorphic genotype for TS3'UTR genetic variation (16.0%, n=17), and del allelic frequency of 41.5% was observed (Table 2).

To evaluate the association between MTHFR C677T, MS A2756G, and TS3'UTR polymorphisms according to the course of cytological abnormalities, we compared genotype distribution of Remission and Persistence groups (Table 2).

No differences were observed between distribution of MTHFR C677T and MS A2756G, and course of cytological abnormalities (Table 2). On the other hand, higher heterozygote and polymorphic genotypic frequencies of TS3'UTR was observed among women presenting persistent lesions if compared with Remission group. Furthermore, ins/del and del/del genotypes increased the risk of persistence at least

three times [OR (IC95%): 3.13 (1.21 – 8.12), p=0.019; OR (IC95%): 5.96 (1.67 – 21.25), p=0.006 - respectively] (Table 2).

To evaluate the presence of MTHFR C677T, MS A2756G, and TS3'UTR simultaneously, the Genetic Risk Score (GRS) were determined, and women with GRS≥3 was classified as presenting high number of genetic variants. Higher frequency of women with GRS≥3 was observed in Persistence group (n=17, 37.0%) if compared with Remission group (n=12, 20.0%), and women with high number of genetic variants presented higher risk of persistent lesions [OR (IC95%): 2.21 (0.89 – 5.48), p=0.086] (Table 2). However, when adjusted for TS3'UTR, the risk of persistence according GRS was modified [OR (IC 95%): 1.26 (0.44-3.61), p=0.669], evidencing that between three polymorphism analyzed, only TS3'UTR was associated with course of cytological abnormality.

Discussion

Although persistent hr-HPV infection is the main cause of cervical cancer development, genetic alterations may alter the risk of this neoplasia. Thus, genetic markers may be useful in the screening of pre-neoplastic and neoplastic cervical lesions, especially in cases of persistence of HPV infections or recurrent cytological abnormalities (7, 32). Moreover, the conventional methods used for screening of cervical cancer are not able to differentiate pre-neoplastic cervical lesions that will regress, or persist and progress. Therefore, the prognosis of individual pre-neoplastic lesions should be predictable, with the purpose of selecting women with higher risk of persistence and progression, which could decrease the number or unnecessarily treatment of lesions (4).

In this study, five genetic polymorphisms in enzymes that act on one-carbon metabolism were evaluated: MTHFR C677T, MS A2756G, MTRR A66G, TSER, and TS3'UTR. However, distribution of MTRR A66G and TSER were not under Hardy-Weinberg Equilibrium (HWE), which led to their exclusion from further analyzes. Some studies with Brazilian population also did not present genotypic distribution of this polymorphisms attending HWE (33, 34).

Many polymorphisms were identified in TS gene, which is located on chromosome 18p11.32. One of most frequently studied is TS3'UTR, that was related to increased *in vitro* degradation of mRNA, leading to decrease of expression protein (35). We observed that the presence of polymorphic allele (del) of TS3'UTR increased twice the risk of persistence of cytological abnormalities. Probably it occurred due to decreased synthesis of thymidylate, catalyzed by TS enzyme whose activity is decreased by TS3'UTR polymorphism, leading to DNA uracil incorporation. It results in DNA instability and chromosome damage, crucial events for carcinogenesis (26, 35, 36).

On the other hands, although MTHFR C677T and MS A2756G polymorphisms have already been associated with the presence of pre-neoplastic and neoplastic lesions in uterine cervix, we did not observe any association of these SNPs with the persistence of cytological abnormalities (11, 13).

This was the first study in which the risk of persistence of pre-neoplastic cervical lesions was evaluated in relation to the presence of genetic polymorphisms in enzymes that act on folate metabolism. Evaluation of TS3'UTR polymorphism as a possible marker of cervical carcinogenesis has shown promising results, and further study should be performed. Some authors have shown an association between this polymorphism and esophageal, gastric and breast cancer, although the results were controversial and varied with ethnicity (37-41).

