# Loss of heterozygosity on chromosomes 2p, 3p, 18q21.3 and 11p15.5 as a poor prognostic factor in stage II and III (FIGO) cervical cancer treated by radiotherapy

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Loss of heterozygosity (LOH) has been shown to be an important prognostic factor in a variety of malignant neoplasm's. Cervical cancer develops as result of multiple genetic alterations. The aim of this study was to analyze presence of LOH in cervical cancer and to identify the correlation between LOH and survival and relapse-free survival time in patients treated with radiotherapy. Studies were performed on tumor specimens and venous blood from 20 patients with cervical cancer (squamous cell carcinoma G2 and G3) in stage II and III (FIGO) treated with radiotherapy. DNA was isolated using organic extraction. Additional microcolumn purification was performed. The fluorescent multiplex polymerase chain reaction (PCR) was used to amplify 10 microsatellite loci included in commercially available human identification kits. Microsatellite marker BAT 26 was amplified in separate PCR reactions. 75% cervical cancers manifested LOH. LOH in BAT 26 analysis (chromosome 2) was present in all these specimens. 60% of the cases showed LOH at one or more of other examined loci (mostly on 3p, 18q21.3, and 11p15.5). Eight of nine cervical cancers in clinical stage III showed LOH. All cases of G3 squamous cell carcinoma of the cervix manifested LOH on 2p. Patients with LOH have worse prognosis for survival and relapse-free survival compared to patients without LOH.

*Key words: cervical cancer, loss of heterozygosity*

Cervical carcinoma is the second most common malignancy among women worldwide. Radiation therapy plays major role in the management of cervical cancer in stage II and III (according to FIGO classification) as a radical or adjuvant treatment. A combination of external beam radiotherapy and intracavitary brachytherapy with respective contribution based upon the initial volume of the disease and individually according to tumor regression and dose distribution to critical organs. The goal of treatment is to maximize local control and disease free survival.

Some prognostic factors in cervical cancer are under investigation. Loss of heterozygosity (LOH) has been shown to be an important prognostic factor in a variety of malignant neoplasm's [1–4]. Cervical cancer develops as a result of multiple genetic alterations. LOH frequently occurs in squamous cell carcinoma of the uterine cervix [5–8]. LOH is one of the most important mechanisms for inactivation of tumor-suppressor genes, thus the genetic abnormalities may play a role in the development of the neoplasm. The effects of such alterations on survival of patients with cervical cancer after radiotherapy has not yet been explain.

The aim of this study was to analyze the presence of LOH in cervical cancer and to determine the correlation between LOH and survival and relapse-free survival in patients treated with radiotherapy.

## Material and methods

Studies were performed on tumor specimens obtained from 20 patients with cervical cancer (squamous cell carcinoma) in stage IIB (8 patients) and III (12 patients) (according to FIGO classification) treated with radiotherapy in the Department of Gynecologic Oncology, Regional Cancer Center in Bialystok, Poland (Tab. 1). Samples of venous blood from these patients were studied as respective controls. The mean patients' age was 64 years (range from 32 to 78 years). The mean follow-up was 23.1 months (range from 4 to 36 months).

All patients were treated by external beam radiation ($\gamma$-$Co^{60}$, X 4MV or X 9MV) to whole pelvis, given with the anterior-posterior portals to the total dose of 45–50 Gy with daily fractionated dose of 2 Gy. The appear fields border was placed at L4/L5. Whole pelvis was shaped following the anatomy of the patient. The target volume included the uterus, the vagina, the parametrium and the lymphatic nodes including common iliac nodes. Guidance was given as to the anatomical limits as determined radiographically. All patients received also intracavitary brachytherapy ($Cs^{137}$, Selectron MDR, Nucletron). Intracavitary applications with Fletcher-Suit applicators were performed twice – in the middle of external radiation and at the end of treatment. The dose was prescribed on the Manchester point A (20 Gy to point A for one application, to total dose 40 Gy to point A). After the first application of brachytherapy a middle block protected the region of high dose from brachytherapy.

