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ORIGINAL ARTICLE

Does Micronucleus Score Significantly Correlate with Dysplasia in Cervical Pap Smears?

Taraknath Mahanta[1], Debarshi Saha[1], Priyanka Roy[1], Isha Agarwal[1], Barnali Maiti[1], Nikhil Kumar[1]

[1]*Department of Pathology, IQ City Medical College, Durgapur, West Bengal India*

Background: The grade of dysplasia on cervical pap smears may be indicated by micronucleus (MN) scoring, much like cancers of the oral cavity, urinary bladder, and esophagus. **Methods:** Cross-sectional study. MN scores of 106 subjects comprising all major diagnostic categories included in "The Bethesda system, 2014 for reporting cervical pap smears" were taken. High grade squamous intraepithelial lesion (HSIL) and Invasive carcinoma (IC) were further grouped as "high-risk" and the rest, "low-risk" to construct receiver operating characteristic (ROC) curve to seek a cutoff delineating the two classes. Analysis of variance was used to determine the significance of differences in MN scoring between the various groups. **Results:** The difference of mean MN scores of HSIL (9.4) and IC (10.7) was significant from the low-risk group but not within themselves. A huge difference in MN scores between low-grade squamous intraepithelial lesions and HSIL is notable. The difference of mean age was significant between high and low-risk groups. ROC curve delivered a cutoff of 5.15 to distinguish between the two categories with 85.7% sensitivity, 97.2% specificity, and 93.3% accuracy. **Conclusions:** Sequential and significant increase of MN score from low- to high-grade dysplasia is established by the current study. A cutoff of 5.15 MN scores adequately detects HSIL and IC. Despite its performance, MN scoring is time-consuming, labor-intensive, and strenuous process, which might make it difficult to impose on laboratories and pathologists.

Key words: High grade squamous intraepithelial lesion, invasive cancer, receiver operating characteristic curve, cut-off

INTRODUCTION

Cervical cancer is the fourth-most common cancer among women worldwide, representing 7.9% of all female cancers, and it is the second leading cause of cancer mortality in Indian women. The incidence of cervical cancer varies widely and about 90% of deaths due to cervical cancer occur in developing nations.[1] In a report published in 2017 from an International agency for research on cancer, 122,844 cases with 67,477 deaths from cervical cancer were reported. In women aged 15–44 years, cancer cervix holds the second position in cancer incidence among women. The highest age-standardized incidence of cervical cancer is 22% in India, compared to 19.3 in southern Asia and 14.0 in the world.[2]

Despite its severity, cervical cancer responds favorably to secondary preventive measures when detected early by effective screening and early diagnostic methods.[1]

HPV testing and p16 immunostaining are applied to detect cervical intraepithelial neoplasia (CIN), which may or may not progress to invasive cancer (IC), thus conducing to the reduction of incidence of cervical carcinoma. However, the cost of HPV testing and p16 immunostaining is high, so, in developing countries, cervical cancer goes undetected at higher frequencies than in developed countries. Hence, a simple procedure, the "Micronucleus test (MNT)" may be used in conjunction with the cervical pap. It has been proven to identify the menace of malignancies of the cervix, oral cavity, esophagus, and urinary bladder.[3]

A micronucleus (MN) is an additional small nucleus in the cytoplasm consequent upon chromosomal breakage or chromosomal loss, formed when chromosomes or chromosomal fragments fail to be incorporated into the nucleus during cell

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Corresponding Author: Dr. Debarshi Saha, IQ City Medical College, Durgapur - 713 206, West Bengal, India. Tel: +91-8147593116; Fax: +91-343-2408000. E-mail: devrishi2006@gmail.com

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division.[4] MN serves as a potential biomarker of genotoxicity.[5] Their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident, and therefore, the MNT predicts cancer risk.[6]

A few studies have been done on MN scoring in cervical preneoplastic and neoplastic conditions. The current study evaluates the MN score in the entire gamut of pap smear outcomes from negative for intraepithelial lesion or malignancy (NILM) to IC, including all the preneoplastic and neoplastic diagnoses mentioned in The Bethesda system, 2014.[7]

METHODS

A total of 106 cases consisting of nonneoplastic, preneoplastic, and neoplastic cases were studied. The smears were collected from the archives of the cytology section of the pathology department of our institution from 2012 to 2014 spanning 2 years. The study is approved by Burdwan medical college, Burdwan - 713104. West Bengal. India. The approval number is BMC/PG/149.