However, this research had some limitations, as the small sample size, the losses of participants during the study, and the short follow-up time. Thus, it is necessary to carry out larger studies, for a longer period and with different population groups to better understand the role of genetic cofactors on cervical carcinogenesis. Besides, considering the different grades of cytological abnormalities separately may be important. In this study, 69.7% (n=32) presented same cervical lesion at T_1 and T_2, while 23.9% (n=11) presented LSIL at T_1 and ASC-US at T_2, and 6.4% (n=3) presented LSIL or ASC-US at T_1 and ASC-H at T_2. However, no differences of characteristics analyzed were observed among these participants (data not showed).

Conclusions

Presence of TS3'UTR polymorphism increased risk of cervical abnormalities persistence. Thus, this genetic variant could be considered as potential marker of cervical carcinogenesis, assisting follow-up of women with persistent pre-neoplastic cervical lesions.

Methods

Study design

From October 2016 to September 2018, 280 women living in Minas Gerais State and attended at Basic Health Units of Ouro Preto, and State Specialized Care Center of Itabirito were selected for this study.

Inclusion criterion was age ≥18 years old. Exclusion criteria were pregnancy in a period <6 months, history of neoplasia, and presence of cervical atypia in glandular cells.

At the first meeting (T_1), an interview was performed for information about sociodemographic and behavioral characteristics, and cervical sample was collected for cytological analysis, genetic polymorphism evaluation, and HPV detection.

After six months of follow-up (T_2), 164 women performed the second cytological analysis. From this group, 50 participants presenting normal cytology at T_1 and T_2, and eight women with normal cytology at T_1 and presence of pre-neoplastic lesion at T_2 were excluded (Figure 1). Thus, sample group (n=106) was divided into:

- Remission (n=60): presence of pre-neoplastic lesion at T_1, and normal cytology at T_2;

- Persistence (n=46): pre-neoplastic lesion detected at T_1 and T_2.

Presence of pre-neoplastic lesion was considered when Atypical Squamous Cells of Undetermined Significance (ASC-US), Low Grade Squamous Intraepithelial Lesion (LSIL), High Grade Squamous Intraepithelial Lesion (HSIL), or Atypical Squamous Cell – cannot exclude HSIL (ASC-H) were detected at two times.

Research Ethics Committee of Federal University of Ouro Preto approved this study (CAAE 57187316.7.0000.5150, and CAAE 88479718.0.0000.5150).

Sample collection

Cervical samples were obtained through conventional double collection by health care professionals, using Ayre spatulas for ectocervical sample, and cylindrical brushes for endocervical sample. After confection of cervical smear for cytological analysis, brush was conditioned in Phosphate-Buffered Saline (PBS) pH 7.2, and stored at -80°C for genetic polymorphisms evaluation.

Cytological analysis

Cervical smears were stained according to Papanicolaou method, and samples were evaluated based on cytomorphological criteria described in Bethesda System 2014 for reporting cervical cytological diagnoses (Nayar and Wilbur, 2015). All samples were evaluated by two cytopathologists. In case of disagreement between results, a third professional evaluated the sample. The analyses were performed in Laboratório de Análises Clínicas (LAPAC) from Federal University of Ouro Preto.

DNA extraction

DNA extraction from cervical samples was performed with illustra blood genomicPrep Mini Spin™ Kit (GE Healthcare, Chicago, Illinois, USA). Evaluation of quality and integrity of DNA was performed by amplification of *β-actin* gene (42).

HPV detection

HPV detection was performed by conventional Polymerase Chain Reaction (PCR) with MY09/MY11 primers, as described by Miranda *et al.* (2013). For positive samples, HPV genotype was analyzed by Restriction Fragment Length Polymorphism (RFLP) (42). HPV negative samples were also analyzed by conventional PCR with GP5+/GP6+ primers (43).