DNA was isolated using organic extraction [9]. Additional microcolumn purification using Microcon-100 microconcentrators was performed. The fluorescent multiplex polymerase chain reaction (PCR) was used to amplify 10 microsatellite loci included in commercially available human identification kits (Tab. 2). Phenotyping was performed with the use of ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). Microsatellite marker BAT 26 was amplified in a separate PCR reaction and electrophoresed using the non-denaturing PAGE with subsequent silver-staining [10]. PCR products were sized according to 100 bp ladder (Gibco, BRL).

## Results

75% cervical cancers manifested LOH. LOH in BAT 26 analysis (chromosome 2) was present in all of LOH positive specimens. 60% of the cases showed LOH on one or more of other examined loci. Eight of nine cervical cancers (83.3%) in clinical stage III showed LOH. The most common sites of LOH, except chromosome 2p, were 3p, 18q21.3, and 11p15.5. LOH was found on 3p in 40% (8/20), on 18q21.3 in 30% (6/20) and on 11p15.5 in 20% (4/20) of the examined cases. All cases of G3 squamous cell carcinoma of cervix manifested LOH on chromosome 2 (BAT 26 analysis). In these samples LOH was also present on two or three other chromosomes (3p, 11p, 12p, 18q and 21q) (Tab. 3).

In addition to LOH, microsatellite instability (MSI) was found in 7 specimens (also on chromosome 3p, 18q and 11p). Five cervical cancers (4 in clinical stage II B, and 1 in clinical stage III A/B) did not demonstrate any LOH or MSI at analyzed microsatellite markers.

Patients with LOH were found to have worse prognosis for survival and relapse-free survival compared to patients without LOH (Fig. 1, 2). This observation was documented for all

**Table 1. Distribution of analyzed cervical cancers according to histologic grade of malignancy and clinical stage (according FIGO classification)**

| | II B | III A/B |
|---|---|---|
| squamous cell carcinoma G2 | 10 | 6 |
| squamous cell carcinoma G3 | 1 | 3 |
| total | 11 | 9 |

**Table 2. Analyzed microsatellite markers and their chromosomal localization**

| Analyzed microsatellite marker | Chromosomal localization |
|---|---|
| D3S1358 | 3p |
| VWA | 12p12pter |
| D16S539 | 16q24-ter |
| rarsid4202484 D2S1338 | 2q35-37.1 |
| D8S1179 | 8 |
| D21S11 | 21q11.2-q21 |
| D18S51 | 18q21.3 |
| D19S433 | 19q12-13.1 |
| TH01 | 11p15.5 |
| FGA | 4q28 |

