Prospective study of apoptotic index and mitotic index as a predictor of response to radiation in subjects of squamous cell carcinoma of uterine cervix treated by radical radiotherapy

Gorijavolu Durgaprasad

Associate Professor, Dept. of Radiotherapy Government Medical College and General Hospital, Anantapuramu, Andhra Pradesh, India

***Corresponding Author:**
Email: durgaoncodr@gmail.com

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Abstract

Introduction: There exists a wide variation in the response of tumors to radiotherapy and prognosis from individual to individual after radiotherapy to carcinoma of uterine cervix in all stages. Before embarking on treatment one would like to be forearmed with the knowledge that the treatment outcome would be good. Apoptosis is an important mechanism in predicting response to radiotherapy in squamous cell carcinoma of uterine cervix.

Objective: Prospective study to correlate the relation between markers of cell proliferation (MI), cell death (AI), and MI to AI ratio as an independent prognostic factors in patients of carcinoma of uterine cervix treated by radical radiotherapy.

Materials and Methods: 56 evaluable subjects of squamous cell carcinoma of uterine cervix during the period from May 1999 to December 1999 were enrolled in the study and patients were followed for 2 years. Measurement of Apoptotic index (AI) and Mitotic Index (MI) was done by microscopic method only. All subjects received radiation to a dose of 70 Gy using standard technique.

Results: Patients with Apoptotic Index (AI) less than median value of 0.75% and Mitotic Index (MI) greater than median value of 0.2% showed better response to radical radiation therapy at 2 years. However the difference is not statistically significant ($p = 0.20$ and 0.25 respectively)**.** Patients with AI/MI less than median value of 0.3 showed better local as well as overall control of disease when compared to patients with AI/MI ratio greater than median value of 0.3. The difference is statistically significant ($p = 0.03$).

Conclusions: Besides traditional prognostic factors like stage, lympho-vasular invasion, AI/MI ratio will provide a guide for response to radiotherapy and prognosis.

Keywords: Apoptosis index, Mitotic index, Apoptosis index to mitotic index ratio, Squamous cell carcinoma of uterine cervix, Response to radiation therapy and prognosis.

Introduction

Carcinoma of uterine cervix is the most common malignancy seen among the female population of India especially in rural areas of south India. Since over 70 per cent of the Indian population resides in the rural areas, cancer cervix still constitutes the number one cancer in either sex. Based on the data of the PBCRs, the estimated number of new cancers during 2007 in India was 90.708. The relative five year survival reported some time earlier averaged 48.7 per cent.[1] 24.77% of women cancers belong to carcinoma of uterine cervix at government medical college and government general hospital, Anantapuramu, Andhra Pradesh, India as per 2016 statistics. Radiotherapy is the most common non surgical treatment for all stages of carcinoma of uterine cervix and it achieves tumor control in 100% of patients with stage IA, 99% with stage IB, 95% with stage IIA, 91% with stage IIB, and IIIA, 74% with IIIB and 69% with stage IVA. Pelvic tumor control rates at 5 years have been 49% to 64% in stage III.[2-4] However there exists a wide variation in the response of tumors to radiotherapy and prognosis from individual to individual. Before embarking on treatment, one would like to be forearmed with the knowledge that the treatment outcome would be good. Apart from traditional prognostic prognostic markers like stage, histological grade and lympho-vascular invasion, the focus is now on markers of proliferation, cell death and intrinsic radio-sensitivity like Apoptosis in squamous cell carcinoma of uterine cervix.[4] If we know that particular tumor is radioresistant we can choose surgery. If possible we can enhance the radiation dose by conformal radiation or by using multiple fractions daily. We may also prefer concurrent chemo radiation or preoperative chemo-radiotherapy followed by surgery. If we know that a particular tumor is radiosensitive we could get away with lesser dose of radiation within the spectrum of doses needed for radiation dose. We can reduce radiation related early and late morbidity and increase overall quality of life. Apoptosis is now of considerable interest in the field of oncology and the study of apoptosis could well be an important logical step in this direction.[6-8]

Materials and Methods

Selection of Subjects:
Inclusion Criteria:
1. Histologically confirmed squamous cell carcinoma by biopsy
2. Age less than 75 years
3. ECOG performance status 0,1,2
4. Stage I to III
5. No evidence of metastases
6. Normal liver and renal function tests.