Genetic polymorphisms

MTHFR C677T (rs1801133), MS A2756G (rs1805087), MTRR A66G (rs1801394), and TS3'UTR (rs151264360) polymorphisms were evaluated by PCR-RFLP (14, 35, 44, 45). TSER (rs34743033) was evaluated by PCR (46).

Sequences of primers, restriction enzymes, and PCR protocols were presented in *Supplementary Tables 1, 2, and 3*.

Table 3 shows the size of DNA fragments that characterize the genotypes of polymorphisms analyzed.

GRS was calculated to evaluate the presence of all polymorphisms simultaneously, as described by Tomita *et al.* (2013). Presence of heterozygosity or polymorphic homozygotes received one or two points, respectively. Non-polymorphic homozygotes were not scored (zero) (13). Thus, the higher the GRS, the higher the frequency of genetic polymorphisms.

Statistical analysis

Data were tabulated by Microsoft Office Excel™ (Microsoft, Redmond, Washington, USA), and analyzed by Statistical Package for the Social Sciences™ 17.0 (International Business Machines, New York, USA).

Descriptive statistics were performed to evaluate the frequency of genotypes. Allelic frequency was calculated by Genepop software (47). HWE of genotypic frequencies was calculated by HWE calculator including analysis for ascertainment bias (48).

Chi-square was used for comparison between groups. Binary logistic regression was used to calculate the relative risk (Odds Ratio), with a 95% confidence interval.

p values <0.05 were considered as evidence of statistically significant association.

List Of Abbreviations

- A: Adenine;
- ASC-H: Atypical Squamous Cell – cannot exclude High Grade Squamous Intraepithelial Lesion;
- ASC-US: Atypical Squamous Cells of Undetermined Significance;
- C: Cytosine;
- dTMP: deoxythymidine monophosphate;
- dUMP: deoxyuridine monophosphate;
- G: Guanine;
- GRS: Genetic Risk Score;
- HPV: Human Papillomavirus;
- hr-HPV: high-risk Human Papillomavirus;
- HSIL: High Grade Squamous Intraepithelial Lesion;
- HWE: Hardy-Weinberg Equilibrium;
- LAPAC: Laboratório de Análises Clínicas;

- LSIL: Low Grade Squamous Intraepithelial Lesion;
- mRNA: messenger RNA;
- MS: Methionine Synthase;
- MS A2756G: polymorphism A2756G of Methionine Synthase;
- MTHFR: Methylenetetrahydrofolate Reductase;
- MTHFR C677T: polymorphism C667T of Methylenetetrahydrofolate Reductase;
- MTRR: Methionine Synthase Reductase;
- MTRR A66G: polymorphism A66G of Methionine Synthase Reductase;
- PBS: Phosphate-Buffered Saline;
- PCR: Polymerase Chain Reaction;
- RFLP: Restriction Fragment Length Polymorphism;
- SNP: Single Nucleotide Polymorphism;
- T: Thymine;
- T_1: First meeting;
- T_2: Second meeting, after six months of follow-up;
- TS: Thymidylate Synthase;
- TS3'UTR: 6 bp deletion at nucleotide1494 in TS 3'-untranslated region;
- TSER: Double or triple 28 bp tandem repeat in 5'-untranslated enhanced region of Thymidylate Synthase.

Declarations

Ethics approval and consent to participate: Research Ethics Committee of Federal University of Ouro Preto approved this study (CAAE 57187316.7.0000.5150, and CAAE 88479718.0.0000.5150). All women invited and who agreed to participate in this study signed the consent form to participate.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: There are no compenting interests.

Funding: This work was supported by grants from Pró-reitoria de Pós-Graduação e Pesquisa - PROPP/UFOP (**082017066**).

Authors' contributions: NNTS, ACSS, CMC, and AAL analyzed and interpreted the patient data regarding the cytological analyses, HPV infection, and genetic polymorphisms. VMN assisted in selection of participants, and collection of cervical samples. All authors read and approved the final manuscript.

Acknowledgements: To Laboratório de Análises Clínicas (LAPAC) for infrastructure.