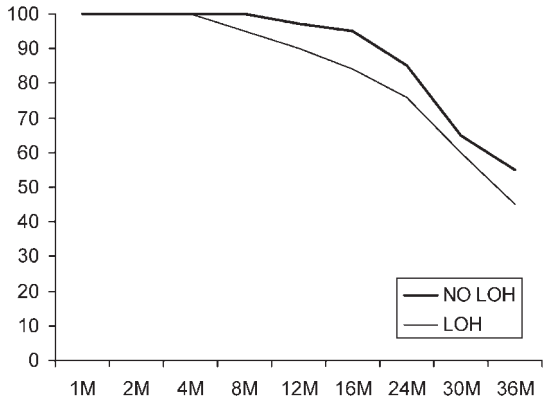


**Figure 1. Overall survival of patients with cervical cancer (squamous cell carcinoma G2).**

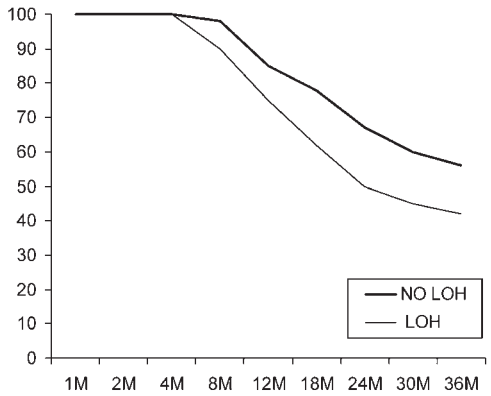


**Figure 2. Overall survival of patients with cervical cancer (squamous cell carcinoma G3).**

**Table 3. Presence of LOH and MSI in cervical cancers**

| Clinical stage | Sample | Analyzed microsatellite markers and their chromosomal localization | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Histologic grade of malignancy | | D3S1358 3p | VWA 12p | D16S539 16q | D8S1179 8q | D21S11 21q | D18S51 18q | TH01 11p | BAT26 2p |
| FIGO II B | | | | | | | | | |
| Squamous cell ca G2 | 1 | | | | | | | | LOH |
| | 2 | MSI | | | | | MSI | LOH | LOH |
| | 3 | | | | | | | | |
| | 4 | | | | | | LOH | | LOH |
| | 5 | LOH | | | | | | | LOH |
| | 6 | | | | | | | | |
| | 7 | LOH | | | | | MSI | MSI | LOH |
| | 8 | | | | | | | | |
| | 9 | MSI | | | | | | | LOH |
| | 10 | | | | | | | | |
| Squamous cell ca G3 | 11 | MSI | | | | | LOH | LOH | LOH |
| FIGO III A/B | | | | | | | | | |
| Squamous cell ca G2 | 12 | LOH | | | | | | | LOH |
| | 13 | LOH | | | | | MSI | LOH | LOH |
| | 14 | | | | | | | | LOH |
| | 15 | LOH | | LOH | | | LOH | | LOH |
| | 16 | LOH | | | LOH | | MSI | | LOH |
| | 17 | | | | | | | | |
| Squamous cell ca G3 | 18 | LOH | | | | | LOH | LOH | LOH |
| | 19 | MSI | LOH | | | | LOH | MSI | LOH |
| | 20 | LOH | | | | LOH | LOH | | LOH |

the examined patients and also in a separate analysis for patients in FIGO stage II B.

## Discussion

Chromosome arm 6p is one of the most frequently involved in loss of heterozygosity in patient with cervical carcinoma [11–13]. Chromosome 6p21.2 presents LOH in about 45% [14]. Other most common sites of LOH are 3p, 11q, 17p, 18q. LOH were frequently found on 3p21.1 (41%) [15]. About 40% of squamous cell carcinoma of uterine cervix had LOH on 11q23.3 and 11q22 [8, 15]. On chromosome 17p13.3 LOH was demonstrated in about 35% of specimens [14, 15].

The present study revealed that 75% cervical cancers (squamous cell carcinoma G2 and G3) manifested LOH. The most frequent site of LOH was chromosome 2p (in BAT 26 analysis). LOH was found also on 3p (40%), on 18q21.3 (30%) and on 11p15.5 (20%). All cases of G3 *squamous cell carcinoma* of the cervix manifested LOH at chromosome 2 (BAT 26 analysis) however in many of them LOH was present also on chromosomes 3p, 18q and 11p. Other authors [15, 16] did not find any correlations between presence of LOH and histological type of cervical cancer. However genetic alterations increasingly appear with the tumor growth. It was demonstrated that bigger tumors and tumors in more advanced stage have a higher percentage of LOH [5, 14]. Patients with metastases in lymph nodes often show LOH [8, 12]. In our study almost all of the cervical cancers in clinical stage III showed LOH.

A correlation between the presence of LOH and cervical cancer prognosis was demonstrated [6. 8. 11]. Patients with LOH on 11q23.3 had significantly more recurrences compared to patients without this abnormality [8]. LOH on chromosome 6p21.2 correlated with high rate of recurrence after radiotherapy [11]. Presence of LOH on chromosome 6q and 18q correlates with poor survival [6, 11, 15]. Patients with LOH on chromosome 6p21.2 and 18q21.2 exhibited overall and disease-free survival after radical radiotherapy [6, 11] significantly shorter as compared with those without LOH on these chromosomes. The results of this study suggest that LOHs on 2p, 3p, 18q and 11p correlate with recurrences of the cervical cancer. A shorter relapse-free survival time and overall survival in the cases with LOH on chromosome 2p, 3p, 18q and 11p was documented in comparison to the cases without LOH.

The data suggest that LOH may reflect genomic instability in squamous cell carcinoma of the uterine cervix and seems to be a connected with poor prognosis in patients with cervical cancers. LOH analysis may represent a value in the assessment of biological activities of the cervical carcinoma.

## References


[1] RUCINSKA M, KOZLOWSKI L, PEPINSKI W, SKAWRONSKA M, JANICA J, WOJTUKIEWICZ MZ. High grade sarcomas are associated with microsatellite instability (chromosom 12) and loss of heterozygosity (chromosom 2). Med Sci Monit 2005; 11(2): 65–68.

[2] CADUFF RF, JOHNSTON CM, SVOBODA-NEWMAN SM, POY EL, MERAJVER SD, FRANK TS. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 1996; 148: 1671–1678.

[3] FUJIWARA Y, CHI DD, WANG H, KELEMAN P, MORTON DL et al. Plasma DNA microsatellites as tumor-specific markers and indicators of tumor progression in melanoma patients. Cancer Res 1999; 59: 1567–1571.

[4] PAULSON TG, WRIGHT FA, PARKER BA, RUSSACK V, WAHL GM. Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res 1996; 56: 4021–4026.

[5] CHEUNG TH, CHUNG TK, POON CS, HAMPTON GM, WANG VW, WONG A. Allelic loss on chromosome 1 is associated with tumor progression of cervical carcinoma. Cancer 1999; 86: 1294–1298.

[6] HARIMA Y, SAWADA S, NAGATA K, SOUGAWA M, OHNISHI T. Chromosome 6p21.2, 18q21.2 and human papilloma virus (HPV) DNA can predict prognosis of cervical cancer after radiotherapy. Int J Cancer 2001; 96: 286–296.

[7] MANOLARAKI MM, ARVANITIS DA, SOURVINOS G, SIFAKIS S, KOUMANTAKIS E, SPANDIDOS DA. Frequent loss of heterozygosity in chromosomal region 9pter-p13 in tumor biopsies and cytological material of uterine cervical cancer. Cancer Lett 2002; 176: 175–181.

[8] O'SULLIVAN MJ, RADER JS, GERHARD DS, LI Y, TRINKAUS KM et al. Loss of heterozygosity at 11q23.3 in vasculoinvasive and metastatic squamous cell carcinoma of the cervix. Hum Pathol 2001; 32: 475–478.

[9] KUNKEL LM, SMITH KD, BOYER SH, BORGAONKER D, WACHTEL SS et al. Analysis of human Y chromosome-specific retired DNA in chromosome variants. Proc Natl Acad Sci 1977; 74: 1245–1249.

[10] BUDOWLE B, ALLEN R. Discontinous polyacrylamide gel electrophoresis of DNA fragments. In: Matthew C, editor. Protocols in Human Molecular Genetics. The Humana Press Inc. 1991.

[11] HARIMA Y, SHIRAHAMA S, HARIMA K, AOKI S, OHNISHI T, TANAKA Y. Genetic alterations on chromosome 17p associated with response to radiotherapy in bulky cervical cancer. Br J Cancer 1999; 81: 108–113.

[12] HUETTNER PC, GERHARD DS, LI L, GERSELL DJ, DUNNIGAN K et al. Loss of heterozygosity in clinical stage IB cervical carcinoma relationship with clinical and histopathologic features. Hum Pathol 1998; 29: 364–370.

[13] KERSEMAEKERS AM, KENTER GG, HERMANS J, FLEUREN GJ, VAN DE VIJVER MJ. Allelic loss and prognosis in carcinoma of the uterine cervix. Int J Cancer 1998; 79: 411–417.

[14] HARIMA Y, HARIMA K, SAWADA S, TANAKA Y, AOKI S, OHNISHI T. Loss of heterozygosity on chromosome 6p21.2 as a potential marker for recurrence after radiotherapy of human cervical cancer. Clin Cancer Res 2000; 6: 1079–1085.

[15] KERSEMAEKERS AM, HERMANS J, FLEUREN GJ, VAN DE VIJVER MJ. Loss of heterozygosity for defined regions on chromosomes 3, 11 and 17 in carcinomas of the uterine cervix. Br J Cancer 1998; 77: 192–200.

[16] NISHIMURA M, FURUMOTO H, KATO T, KAMADA M, AONO T. Microsatellite instability is a late event in the cercinogenesis of uterine cervical cancer. Gynecol Oncol 2000; 79: 201–206.