Exclusion Criteria:
1. Uraemia
2. Active tuberculosis
3. Involvement of urinary bladder and Rectum

Quantification of Apoptosis and Mitosis: All formalin fixed paraffin embedded sections were stained with H&E stain. Ten high power fields were selected avoiding areas of necrosis within the sections and the total number of tumor cells, Apoptotic cells and cells showing Mitosis were recorded. Cells were scored as Apoptotic, only if they contained pyknotic nuclei showing evidence of nuclear condensation and eosinophilic condensed cytoplasm with or without apoptotic bodies.[9] 1000 tumor cells were scored per specimen and the percentage of Apoptotic cells were determined as Apoptotic index (AI) and the number of cells showing Mitosis was scored as Mitotic Index (MI).

Radiation Therapy technique: External beam radiation (EBRT) was delivered to a dose of 50 Gy to whole pelvis, followed by intracavitary radiation to a dose of 30 Gy by low dose rate Brachytherapy. All subjects received 70 Gy to target volume by combination of EBRT and Brachytherapy[4]. Weekly cis-platin based chemotherapy was given concurrently along with radical radiotherapy.

Assessment and follow up: All subjects were followed up every 3 months for 2years. Those subjects having growth, significant indurated lesion with bleeding on pelvic examination were considered as having residual disease and poor response to radiotherapy. Those having no evidence of disease either clinically or radiologically were considered as having good response to radiotherapy. Those patients having paraaortic, mediastinal, supraclavicular nodes or lung, liver, bone, brain metastasis identified either by clinical or radiologically, without evidence of local disease were considered as systemic failures.

Observations and Results

Out of 67patients entered in to the study only 56 patients were evaluable. Five patients did not receive EBRT and seven patients did not receive intracavitary application were excluded from the study.

AI by morphology ranges from 0.1% to 5% with a median value of 0.75% in the 56 evaluable subjects. MI ranges from 0 to 10% with a median value of 0.2%. Twenty subjects had MI greater than median value of 0.2% and 34 subjects had MI less than median value. AI/MI ratio in our subjects had a median value of 0.3%. Out of 56 patients 27 patients had a AI/MI ratio less than median value. The patient and tumor characteristics of prognostic significance were found to be well balanced in both the groups with AI greater than and less than median value of 0.75% is shown in (Table 1).

A**poptotic Index (AI) and Response to radiation:** Patients with Apoptotic Index (AI) greater than median value of 0.75% showed local control of 75% (7 patients out of 28 patients had residual disease). Patients with AI less than median value of 0.75% had local control of 89.28% (3 patients out of 28 patients had residual disease). However difference was not statistically different (P=0.20). Two patients developed systemic failures and both of them had an AI greater than median value of 0.75%. Overall tumor control rate for patients with AI<0.75% was 89.28%, when compared with 67.85% in patients with AI>0.75%. Out of two systemic failures one patient had disease in para-aortic nodes and another patient developed lung metastases which were detected by CT scan (Table 2).

Mitotic index and (MI) and Response to Radiation: Out of 56 patients, 20 patients had mitotic index greater than median value of 0.2% and 34 patients had MI less than median value of 0.2%. Patients with MI>0.2% had a local control of 90% (18/20) where as patients with MI<0.2% had local control of only 76.47% (26/34). However the difference was not statistically significant (P=0.25) (Table 2).

Apoptotic Index to Mitotic Index Ratio (AI/MI) as Predictor of Response to Radiation: Patients with AI/MI ratio of greater than median value of 0.3% had local control of 70.37% (8/27 had residual disease) where as in patients with AI/MI less than median value of 0.3% had local control of 93.1% (2/29 patients had residual disease). Two systemic failures were seen with AI/MI>0.3% bringing down the overall tumor control rate to 62.96%. AI/MI ratio as prognostic factor showed greater significance for local control and over all control than AI alone. The difference seen in local control was statistically significant (p=0.03). Hence we conclude that AI/MI ratio is a better prognostic factor than AI or MI alone (Table 2).

Multivariate Analysis: A multivariate analysis of the results was done to assess the response patterns in relation to the age of the patients, stage, size of the tumor at diagnosis and differentiation by histopathology.

Age: In our study, patients with age greater than 60 years had a better local control amounting to 100% irrespective of AI whether it was less than median value or greater than median value. Younger age group

patients had poorer response to radiotherapy in both the AI groups. When AI was greater than 0.75%, though the local control was good (2 out of 2 patients) in older age group, but one patient had systemic failure resulting in overall response rate of 50%only. In age group of 41-60 years, those patients with AI<0.75% had a local control of 92.3% (12/13) compared to those patients AI>0.75% who had local control of 66.6% (10/15 patients) one out of 10 patient had a systemic failure in lungs in 3 months of follow up. In the age group of 21-40 years local control was comparable whether the AI was less than or greater than median value (84.6 vs 81.8%) (Table 3).