References

1. Schiffman M, Wentzensen N. Human papillomavirus infection and the multistage carcinogenesis of cervical cancer. Cancer Epidemiol Biomarkers Prev. 2013;22(4):553-60.
2. Sasagawa T, Takagi H, Makinoda S. Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer. J Infect Chemother. 2012;18(6):807-15.
3. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2(5):342-50.
4. Koeneman MM, Ovestad IT, Janssen EAM, Ummelen M, Kruitwagen RFPM, Hopman AH, et al. Gain of Chromosomal Region 3q26 as a Prognostic Biomarker for High-Grade Cervical Intraepithelial Neoplasia: Literature Overview and Pilot Study. Pathol Oncol Res. 2018.
5. Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14(6):395-405.
6. Torres-Poveda K, Bahena-Román M, Delgado-Romero K, Madrid-Marina V. A prospective cohort study to evaluate immunosuppressive cytokines as predictors of viral persistence and progression to pre-malignant lesion in the cervix in women infected with HR-HPV: study protocol. BMC Infect Dis. 2018;18(1):582.
7. Bahrami A, Hasanzadeh M, Shahidsales S, Farazestanian M, Hassanian SM, Moetamani Ahmadi M, et al. Genetic susceptibility in cervical cancer: From bench to bedside. J Cell Physiol. 2018;233(3):1929-39.
8. Castellsagué X, Muñoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis–role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr. 2003(31):20-8.
9. Fang DH, Ji Q, Fan CH, An Q, Li J. Methionine synthase reductase A66G polymorphism and leukemia risk: evidence from published studies. Leuk Lymphoma. 2014;55(8):1910-4.
10. Szalmás A, Kónya J. Epigenetic alterations in cervical carcinogenesis. Semin Cancer Biol. 2009;19(3):144-52.
11. Zhu J, Wu L, Kohlmeier M, Ye F, Cai W. Association between MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms and risk of cervical intraepithelial neoplasia II/III and cervical cancer: a meta-analysis. Mol Med Rep. 2013;8(3):919-27.
12. Tong SY, Kim MK, Lee JK, Lee JM, Choi SW, Friso S, et al. Common polymorphisms in methylenetetrahydrofolate reductase gene are associated with risks of cervical intraepithelial neoplasia and cervical cancer in women with low serum folate and vitamin B12. Cancer Causes Control. 2011;22(1):63-72.
13. Tomita LY, D'Almeida V, Villa LL, Franco EL, Cardoso MA, Group BS. Polymorphisms in genes involved in folate metabolism modify the association of dietary and circulating folate and vitamin B-6 with cervical neoplasia. J Nutr. 2013;143(12):2007-14.

14. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111-3.
15. Trimmer EE. Methylenetetrahydrofolate reductase: biochemical characterization and medical significance. Curr Pharm Des. 2013;19(14):2574-93.
16. Henao OL, Piyathilake CJ, Waterbor JW, Funkhouser E, Johanning GL, Heimburger DC, et al. Women with polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS) are less likely to have cervical intraepithelial neoplasia (CIN) 2 or 3. Int J Cancer. 2005;113(6):991-7.
17. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol. 2004;159(5):423-43.
18. Fowler B. The folate cycle and disease in humans. Kidney Int Suppl. 2001;78:S221-9.
19. van der Put NM, van der Molen EF, Kluijtmans LA, Heil SG, Trijbels JM, Eskes TK, et al. Sequence analysis of the coding region of human methionine synthase: relevance to hyperhomocysteinaemia in neural-tube defects and vascular disease. QJM. 1997;90(8):511-7.
20. Chen LH, Liu ML, Hwang HY, Chen LS, Korenberg J, Shane B. Human methionine synthase. cDNA cloning, gene localization, and expression. J Biol Chem. 1997;272(6):3628-34.
21. Paz MF, Avila S, Fraga MF, Pollan M, Capella G, Peinado MA, et al. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. Cancer Res. 2002;62(15):4519-24.
22. Weiner AS, Beresina OV, Voronina EN, Voropaeva EN, Boyarskih UA, Pospelova TI, et al. Polymorphisms in folate-metabolizing genes and risk of non-Hodgkin's lymphoma. Leuk Res. 2011;35(4):508-15.
23. Wang P, Li S, Wang M, He J, Xi S. Association of MTRR A66G polymorphism with cancer susceptibility: Evidence from 85 studies. J Cancer. 2017;8(2):266-77.
24. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, et al. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. Proc Natl Acad Sci U S A. 1998;95(6):3059-64.
25. Gaughan DJ, Kluijtmans LA, Barbaux S, McMaster D, Young IS, Yarnell JW, et al. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. Atherosclerosis. 2001;157(2):451-6.
26. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr. 2000;130(2):129-32.
27. Voeller D, Rahman L, Zajac-Kaye M. Elevated levels of thymidylate synthase linked to neoplastic transformation of mammalian cells. Cell Cycle. 2004;3(8):1005-7.
28. Zhou JY, Shi R, Yu HL, Zeng Y, Zheng WL, Ma WL. The association between two polymorphisms in the TS gene and risk of cancer: a systematic review and pooled analysis. Int J Cancer. 2012;131(9):2103-16.