As a part of a routine check-up, the pap smears were taken in the outpatient department by the gynecologists and sent to the cytology laboratory where 30 min were allocated for fixation in 95% ethanol before staining with Papanicolaou's stain. The microscopic examination followed to evaluate for cytoplasmic and nuclear characteristics so that the smear could be categorized into one of the diagnostic classes as propounded by "The Bethesda system, 2014." Review was conducted for all smears for micronuclei scoring. The original diagnoses were taken into account while calculating the statistics.

Cells tangled in clusters and overlapped cells where their delineation of cytoplasmic and nuclear borders were compromised were debarred, just individual cells lying in a clean background were included to be counted for MN. Cytoplasmic remnants, apoptotic, and degenerated cells also made the exclusion list while counting and scoring MN. The zigzag method was used to screen the slide.[8]

The MN diameter varied from 1/16 to 1/3 nuclear diameter while retaining the nucleus' shape, color and texture with similar or sometimes, trifle lower staining intensity. The round-to-oval-shaped MN was found to lie near the nucleus without attaching to it and could be focused on the same plane. A score of 1 was bestowed on cells harboring single/multiple MN. After being screened first by two pathologists, it was subjected to review by a third observer and the final scores decided in consensus. A total of 2000–3000 cells were counted per smear, and the MN score was conveyed per 1000 cells.[9]

The results were then subjected to statistical evaluation using 'SPSS software for WINDOWS v.20, IBM, New York, USA. The significance of variance between the mean MN scores of the diagnostic groups was analyzed by one-way analysis of variance (ANOVA). The least significant difference/least square deviation test was used to calculate the *P* value. The diagnostic groups were divided into two classes – the low/very low and the high risk. The low risk comprised of NILM, REA, atypical squamous cells of undetermined significance (ASC-US) and low grade squamous intraepithelial lesion (LSIL). The high grade squamous intraepithelial lesion (HSIL) and IC together defined the high-risk class because of a significant 33%–50% of HSILs progress to IC. In contrast, most LSILs revert to normal, just 16%–25% advance toward HSIL.[10]

In yet another reference, we find that 30% of the HSILs regress, 60% persist and only 10% progress to frank invasive carcinoma.[11] Nevertheless, patients were found to be 25 times at risk of cancer on a long-term (5-year) follow-up after initial detection and conservative therapy of HSIL.[12]

A receiver operating characteristic (ROC) curve was constructed to test the efficacy of MN score to distinguish between the two groups or, rather, detect HSIL and IC. The sensitivity added to specificity subtracted from 100 constitutes the Youden's index (YI), i.e., YI = (sensitivity + specificity) – 100. The cutoff with the highest YI is accepted as the best achievable with any particular ROC curve.

RESULTS

Of the 106 patients, 20 were diagnosed cytologically as NILM having mean age of 41 years, 20 as "reactive cellular changes associated with inflammation" (REA) with mean

Table 1: Age distribution of cases selected for micronucleus scoring stepwise gradual increase in micronucleus score from inflammatory to atypical squamous cell of undetermined significance to low grade squamous intraepithelial lesion to high grade squamous intraepithelial lesion group, followed by a slight increase in invasive carcinoma

Group	Number of cases	Age range (years)	Mean age (years)	MN score±SD
NILM	20	24-57	41	0.5069±0.3501
REA	20	20-62	43	0.5186±0.4324
ASCUS	16	28-64	45	1.9607±0.9689
LSIL	15	23-55	43.2	3.6585±1.3708
HSIL	17	26-69	48.4	9.3931±4.550
IC	18	35-70	52	10.7008±4.1946
Total	106	-	-	

LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, MN: Micronucleus, SD: Standard deviation, NILM: Negative for intraepithelial lesion or malignancy, ASCUS: Atypical squamous cell of undetermined significance, IC: Invasive carcinoma, REA: Reactive changes associated with inflammation

age of 43 years and 16 as ASC-US. The LSIL comprised of 15 cases, the HSIL encompassed a further 17 cases with a mean age of 48.4 years and the "IC," 18 cases with a mean age of 52 years [Table 1]. The difference of mean age was insignificant with ANOVA except between high risk and the low-risk groups since most of the low risk groups were in the early 40s. The HSIL patients were in the late 40 s and IC, in early 50 s. Again, the mean age difference between IC and HSIL cases was not significant.

We received biopsy specimens for four cases of ASCUS and all HSIL and IC cases for histological correlation. The biopsy was not available in NILM, REA, and LSIL categories. While the biopsy of the HSIL cases showed either a CIN II/III, the IC cases showed invasive squamous cell carcinomas in all of their biopsies. Thus, no discordance between cytological and histological findings was found in the HSIL and IC categories. Of the four available biopsies of the ASC-US cases, three showed chronic cervicitis and one, (CIN) I.

Two observers separately and independently counted the number of micronucleated cells per 1,000 of epithelial cells in oil immersion magnification (×100 objective).

The mean MN score was found to be 0.5069 ± 0.3501 in NILM cases, 0.5186 ± 0.4324 in REA, 1.9607 ± 0.9689 in ASCUS, 3.6585 ± 1.3708 in LSIL, 9.3931 ± 4.550 in HSIL and 10.7008 ± 4.1946 in IC [Table 1]. The MN score went gradually and steadily uphill from NILM to ASC-US, a slight step-up to LSIL, and thence, a huge leap to HSIL, followed by a casual canter to IC as shown in Figure 1a.

The key facts revealed from MN scores found in the different diagnostic groups are obtained by ANOVA [Table 2]. They are:

1. The MN score was significantly higher in IC compared to all the groups ($P = 0.000$) except HSIL ($P = 0.143$)
2. The difference in MN score between that of HSIL and other groups was significant ($P = 0.000$) except IC ($P = 0.143$)
3. The MN score of LSIL was significantly different from that of other groups ($P = 0.000$) except ASC-US ($P = 0.204$)
4. The difference of MN scores between that of NILM and REA was insignificant ($P = 0.989$).

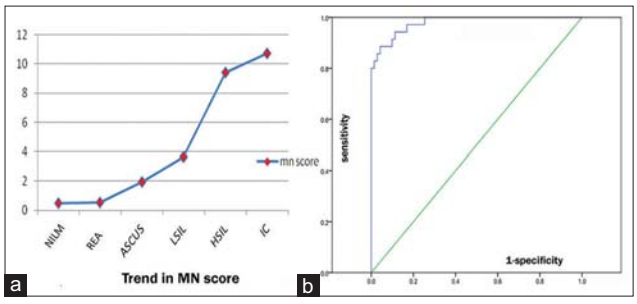

Figure 1: (a) Trend of micronuclear score in cervical lesions. (b) Receiver operating characteristic curve

The Area under the curve is 0.979 [Figure 1b]. The MN count of 5.15 detects HSIL + IC with 85.7% sensitivity and 97.2% specificity yielding a YI of 82.9. At a lower MN count of 3.86, with sensitivity and specificity of 94.3% and 88.7%, the YI was 83. Since the distribution was skewed with 71 cases in the low-risk group and 35 in the high-risk group, accuracy came to play a bigger role. At an MN score of 5.15, the accuracy was 93.3%, while a 3.86 cutoff achieved just 90.5% accuracy. Thus, an MN score of 5.15 was considered to be the cutoff in the current study.

Figure 2 demonstrates the micronuclei across all the Bethesda, 2014 diagnostic categories. The nuclear enlargements in ASCUS, mild nuclear irregularity in LSIL contrast well with the marked nuclear abnormalities in HSIL and IC.

DISCUSSION

Studies involving oral squamous cell carcinoma (OSCC) reveal that MN in oral mucosal with OSCC was threefold to fourfold more frequent than that of controls.[13] Exfoliated cervical cells exposed to few risk factors for cancer cervix are more likely to express a greater prevalence rate of MN than those without exposure.[14]

From previous studies, we know that MN indicates chromosomal damage or aberrations rather than the risk of cancer and since, chromosomal defects underlie most cancers, the MNT is used as a biomarker for predicting cancer risk. MN scoring has been used to assess the risk of malignant transformation in the uterine cervix.[15]

Guzmán *et al*.[16] in 2003 noted that HSIL smears had the highest MN frequency of 33% followed by LSIL at 18%. IC showed a modest 16%. The MN frequency of irradiated cells was expected to bear the highest magnitude of chromosomal aberrations induced by the radiation itself and thus were

Table 2: Result of analysis of variance (*P*) (*post hoc* test); *P* value significant at the level of ≤0.05

	NILM	REA	ASCUS	LSIL	HSIL	IC
NILM		0.989	0.015	0.000	0.000	0.000
REA	0.989		0.016	0.000	0.000	0.000
ASCUS	0.015	0.016		0.204	0.000	0.000
LSIL	0.000	0.000	0.204		0.000	0.000
HSIL	0.000	0.000	0.000	0.000		0.143
IC	0.000	0.000	0.000	0.000	0.143	

LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, NILM: Negative for intraepithelial lesion or malignancy, ASCUS: Atypical squamous cell of undetermined significance, IC: Invasive carcinoma, REA: Reactive changes associated with inflammation

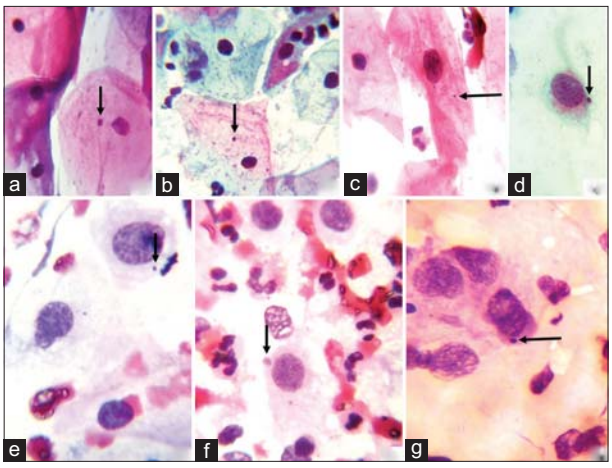

Figure 2: (a) Showing micronucleus (arrow) in cytologically negative for intraepithelial lesion or malignancy smears (Papanicolaou stain, ×1000). (b) Showing micronucleus (arrow) in cytologically REA smears (Papanicolaou stain, ×1000). (c) Showing micronucleus (arrow) in cytologically atypical squamous cells of undetermined significance smears (Papanicolaou stain, ×1000). (d) Showing micronucleus (arrow) in cytologically low grade squamous intraepithelial lesion smears. (Papanicolaou stain, ×1000). (e and f) Showing micronucleus (arrow) in cytologically high grade squamous intraepithelial lesion smears. (Papanicolou stain, ×1000). (g) Showing micronucleus (arrow) in cytologically IC smears (Papanicolaou stain, ×1000)

considered positive controls. However, the MN frequency of HSIL was the highest but not significantly higher than that of LSIL. The LSIL, HSIL, and IC showed a significantly higher MN frequency than smears within normal limits. In contrast, the present study showed that the MN score of HSIL was significantly higher than LSIL ($P < 0.000$).

A similar study by Gayathri *et al*.[17] in 2012 also found that there was a gradual increase in MN frequency from NILM to LSIL as in our study, and there is also a significant difference in MN frequency between LSIL and HSIL. Their MN scores were comparable to ours in the precancerous and cancerous lesions, i.e., 8.03 and 10.05 in HSIL and IC respectively versus 9.39 and 10.7 correspondingly in our study. The quantum leap in the trend of MN scores from LSIL to HSIL found in our study is similar to that of Gayathri *et al*. study. Their conclusion that determination of MN scores serves as an ancillary tool for cancer screening, earnestly begs for additional or buttressing evidence since the score at which cancer or high-risk cases may be suspected has not been determined, nor any method about how the scores should be used, described. Gayathri *et al*. went merely as far as to establish that grade of dysplasia are proportional to the MN score and nothing else.

A study by Bueno *et al*.[18] in 2014 showed that the MN frequencies in the different groups were 0.95 ± 1.12 ($n = 223$) in the control group (NILM), 2.98 ± 1.20 ($n = 50$) in ASCUS, 4.04 ± 1.45 ($n = 52$) in CIN I, 5.97 ± 1.83 ($n = 30$) in CIN II, 7.29 ± 1.55 ($n = 17$) in CIN III, and 8.64 ± 1.55 ($n = 25$) in cervical cancer.[18]

These frequencies were significantly higher in precancerous and cancer groups than that of the control group ($P < 0.001$). It should, however, be noted that CIN is a diagnosis on histopathological sections. The definition of CIN on cytological pap smears is at best sketchy, and therefore, the outcome purported in the said study is speculative. Compared to our study, their MN score was tad low (9.39 and 10.7 in HSIL and IC respectively) versus 7.29 (CIN III) and 8.64 correspondingly in Bueno *et al*. study.[18]

Samanta *et al*.[8] in 2011 performed a study correlating MN scores with the different cytologic diagnostic groups proposed by the Bethesda system, 2001. They also found that a gradual increment of MN scores with an upward trend was noted from the NILM to LSIL. A huge leap was evidenced from LSIL to HSIL, as observed in the current study.

MN antigen (MnAg) expression by immunohistochemistry was studied by Liao and Stanbridge in SIL as well as adenocarcinoma-in-situ cases. All dysplastic cells in both the categories stained positively. They proposed that MnAg expression in conjunction with routine cytology will discriminate between REA and dysplastic cells, both of which might be morphologically atypical.[19]

In yet another study by Shi *et al*.[20] MN scoring was correlated with cytologic diagnoses as well as high-risk HPV infection. They used Kruskal–Wallis test as their statistical tool to determine the mean rank of MN count, which showed a significant difference among the diagnostic groups. From their ROC curve, they determined that an MN count of 7.5 could determine HSIL and IC with 85% and 66% sensitivity and specificity, respectively. The MN scores displayed a similar tendency in the current study. However, our cutoff was tad less, 5.15 versus their 7.5 to distinguish between the high- and the low-risk groups. We achieved a sensitivity and specificity of 85.7% and 97.2%, respectively, yielding a YI of 82.9, whereas their YI was 51 only. At a cutoff of 7.53, our study generates sensitivity approximately 50% and specificity of 100% and an accuracy of 88.6%.

In this context, it should be noted that some oncogenic strains of HPV (16, 18, 31, and 33), which predispose to cervical cancer, elicit HPV proteins, namely E6 and E7 that disrupt cytokinesis and centrosome duplication thereby resulting in chromosome instability. The chromosomal abnormalities arising therein produce the micronuclei.[21,22] Since the chromosomal aberrations accrue as the dysplasia progresses in stages to invasive carcinoma, the HSILs and ICs may exhibit a higher MN frequency.

While performing our study, it was observed that MN scoring puts strain on available time for diagnostics and is immensely labor intensive. It takes three pathologists to arrive

at any concluding score to be effective on any particular case. Moreover, counting 1000 available cells per case is stressful to psyche and sight, particularly to pathologists with weaker eyesight. For all the labor it demands, MN scoring by no way is confirmatory. It may indicate an increased risk, much the same way as morphology does.

In a nutshell, we have observed a significant difference between MN scores of the high-risk group comprising HSIL (9.4) and IC (10.7) and the low-risk group with a cutoff of 5.15 segregating the two. The MN scores of LSIL and ASCUS also differed significantly from normal and inflammatory lesions but not between themselves. However, HSIL and IC reveal insignificant differences. The sequential and significant increase of MN score from the low-grade dysplasia to a higher grade is well established in other studies[8,17,20] and also endorsed by the current study. Only one other study[20] mentioned a cutoff of 7.5 to discriminate between the two groups, albeit with much less sensitivity and specificity than the current study.

CONCLUSIONS

Sequential and significant increase of MN score from low- to high-grade dysplasia in Pap smears is established by the current study. A cutoff of 5.15 MN score adequately detects HSIL and IC with an accuracy of 93.3%, sensitivity of 85.7% and specificity of 97.2% yielding a YI of 82.9. Despite its performance, MN scoring is time-consuming, labor-intensive, and strenuous process, which might make it difficult to impose on laboratories and pathologists alike.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. WHO.(n. d.). Cervical Cancer. Available from: http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/. [Last Retrieved 2018 May 22].
2. ICO Information Centre on HPV and Cancer. Human Papillomavirus and Related Diseases in India; 2017. Available from: http://www.hpvcentre.net/statistics/reports/IND.pdf. [Last accessed on 2018 Oct 25].
3. Gandhi G, Kaur B. Elevated frequency of micronuclei in uterine smears of cervix cancer patients. Caryologia 2003;56:217-22.
4. Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The HUman micronucleus project-an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res 1999;428:271-83.
5. Olaharski AJ, Sotelo R, Solorza-Luna G, Gonsebatt ME, Guzman P, Mohar A, *et al*. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. Carcinogenesis 2006;27:337-43.
6. Aires GM, Meireles JR, Oliveira PC, Oliveira JL, Araújo EL, Pires BC, *et al*. Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix. Genet Mol Res 2011;10:1558-64.
7. Nayar R, Wilbur DC, editors. The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes. ed. 3. New York: Springer; 2015.
8. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta Cytol 2011;55:42-7.
9. Ambroise MM, Balasundaram K, Phansalkar M. Predictive value of micronucleus count in cervical intraepithelial neoplasia and carcinoma. Turk Patoloji Derg 2013;29:171-8.
10. Milde-Langosch K, Riethdorf S, Löning T. Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications. Virchows Arch 2000;437:227-33.
11. Ellenson LH, Pirog EC. The Female Genital Tract. In: Kumar V, Abbas A, Aster J, editors. Robbins and Cotran Pathologic Basis of Disease. ed. 9. Philadelphia: Elsevier Saunders; 2015. p. 1005.
12. McIndoe WA, McLean MR, Jones RW, Mullins PR. The invasive potential of carcinoma *in situ* of the cervix. Obstet Gynecol 1984;64:451-8.
13. Jadhav K, Gupta N, Ahmed MB. Micronuclei: An essential biomarker in oral exfoliated cells for grading of oral squamous cell carcinoma. J Cytol 2011;28:7-12.
14. Reis Campos LM, Luz Dias FD, Antunes LM, Murta EF. Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer. Sao Paulo Med J 2008;126:323-8.
15. Nersesyan AK. Possible role of the micronucleus assay in diagnostics and secondary prevention of cervix cancer: A minireview. Cytology Genetics 2007;41:317-18.
16. Guzmán P, Sotelo-Regil RC, Mohar A, Gonsebatt ME. Positive correlation between the frequency of micronucleated cells and dysplasia in Papanicolaou smears. Environ Mol Mutagen 2003;41:339-43.
17. Gayathri B, Kalyani R, Hemalatha A, Vasavi B. Significance of micronucleus in cervical intraepithelial lesions and carcinoma. J Cytol 2012;29:236-40.
18. Bueno CT, Dornelles da Silva CM, Barcellos RB, da

Silva J, Dos Santos CR, Menezes JE, *et al*. Association between cervical lesion grade and micronucleus frequency in the Papanicolaou test. Genet Mol Biol 2014;37:496-9.
19. Liao SY, Stanbridge EJ. Expression of the MN antigen in cervical papanicolaou smears is an early diagnostic biomarker of cervical dysplasia. Cancer Epidemiol Biomarkers Prev 1996;5:549-57.
20. Shi YH, Wang BW, Tuokan T, Li QZ, Zhang YJ. Association between micronucleus frequency and cervical intraepithelial neoplasia grade in Thinprep cytological test and its significance. Int J Clin Exp Pathol 2015;8:8426-32.
21. Duensing S, Lee LY, Duensing A, Basile J, Piboonniyom S, Gonzalez S, *et al*. The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. Proc Natl Acad Sci U S A 2000;97:10002-7.
22. Duensing S, Duensing A, Crum CP, Münger K. Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. Cancer Res 2001;61:2356-60.