Stage: The response to radiotherapy in relation to the stage of disease was analysed between the two groups of AI less than and greater than median value (Table 4). Stage IB1 patients in both groups had 100% local control (2/2 in AI>0.75%and 4/4 in <0.75%) but in stage IB2 the local control was 100% when AI was <0.75%where as only 60% (3 out of 5 subjects) in patients with AI >0.75%. Among these 3 subjects with local control one had systemic failure (para- aortic lymphadenopathy by CT scan. Stage IIA and IIB patients with AI<0.75 had 100% local control (4/4) and 71.4% local control (5/7) respectively, in patients with AI>0.75% IIA patients had 80% (4/5) and IIB patients had 50% (2/4) local control. Only one subject was presented with stage IIIA and AI > 0.75% had good local control by radiotherapy. In stage IIIB 10/11 patients with AI < 0.75% had local control of 91%, where as 9 out of 11 patients with AI > 0.75% had local control of 81%. One of these patients with local control had lung metastasis at 4 months after ICR (by CT scan). Better local control was achieved with radiotherapy when AI was less than 0.75% in early as well as in late stages. In patients with greater than 0.75% of AI there was a trend towards poorer local control and systemic control irrespective of stage (stage I –III).

Hemoglobin (Hb%): Local control in this study was analysed in both the groups of < 0.75% and > 0.75% of AI with Hb% less than 70% and more than 70% (Table 5). Subjects with < 70% of Hb% had 77% local control (10/13) in group with AI<0.75% compared to 60% (11/15) in subjects with AI>0.75% two out of eleven subjects with local control in the later group had systemic failure bringing down the overall control to 60%. In subjects with >70% of Hb% had better local control, however subjects with AI <0.75% group had 100% response (15/15) and subjects with >0.75% had 77% (10/13) local control but none had systemic failure.

Tumor Size: Tumor size was assessed by Ultrasonography, and correlation between size of the tumor and local control in both the groups when AI <0.75% and >0.75% were analysed in Table 6. In subjects with tumor size less than 4 cm, better response to RT was seen when AI was < 0.75% (100%) than when AI was >0.75% (62.5%). In large tumors with >4cm there was no significant difference in the response rates whether AI was >0.75% or <0.75% (68.75% vs 65.8%). AI becomes less significant if the size is increasing.

Mitotic Index: Patients with MI<0.2% and >0.2% were analysed with respect to AI>0.75% and <0.75% for local and systemic failures. When MI was <0.2%, subjects with AI>0.75% showed local control of 68.4% and 2 systemic failures, compared to subjects with AI<0.755, who showed a local control of 88.2% and no systemic failures. When MI was >0.2%, there was no difference in local and systemic failure rates whether AI was less than or greater than 0.75%. AI is useful as a prognostic factor only when MI was <0.2% (Table 7).

Table 1: Patient and Tumor characteristics

Characteristic feature		**AI >0.75%**	**AI<0.75%**
Stage	IB1	2	4
	IB2	5	2
	IIA	5	4
	IIB	4	7
	IIIA	1	0
	IIIB	11	11
AGE	20-40	11	13
	41-60	15	13
	>61	2	2
HB%	<50	2	1
	51-60	2	3
	61-70	11	9
	>71	13	15
Size in cm by USG	2.0-4.0	8	14

	4.1-6.0	16	11
	>6.1	4	3
HPE	WD	3	1
	MD	5	6
	PD	2	7
	Unknown	18	14
MI < 3	<3	19	17
	>= 3	9	11

Table 2: Apoptotic Index (AI), Mitotic Index (MI) and AI/MI ratio as predictor of response to radiation

Median value	**Greater than medial value**			**Less than medial value**		
	Local control	%	Systemic failure	Local control	%	Systemic Failures
AI: 0.75	21/28	75.0	2	25/28	89.28	0
MI: 0.2	18/20	90.0	0	26/34	76.47	2
AI/MI	19/27	70.3	2	27/29	93.1	0

Table: 3 Age vs Apoptotic Index (AI)

	AI	**>0.75**			**<0.75%**			
Age	**n**	**Local/ systemic control**	**%**	**Systemic failure**	**n**	**Local/ systemic control**	**%**	**Systemic failure**
20-40	11	9/11	81.81	0	13	11/13	84.6	0
41-60	15	10/15	66.67	2	13	12/13	92.3	0
>61	02	2/2	100	0	02	2/2	100	0

Table 4: Response to radiotherapy in relation to the stage of disease

	AI>0.75%				**AI<0.75%**			
Stage	**n**	**Local control**	**%**	**Systemic Failure**	**n**	**Local control**	**%**	**Systemic failure**
IB1	2	2/2	100	0	4	4/4	100	0
IB2	5	3/5	60	1	2	2/2	100	0
IIA	5	4/5	80	0	4	4/4	100	0
IIB	4	2/4	50	0	7	5/7	71.5	0
IIIA	1	1/1	100	0	0	-	-	-
IIIB	11	9/11	81.8	1	11	10/11	90.9	0

Table 5: Correlation between Haemoglobin and Apoptotic Index (AI) in tumor control

	AI>0.75%				**AI<0.75%**			
Hb%	**n**	**Local control**	**%**	**Systemic failure**	**n**	**Local control**	**%**	**Systemic failure**
<70%	15	11/15	73.3	2	13	10/13	76.9	0
>70%	13	10/13	76.9	0	15	15/15	100	0

Table 6: Correlation between the size of tumor and apoptotic index in control of tumor

	AI>0.75%				**AI<0.75%**			
Size in Cm	**n**	**Local control**	**%**	**Systemic failure**	**n**	**Local control**	**%**	**Systemic Failure**
2 to 4	08	5/8	62.5	0	14	14/14	100	0
4.1 to 6.0	16	12/16	75.0	1	11	9/11	81.8	0
>6.1	04	4/4	100	1	03	2/3	66.7	0

Table7: Mitotic index vs Apoptotic index in tumor control

	AI>0.75%				AI<0.75%			
MI	**n**	**Local control**	**%**	**Systemic failure**	**n**	**Local control**	**%**	**Systemic failure**
<=0.2	19	13/19	68.4	2	17	15/17	100	0
>0.2	09	8/9	88.9	0	11	9/11	81.8	0

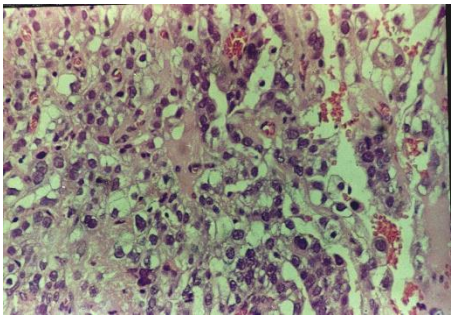

Fig. 1: Field showing malignant cells with pleomorphic nuclei and comet shaped Apoptotic cells (H&E X 250)

Discussion

Radiotherapy is the most common non-surgical treatment of carcinoma cervix in all stages.[4] However, there exists a variable response to radiation therapy. The various studies to predict the outcome, apart from clinical stage, Histological variety, tumor grade and lympho-vascular Invasion include markers of tumor proliferation (S-Phase fraction, Labeling index- Br.dU, Flow- cytometry, mitotic index, staining for Ki-67 antigen and potential doubling time) and markers of cell death SF2 and APOPTOSIS.[6-8] Assessment of Apoptosis is a simple, time conserving and reliable method that can be done in routine pathological laboratory without any delay in initiation the treatment modality.

Keeping all these factors in mind we have conducted a prospective study to asses the significance of AI and AI/MI ratio in predicting biological behavior of the tumor and response of Radiation and ultimately the prognosis.

We assessed the AI by morphological criteria, and we found that AI ranges from 0-5% with a median value of 0.75%. Almost all the studies on Apoptosis used morphological criteria only to assess the AI except SHEREDIAN, et al who used TUNEL method.[10]

Levine et al studied AI by morphology with values ranging from 0-4% with a mean and median of 0.91% and 0.71% respectively.[17] Wheeler et al observed a median value of AI 2% (range 0.2% - 10.9% with a mean of 2.5%).[18] Tsang et al AI ranges from 0 - 6.8% with a median of 1% and mean of 1.6%.[19] Sheridian et al assessed AI by morphology as well as by TUNEL method and combination of morphology and TUNEL method for 38 patients. By morphology only they found the AI range from 0.1% to 3.8% with a median of 1.05%. When AI was quantified by TUNEL assay alone, they found AI ranges from 0 - 3.05% with a median of 1.1% with combination of morphology and TUNEL method AI ranges from 0 – 3.55% with a median of 1.58%. They concluded that the mean number of Apoptotic events increases when AI was quantified by a combination of TUNEL method and morphology. This was due to high incidence of false negatives seen with TUNEL method (TUNEL negative with clear Apoptotic morphology).[10]

We analyzed the patients with AI with < 0.75% and >0.75% with respective age, stage, tumor size, Hb% and MI. In patients with more than 60 years of age AI had no prognostic significance in our study. In the age group of 41 – 60 years AI < 0.75% showed better prognosis. In the 22-40 years age group AI showed no prognostic significance. No other study had correlated AI with age with regard to local control. With regard to stage, better local control was achieved in all stages when AI was < 0.75%. There was a trend towards poorer local and systemic control in our study when AI was > 0.75%. Levine et al in his study concluded that after allowing for the stage, the AI was still a significant predictor of patient survival (P=0.05) suggesting independence of the two parameters.[17, 22] In our study Hb% and AI showed no correlation with local or systemic control in patients with Hb% < 70%. Those patients with > 70% Hb% better local control was seen when AI < 0.75%. Various studies have shown that low Hb% is associated with poorer prognosis.[11, 16] Wheeler et al have analyzed Hb% along with AI. However they could not show any relationship with regard to local control or survival, as their findings were inconclusive.[18] We analyzed the relationship between tumor volume, AI and response to Radiation. AI was of less prognostic significance in larger tumors. This may be due to higher proliferation rate in larger tumors. Tsang et al have also shown that AI was prognostic significance in smaller tumors only.[19, 20] In our study AI was of no prognostic significance when MI was greater than 0.2%. Sheridan et al have shown that high AI and low MI predicted better survival.

We have considered AI as a sole prognostic factor in our study. Patients with AI> 0.75% had poorer local control (75%) and higher systemic failures (2 subjects) when compared to patients with AI < 0.75% who had 89% local control and no systemic failures. However the differences were not statistically significant (p=0.20). Levine et al have concluded in their study that a high level of Apoptosis is a poor prognostic

indicator.[17] However Tsang et al failed to show correlation between Apoptosis and clinical outcome.[19,20] The correlation between increased Apoptosis and poorer treatment outcome may be due to increased tumor proliferation.

Mitotic index (MI) is one of the morphological indicators of tumor proliferation. We have analyzed MI as a sole prognostic factor and found that MI> 0.2% had better local control than when MI was < 0.2%. This may be due to a higher number of patients in the group of > 0.2% MI. It may also indicate the better response to Radiation therapy in rapidly proliferative tumors. The difference in response has not achieved statistical significance (p=0.25). Tsang et al have taken a median of 0.7% and showed better prognosis when MI is < 0.7%. However the difference was not statistically significant.[19, 20] Levine et al showed a correlation between AI and MI but failed to show a relationship between MI and patient outcome.[17] Sheridan measured MI as part of TUNEL method and took median of 0.75%. They showed a poor survival when MI was > 0.75%, but failed to achieve statistical significance.[10]

The AI/MI ratio was analyzed for local control in our study. We have taken a median of 0.3. The patients who had AI/MI ratio > 0.3 fared significantly worse. The local control rate was only 70% when AI/MI was 0.3, where as it was 93% when AI/MI was < 0.3%. The differences are statistically significant (0.03). Sheridan et al have shown a high ratio of AI/MI in Adinocarcinoma of cervix indicates good prognosis.[10] Various other studies have also shown a correlation between Apoptosis and tumor proliferation but failed to analyze AI/MI ratio with relation to patient outcome.

Conclusions

1. Patients with Apoptotic Index (AI) less than median value of 0.75% and Mitotic Index (MI) greater than median value of 0.2% showed better response to radical radiation therapy at 2 months. However the difference is not statistically significant (p = 0.20 and 0.25 respectively).
2. Patients with AI/MI less than median value of 0.3 showed better local as well as overall control of disease when compared to patients with AI/MI ratio greater than median value of 0.3. The difference is statistically significant (p = 0.03).
3. In multivariate analysis patients with AI less than median value showed better response to radiation in each sub-category of age, stage and hemoglobin levels.
4. Patients with AI greater than 0.75% and MI less than 0.2% showed systemic failures with local control by Radiation therapy. Hence these patients may be benefited by systemic chemotherapy.

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