29. Iida M, Banno K, Yanokura M, Nakamura K, Adachi M, Nogami Y, et al. Candidate biomarkers for cervical cancer treatment: Potential for clinical practice (Review). Mol Clin Oncol. 2014;2(5):647-55.
30. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis. 2011;34(1):101-9.
31. Anderson OS, Sant KE, Dolinoy DC. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. J Nutr Biochem. 2012;23(8):853-9.
32. Cardoso MFS, Castelletti CHM, Lima-Filho JL, Martins DBG, Teixeira JAC. Putative biomarkers for cervical cancer: SNVs, methylation and expression profiles. Mutat Res. 2017;773:161-73.
33. Nogueira Junior JdS, Marson FAdL, Bertuzzo CS. Thymidylate synthase gene (TYMS) polymorphisms in sporadic and hereditary breast cancer. BioMed Central Research Journal; 2012. p. 676-81.
34. Bezerra AM, Sant'Ana TA, Gomes AV, de Lacerda Vidal AK, Muniz MT. Tyms double (2R) and triple repeat (3R) confers risk for human oral squamous cell carcinoma. Mol Biol Rep. 2014;41(12):7737-42.
35. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol Biomarkers Prev. 2000;9(12):1381-5.
36. Mandola MV, Stoehlmacher J, Zhang W, Groshen S, Yu MC, Iqbal S, et al. A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics. 2004;14(5):319-27.
37. Tang J, Wang PP, Zhuang YY, Chen WJ, Huang FT, Zhang SN. Thymidylate synthase genetic polymorphisms and cancer risk: a meta-analysis of 37 case-control studies. Chin Med J (Engl). 2012;125(14):2582-8.
38. Wang J, Wang B, Bi J, Di J. The association between two polymorphisms in the TYMS gene and breast cancer risk: a meta-analysis. Breast Cancer Res Treat. 2011;128(1):203-9.
39. Guan X, Liu H, Ju J, Li Y, Li P, Wang LE, et al. Genetic variant rs16430 6bp > 0bp at the microRNA-binding site in TYMS and risk of sporadic breast cancer risk in non-Hispanic white women aged ≤ 55 years. Mol Carcinog. 2015;54(4):281-90.
40. Gao CM, Takezaki T, Wu JZ, Liu YT, Ding JH, Li SP, et al. Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. Asian Pac J Cancer Prev. 2004;5(2):133-8.
41. Mo A, Zhao Y, Shi Y, Qian F, Hao Y, Chen J, et al. Association between polymorphisms of thymidylate synthase gene 5'- and 3'-UTR and gastric cancer risk: meta-analysis. Biosci Rep. 2016;36(6).
42. Miranda PM, Silva NN, Pitol BC, Silva ID, Lima-Filho JL, Carvalho RF, et al. Persistence or clearance of human papillomavirus infections in women in Ouro Preto, Brazil. Biomed Res Int. 2013;2013:578276.
43. Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk

and 6 low-risk human papillomavirus genotypes in cervical scrapings. J Clin Microbiol. 1997;35(3):791-5.

44. Shekari M, Sobti RC, Kordi Tamandani DM, Suri V. Impact of methylenetetrahydrofolate reductase (MTHFR) codon (677) and methionine synthase (MS) codon (2756) on risk of cervical carcinogenesis in North Indian population. Arch Gynecol Obstet. 2008;278(6):517-24.
45. Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, Yang H, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. Mol Genet Metab. 1999;67(4):317-23.
46. Villafranca E, Okruzhnov Y, Dominguez MA, García-Foncillas J, Azinovic I, Martínez E, et al. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J Clin Oncol. 2001;19(6):1779-86.
47. Rousset F. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 2008;8(1):103-6.
48. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol. 2009;169(4):505-14.

Tables

TABLE 1: Sociodemographic and behavioral characteristics

Characteristics	Total n (%)	Cytological abnormality		p
		Remission n (%)	Persistence n (%)	
Age (years)				
<25	12 (11.3)	7 (11.7)	5 (10.9)	0.302
25-34	21 (19.8)	9 (15.0)	12 (26.1)	
35-44	34 (32.1)	18 (30.0)	16 (34.8)	
45-54	28 (26.4)	17 (28.3)	11 (23.9)	
≥55	11 (10.4)	9 (15.0)	2 (4.3)	
Area				
Urban	97 (91.5)	54 (90.0)	43 (93.5)	0.524
Countryside	9 (8.5)	6 (10.0)	3 (6.5)	
Income per person (US$/month)[1]				
<250	70 (74.5)	40 (72.7)	30 (76.9)	
250-500	21 (22.3)	12 (21.8)	9 (23.1)	0.333
≥500	3 (3.2)	3 (5.5)	0	
Education[2]				
University	6 (6.3)	5 (9.1)	1 (2.4)	0.341
High school	46 (47.9)	27 (49.1)	19 (46.3)	
Elementary school/Illiterate	44 (45.8)	23 (41.8)	21 (51.2)	
Smoker[2]				
No	81 (84.4)	48 (87.3)	33 (80.5)	0.365
Yes[a]	15 (15.6)	7 (12.7)	8 (19.5)	
Use of alcoholic beverage[2]				
No	39 (40.6)	22 (40.0)	17 (41.5)	0.885
Yes[a]	57 (59.4)	33 (60.0)	24 (58.5)	
Marital status[2]				
Married/Fixed Partner	77 (80.2)	40 (72.7)	37 (90.2)	0.073

Single	9 (9.4)	8 (14.5)	1 (2.4)	
Widow/Divorced	10 (10.4)	7 (12.7)	3 (7.3)	
Age at first vaginal intercourse (years)[1]				
≥18	53 (56.4)	30 (54.5)	23 (59.0)	0.670
<18	41 (43.6)	25 (45.5)	16 (41.0)	
Lifetime sexual partners[2]				
1	23 (23.9)	13 (23.6)	10 (24.4)	0.936
2	18 (18.8)	11 (20.0)	7 (17.1)	
≥3	55 (57.3)	31 (56.4)	24 (58.5)	
Use of hormonal contraceptive[2]				
No	61 (63.5)	39 (70.9)	22 (53.7)	0.082
Yes	35 (36.5)	16 (29.1)	19 (46.3)	
Pregnancies[2]				
0	15 (15.6)	9 (16.4)	6 (14.6)	0.130
1	19 (19.8)	14 (25.5)	5 (12.2)	
2	26 (27.1)	10 (18.2)	16 (39.0)	
3	22 (22.9)	12 (21.8)	10 (24.4)	
≥4	14 (14.6)	10 (18.2)	4 (9.8)	
HPV infection	52 (49.1)			
Negative	54 (50.9)	37 (61.7)	15 (32.6)	0.003
Positive		23 (38.3)	31 (67.4)	

Participants excluded due to absence of information: [1]Twelve; [2]Ten. [a]Amount or frequency not determined. Remission: presence of pre-neoplastic lesion at T_1, and normal cytology at T_2; Persistence: pre-neoplastic lesion detected at T_1 and T_2.

TABLE 2: Genotypic and allelic frequencies, and GRS, according Remission or Persistence of pre-neoplastic cervical lesions.

Polymorphisms		Total	Cytological abnormality		OR (IC95%)[a]	p
			Remission (n=60)	Persistence (n=46)		
MTHFR C677T[1]	*Genotype n (%)*					
	CC	53 (50.0)	28 (46.7)	25 (54.3)	1.0	
	CT	48 (45.3)	28 (46.7)	20 (43.5)	0.93 (0.40 – 2.12)	0.856
	TT	5 (2.2)	4 (6.6)	1 (2.2)	0.25 (0.02 – 2.48)	0.245
	Allele %					
	C	72.6	70.0	76.1	1.0	
	T	27.4	30.0	23.9	0.71 (0.34 – 2.11)	0.842
MS A2756G[2]	*Genotype n (%)*					
	AA	69 (65.4)	42 (70.0)	27 (58.7)	1.0	
	AG	33 (31.1)	17 (28.3)	16 (34.8)	1.20 (0.50 – 2.89)	0.690
	GG	4 (3.8)	1 (1.7)	3 (6.5)	4.99 (0.46 – 54.57)	0.188
	Allele %					
	A	80.2	83.3	76.1	1.0	
	G	19.8	16.7	23.9	1.63 (0.59 – 4.53)	0.349
TS3'UTR[3]	*Genotype n (%)*					
	ins/ins	34 (32.1)	26 (43.3)	8 (17.4)	1.0	
	ins/del	55	28 (46.7)	27 (58.7)	3.22 (1.19 – 8.69)	0.021

		(51.9)				
	del/del	17 (16.0)	6 (10.0)	11 (23.9)	6.50 (1.71 – 24,70)	0.006
	Allele %					
	ins	58.5	66.7	47.8	1.0	
	del	41.5	33.3	52.2	2.28 (1.00 – 5.22)	0.051
GRS n(%)	≤2	77 (72.6)	48 (80.0)	29 (63.0)	1.00	
	≥3	29 (27.4)	12 (20.0)	17 (37.0)	2.21 (0.89 – 5.48)	0.086

Hardy-Weinberg Equilibrium (HWE): [1]p=0.389; [2]p=0.625; [3]p=0.699. [a]Adjusted for HPV infection. Remission: presence of pre-neoplastic lesion at T_1, and normal cytology at T_2; Persistence: pre-neoplastic lesion detected at T_1 and T_2.

TABLE 3: Size of DNA fragments for genetic polymorphisms analysis

	Genotypes		
Polymorphisms	**No polymorphic**	**Heterozygote**	**Polymorphic**
MTHFR C677T	198 bp	23 bp, 175 bp and 198 bp	175 bp and 198 bp
MS A2756G	211bp	80 bp, 131 bp and 211 bp	80 bp and 131bp
MTRR A66G	22 bp and 44 bp	22 bp, 44 bp and 66 bp	66 bp
TS3’UTR	70 bp and 88 bp	70 bp, 88 bp and 152 bp	152 bp
TSER	220 bp	220 bp and 248 bp	248 bp

GRS was calculated to evaluate the presence of all polymorphisms simultaneously, as described by Tomita *et al.* (2013). Presence of heterozygosity or polymorphic homozygotes received one or two points, respectively. Non-polymorphic homozygotes were not scored (zero) (13). Thus, the higher the GRS, the higher the frequency of genetic polymorphisms.

Figures

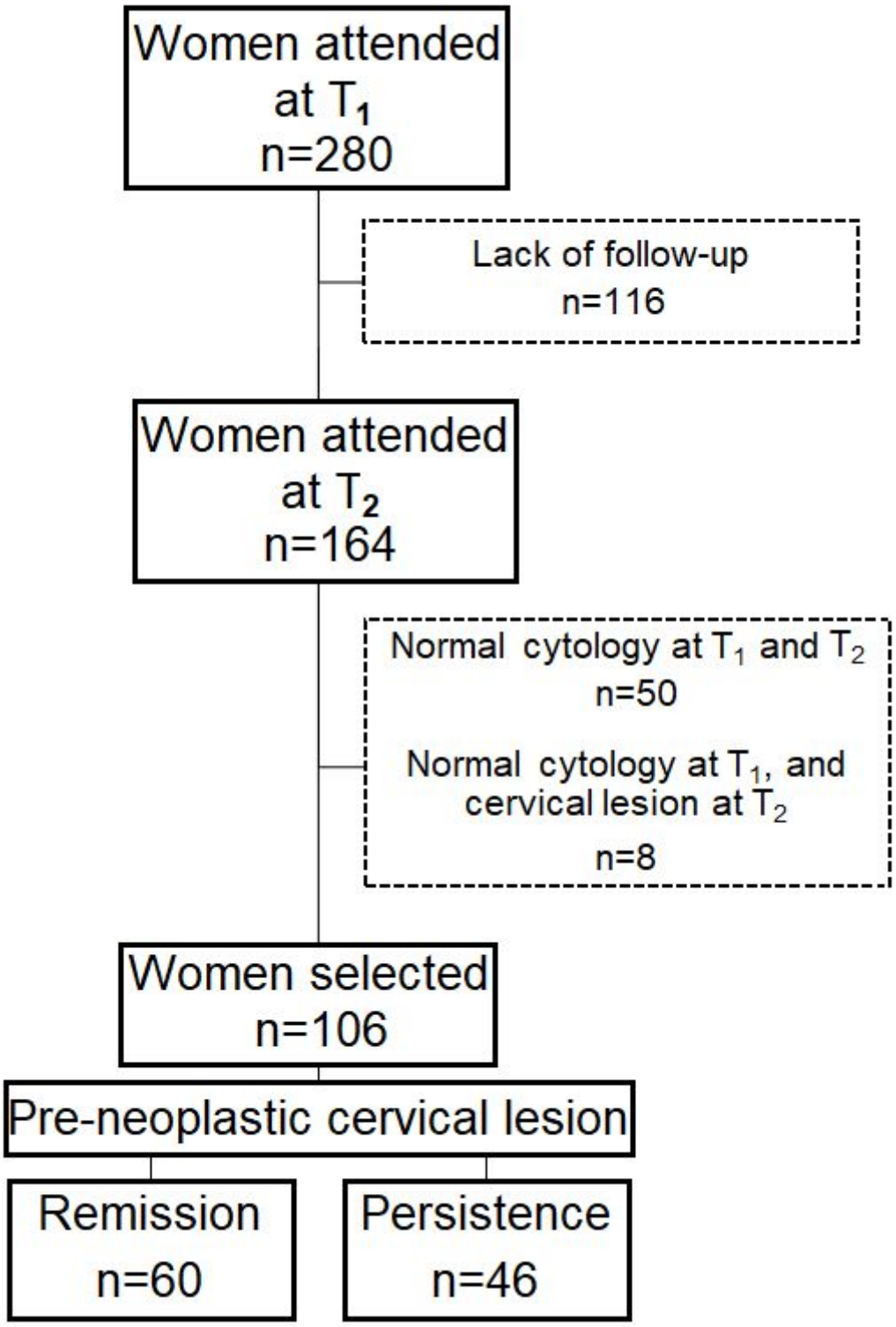

Figure 1

Study flow diagram. T1: First meeting; T2: Second meeting; Remission: Presence of pre-neoplastic cervical lesion at T1, and normal cytology at T2; Persistence: Pre-neoplastic cervical lesion detected at T1 and T2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx