Review Article

Prognostic significance of Bcl-2 expression in carcinoma of the uterine cervix: A systematic review and meta-analysis

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ABSTRACT

Apoptosis is the final common pathway in cellular death induced by radiation and chemotherapy. Antiapoptotic protein bcl-2 plays an essential role in the determination of cellular threshold in the process of apoptosis. Immunohistochemical evaluation of bcl-2 has been one of the most widely investigated prognostic markers in cervical cancer. Given the lack of conclusive evidence in the literature, we aimed to systematically review the evidence to use bcl-2 as a prognostic marker for overall (OS) and disease-free survival (DFS) in cervical cancer. We reviewed the studies after a systematic literature search, reporting either OS or DFS. Estimates were extracted from these studies, and a meta-analysis was done. Positive bcl-2 expression was associated with a decreased risk ratio (RR) for OS. The estimated log RR was −0.52 (confidence interval = −0.91−−0.13, RR = 0.60\0.40, 0.88], $P < 0.001$). No significant association was found with DFS. There was significant heterogeneity among the studies. Bcl-2 can be used as a molecular marker for OS in cervical cancer. It can be helpful to identify a group of patients who might be good responders in locally advanced cervical cancer and help in clinical decision-making to prognosticate the disease.

Keywords: Uterine neoplasms, bcl-2, Apoptosis, Cervical cancer, Chemoradiotherapy

INTRODUCTION

Cervical cancer is one of the most common malignancies in low and middle-income countries.[1] Given the high disease burden and scarcity of available treatment facilities, prognostication of disease is essential. Recently, Noordhuis *et al.* reviewed the prognostic significance of various cell biological markers for patients treated with radiation with or without chemotherapy.[2] Radiation or chemotherapy leads to the induction of multiple molecular pathways, which ultimately culminate in cell death through apoptosis.[3] The apoptotic threshold is related to the balance between proapoptotic (BAX or Bcl-2 associated X protein, BAK or Bcl-2 antagonist killer) and anti-apoptotic group of proteins (FLICE inhibitory proteins, Bcl-2, and IAP or inhibitors of apoptosis protein).[4] Literature evidence suggests that biomarkers for apoptosis are related to prognosis in breast cancer, lung cancer, and lymphoma.[5,6] Among all these markers, the Bcl-2 homology domain (BH) containing proapoptotic (BAX) and anti-apoptotic proteins (Bcl-2) are the primary determinants of the cellular apoptotic process.[4] The biological activity of

these proteins is determined by their ability to form homo or heterodimers and regulate the mitochondrial membrane permeability and electrical conductance.

Bcl-2 is an antiapoptotic protein or death antagonist in the mitochondrial membrane, endoplasmic reticulum, and nuclear envelope.[7] In normal cervical epithelium, it is shown to be localized in the basal layer, and increased expression has been reported in cervical intraepithelial neoplasia (CIN).[8,9] Expression of bcl-2 has been linked to the progression of premalignant cervical lesions to invasive malignancy.[10] Various studies have evaluated the prognostic value of bcl-2 as a predictor of treatment outcomes in cervical cancer, and the immunohistochemical method has been used in most of the studies.[11-13] The prognostic role of bcl-2 expression on treatment outcome is still controversial.[14] There is significant heterogeneity concerning the biomarker evaluation, threshold value, and quantitative or qualitative assessment method. Here, we report a systematic review and meta-analysis of the role of bcl-2 in the prognosis of cervical cancer based on available literature.

MATERIALS AND METHODS

Search strategy

This systematic review and meta-analysis were aimed to address the research question "What is the prognostic significance of immunohistochemical expression of bcl-2 in cervical cancer?" and were conducted as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[15] The search strategy and keywords used are tabulated in Table 1. The following medical literature databases were searched: PubMed, Scopus, Cochrane Library, and ScienceDirect (the last search was done on 12th January 2024). We manually checked the reference list of all the identified articles and were manually searched for any additional studies.

Inclusion and exclusion criteria

For inclusion in the meta-analysis, the following inclusion criteria were laid down: (1) Studies that described the treatment outcome of patients with invasive cervical cancer (treatment could be surgery, radiotherapy, and or chemotherapy), (2) studies were data on pre-treatment expression level of Bcl-2 from the primary tumor was available, (3) retrospective or prospective study (cohort, randomized studies were included), (4) data should be sufficient to allow estimation of risk ratio (RR), and (5) studies published in English language only were included. Letters to editors, review articles, papers published in languages other than English, and papers published in books were excluded from the meta-analysis.

Table 1: Search criteria used to identify the studies (PubMed).

Search set	PubMed
1	Bcl-2
2	BCL-2
3	Bcl
4	bcl*
5	B-cell CLL/lymphoma 2
6	APOPTOSIS REGULATORY PROTEINS
7	APOPTOSIS
8	UTERINE CERVICAL NEOPLASM
9	1–7/OR
10	8 AND 9
11	prognos*
12	10 AND 11

CLL: Chronic lymphoid leukemia. Words in capital letters are MeSH terms.
SCOPUS SEARCH: (TITLE-ABS-KEY ("bcl") AND TITLE-ABS-KEY ("cervical cancer")) AND ((prognos*)) AND (LIMIT-TO (EXACTKEYWORD, "Human") OR LIMIT-TO (EXACTKEYWORD, "Female") OR LIMIT-TO (EXACTKEYWORD, "Humans") OR LIMIT-TO (EXACTKEYWORD, "Protein bcl 2") OR LIMIT-TO (EXACTKEYWORD, "Uterine Cervical Neoplasms") OR LIMIT-TO (EXACTKEYWORD, "Uterine cervix cancer")), *wildcard symbol that broadens a search by finding words that start with the same letters.

Data extraction

The data collection form was designed to collect relevant information from the selected studies. If an article was not selected, then the reason for exclusion was documented. The following data were extracted from each study: the surname of the first author, year of publication, period of recruitment, the country where the study was conducted, the total number of patients, stage and grade of the tumor, and the treatment given. Data regarding the method of assessment of prognostic factor (bcl-2) were collected, as well as criteria for determining the positivity of the biomarker wherever available. The primary outcomes were entered in the form of a table showing the overall (OS) and disease-free survival (DFS) with respect to bcl-2 expression.

Statistical analysis

The outcome measure considered for the data pooling was the log risk ratio (lnRR) and its variance. The lnRR was calculated for each study based on the deaths in each group and the total number of subjects studied in each group. When the number of deaths was not given directly, it was estimated using the survival probability provided in each group. The random effects model approach was used to calculate a weighted estimate of the treatment effect across the studies. The heterogeneity among studies was assessed through a χ^2 statistic with n-1 degrees of freedom (I^2), and if the results of studies were heterogeneous ($I^2 > 70\%$), the effects of possible explaining factors were explored. All calculations and statistical tests were done using R (Version 3.2.0) software.

RESULTS

Eligible studies

A total of 292 articles were retrieved by electronic search. After removing duplicates using the search criteria as shown in Table 1. A total of 24 studies were assessed for eligibility after initial screening and reviewing the abstract. Among these studies, 16 were included in the meta-analysis[10,13,16-29] [Figure 1]. The sixteen studies' characteristics are summarized in Table 2. These studies reported at least one of the survival outcomes of interest (14 studies reported overall, and eight studies reported DFS) and were selected for meta-analysis. The sample size of these included studies ranged from 29 to 259 (mean 101). Total number of patients included in all the studies was 1612. Immunohistochemistry (IHC) to detect bcl-2 was done upfront before starting treatment in all these studies. The staining pattern, percentage of patients who were positive for bcl-2, and the

cutoff value, as reported in the studies, are presented in Table 3. The average prevalence of bcl-2 positivity in the entire population was 49.2% (10–75%).

Data and analysis evidence synthesis

Overall Survival

A total of 14 studies ($n = 1509$ patients) reported OS. Positive bcl-2 expression was associated with the decreased RR for OS (lnRR = −0.52; confidence interval: [C.I.] −0.91–−0.13; $I^2 = 76.89$; RR = 0.60 [0.40, 0.88]; $P < 0.001$). The forest plot and corresponding funnel plot are shown in Figure 1. Six of the included studies showed decreased risk with bcl-2 expression, seven were equivocal, and one study showed expression of bcl-2 to be associated with poor OS. The Egger test showed that there was no evidence of publication bias ($P = 0.473$) [Figure 2].
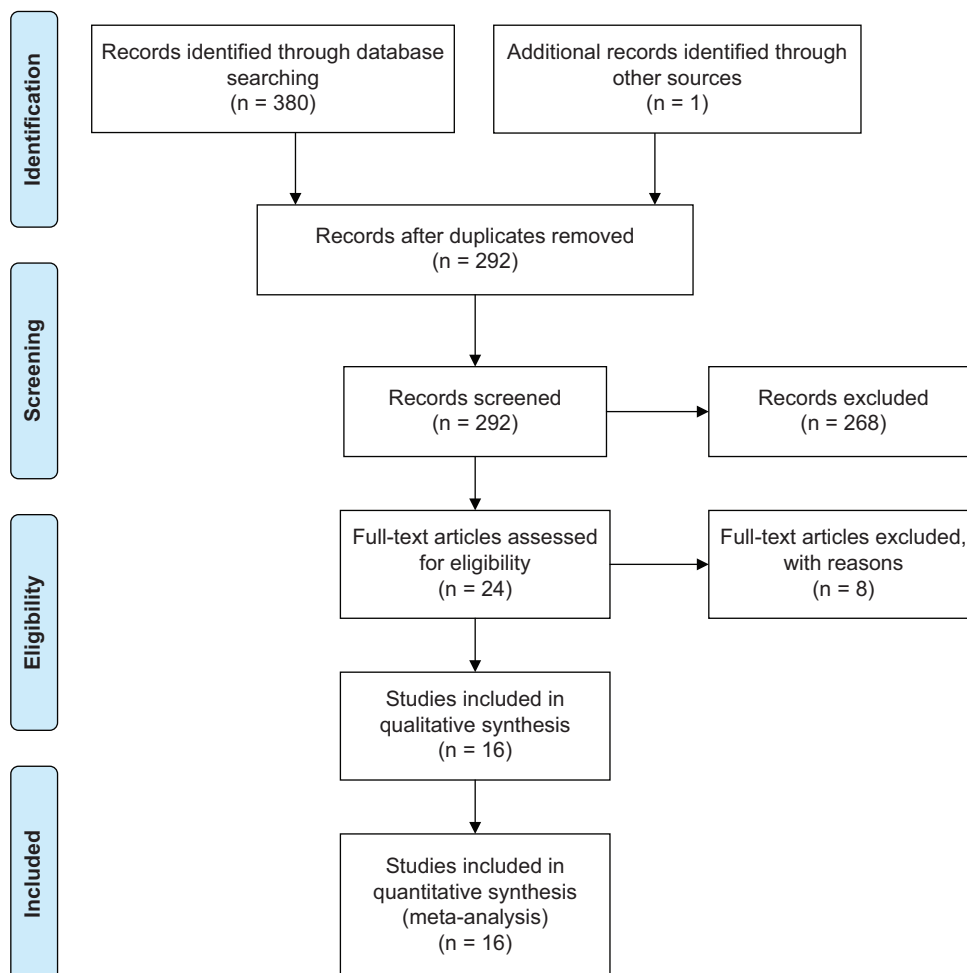

Figure 1: Preferred reporting items for systematic reviews and meta-analyses chart of included studies. #: number of.

Table 2: Description of the studies included in meta-analysis.

Authors	Country	Number	% of bcl-2 positivity	Cut off value of IHC	Stage	Method of IHC (1=semiquantitative, 2 = quantitative)	Treatment	Outcome measure
Uehara, 1995	Japan	259	33	NA	Ib, IIa, IIb	1	Surgery (Radiation for LN + cases)	OS
Tjalma, 1997	Belgium	76	63	5	Ia–IIb	2	Wertheim's radical Hysterectomy (RT for LN/LVSI+)	OS
Crawford, 1998	England	44		10	I–IV	2	-	OS
Harima, 1998	Japan	44	61	30	Ib–IVb	2	RT	OS
Rajkumar, 1998	India	40	65		IIB and IIIB	1	RT	OS, DFS
Padovan, 2000	Italy	86	60	5	Ib1–IVb	2	Surgery	OS
Tjalma, 2001	Belgium	111	68	5	Ia–IVb	2	NM	OS
Chung, 2002	Hong Kong, China	43	65	10	I–IV	2	-	DFS
Grauflund, 2002	Sweden	168	44	30	I–II	2	Surgery (adjuvant RT if LVI, para+, LN+)	OS, DFS
Jain, 2003	India	76	38	NA	IIb–III	1	RT	OS, DFS
Wootipoom, 2004	Thailand	174	10	50	Ib–IV	2	RT	OS, DFS
Saito, 2004	Japan	29	48	10	Ib–IVb	2	Surgery/RT	OS
Munakata, 2005	Japan	125	75	10	I–IV	2	Surgery ± RT ± Chemo	OS, DFS
Van de Putte, 2005	Norway	220	27	5	Ib	2	RT	OS, DFS
Yamasita, 2009	Japan	57	19	NA	II, III, IVA	2	RT	OS
Henriquez, 2011	Spain	50	62	NA	I–IVA	1	RT	DFS

IHC: Immunohistochemistry, LN: Lymph node, LVI: Lymphovascular invasion, LVSI: Lymphovascular space invasion, OS: Overall survival, DFS: Disease-free survival, RT: Radiotherapy, NA: Not available
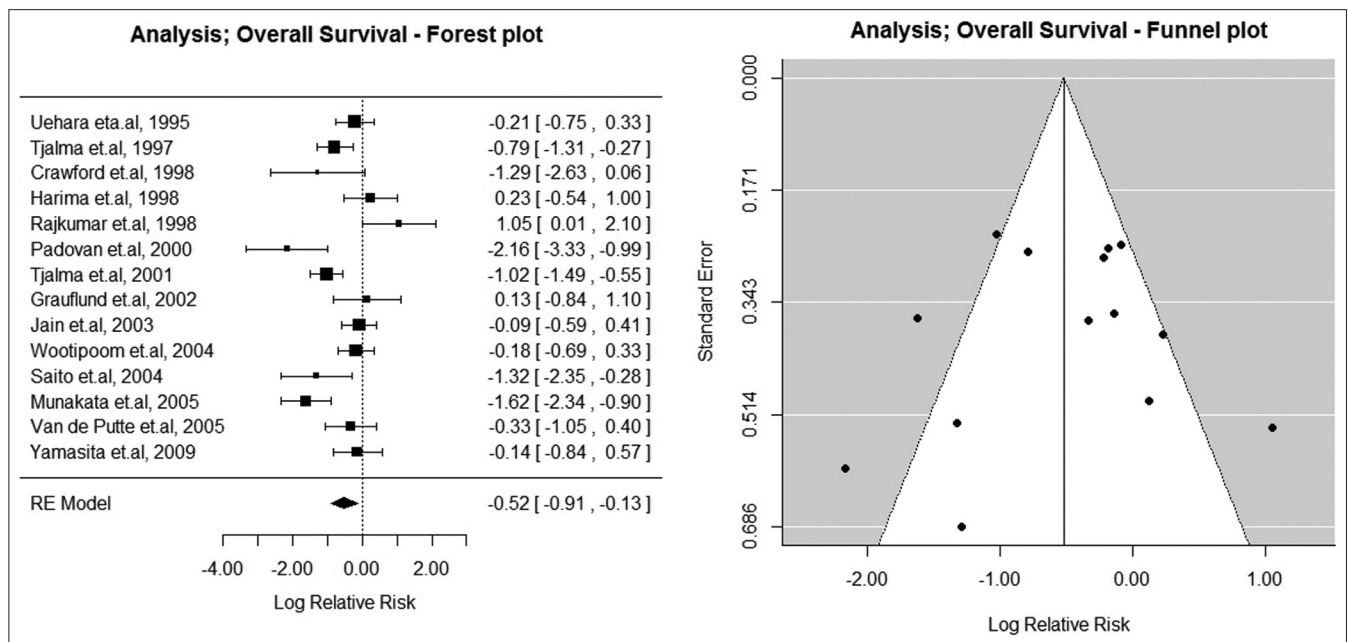
Figure 2: Overall survival: Forest and funnel plot. Random effects (RE) model (*n* = 14 studies).

Table 3: Immunohistochemistry details of the studies.

Authors	Method used	Antibody	Pretreatment	Titer	Incubation	Control	Quality
Uehara, 1995	AB complex	DAKO	-	1:300	12 h	Infiltrating lymphocyte	2
Tjalma, 1997	AB Complex	DAKO	0.01 M Citrate microwave	1:40	30 min	Normal lymphocyte	3
Crawford, 1998	AB complex	DAKO	Pressure cooker	1:50	40 min	Tonsil	3
Harima, 1998	AB complex	DAKO	Microwave	-	-	-	2
Rajkumar, 1998	AB complex	DAKO	0.1 M citrate autoclave	1:75	35 min	Previous known positive sections	3
Padovan, 2000	AB complex	DAKO	Microwave	-	Overnight	Normal endometrium	2
Tjalma, 2001	Streptavidin	DAKO	0.01 M citrate buffer, autoclave	1:40	30 min	Tonsil	4
Chung, 2002	Streptavidin	Burnham	-	-	-	Previous known positive sections	2
Grauflund, 2002	AB complex	DAKO	Microwave, 0.01 M citrate	1:0	30 min	Bladder cancer	3
Jain, 2003	AB complex	Novocastra UK	0.01 M Citrate	1:80	-	-	3
Wootipoom, 2004	Streptavidin	Novocastra UK	Microwave, 0.01 M citrate	1:200	Overnight	Endometrial hyperplasia	3
Saito, 2004	AB complex	BD-Biosciences	Microwave, 0.01M citrate	1:500	Overnight	-	2
Munakata, 2005	-	DAKO	Microwave, 0.01 M citrate	1:100	-	Tonsil	2
Van de Putte, 2005	Streptavidin	DAKO	Microwave, 0.01 M citrate	1:20	30 min	-	3
Yamasita, 2009	Mouse MC	DAKO	Microwave, 1 mM EDTA	1:80	30 min	Lymphoma	4
Henriquez, 2011	Streptavidin	DAKO	-	-	1 h	Positive tumor	3

EDTA: Ethylenediaminetetraacetic acid

Heterogeneity analysis

We observed three studies contributing to the heterogeneity.[10,21,28] We repeated the meta-analysis by excluding the abovementioned studies. The lnRR was −0.46 (−0.75−−0.18), and there was a significant reduction of heterogeneity (I^2 = 46.79). Our results indicate that bcl-2 expression has a potential predictive role for overall survival. There was, however, significant heterogeneity in the meta-analysis for OS. The Egger test showed that there was no evidence of publication bias (P = 0.474).

We investigated the factors contributing to the heterogeneity. We identified the patient, tumor, and treatment-related variables that could have contributed to the heterogeneity. These included stage (early vs. advanced), IHC quality, treatment (radiotherapy ± chemotherapy), and origin (Asian or non-Asian country). The stage-wise distribution of bcl-2 positivity mentioned in the studies is summarized in Table 4. Differences in treatment contributed to the heterogeneity of the study. There was a difference in results between Asian and non-Asian countries, but it was not statistically significant. The IHC method did not significantly contribute to the heterogeneity across the studies.

Disease Free Survival

Eight studies described the outcome of DFS.[10,16,18-21,26,29] There was no significant association between the expression of bcl-2 and DFS (lnRR: 0.05 C.I. (−0.30–0.39) [Figure 3].
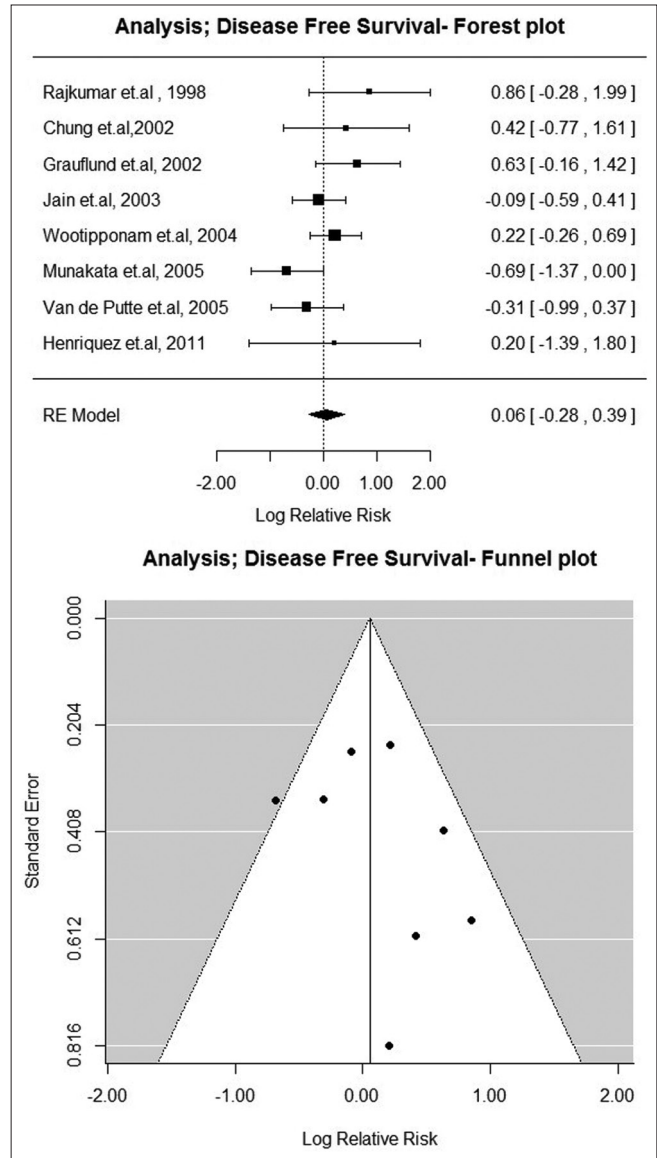
DISCUSSION

In locally advanced cervical cancer, the stage is the most important prognostic factor. In addition to the stage, various other patient, tumor, and treatment-related factors can affect the outcome. These factors include the patient's age, performance status, anemia, lymph node involvement, lymphovascular space invasion, tumor bulk, and hypoxia.[30,31] Although chemoradiation is the current standard of care in locally advanced cervical cancer, the evidence supporting the addition of chemotherapy concurrent with radiotherapy in locally advanced disease, especially in developing countries, has been questioned.[32] Various studies have evaluated the role of the addition of chemotherapy in neo-adjuvant settings.[33,34] In spite of the advancement of radiation therapy and chemotherapy, survival in locally advanced cervical cancer (especially stage III-IV) remains dismal in countries where the disease is highly prevalent.[1] Therefore, the evaluation of prognostic markers is highly relevant in locally advanced cervical cancer.

Table 4: Bcl-2 positivity and FIGO stage.

Authors (Year)	Bcl-2 positivity and FIGO stage distribution
Chung, 2002	I and II: 25/39 III and IV: 3/4
Crawford, 1998	N/A
Grauflund, 2002	IA: 18 (24.7%) IB: 48 (65.8%) IIA: 5 (6.9%) IIB: 2 (2.7%)
Harima, 1998	Stage I: 2/27 Stage II: 3/27 Stage III: 15/27 Stage IV: 4/27
Henriquez, 2011	N/A
Jain, 2003	NA
Munakata, 2005	NA
Padovan, 2000	Stage Ib: 34/39 II: 9/17 III: 9/25 IV: 0/5
Rajkumar, 1998	Stage IIB: 7 (29.2%) Stage IIIB: 17 (70.8%)
Saito, 2004	I: 6/14 (43%) II: 6/14 (43%) III: 2/14 (14.3%) IV: 0
Tjalma, 1997	NA
Tjalma, 2001	Ia: 20 (26%) Ib: 24 (32%) IIa: 24 (32%) IIb: 4 (5%) IIIa: 1 (1%) IIIb: 2 (3%) IVa: 1 (1%) IVb: 0 (0%)
Uehara, 1995	Stage Ib: 43/85 (50.5%) Stage IIa: 18/85 (21.17%) Stage IIb: 24/85 (28.23%)
Van de Putte, 2005	Only IB stage included
Wootipoom, 2004	I: 5 (21.7%) II: 13 (13.1%) III and IV: 2 (3.9%)
Yamasita, 2009	Not specified

FIGO: Federation Internationale de Gynecologie et d'Obstetrique

Figure 3: Disease-free survival: Forest and funnel plot.

Apoptosis is one of the important mechanisms of radiation and chemotherapy-induced cell death. Therefore, apoptotic markers have high predictive value in the progression and outcome of various malignancies.[4,35] Apoptosis is evolutionally conserved, highly regulated, non-lytic, programmed cell death. Broadly, it is mediated through intrinsic (or mitochondrial) pathways and extrinsic (or death receptor-mediated) pathways.[36] The bcl-2 family of proteins plays a crucial role in the process of apoptosis and contains one or more bc-2 homology (BH) domains.[37] The family includes proapoptotic proteins (BIM, BID, and PUMA) and anti-apoptotic proteins (BCL-2, BCL-XL), and the apoptotic process is influenced by the balance of these proteins.[38] Activated proapoptotic proteins enable the release of mitochondrial cytochrome c, activating caspase 9 through the formation of apoptosomes.[39]

Although there have been many reports to correlate bcl-2 with outcomes in cervical cancer, the results are still controversial. The difference in results could be due to heterogeneity of the population, IHC technique and threshold value of interpretation, and various treatment modalities.[40] Our systematic review and meta-analysis show that positive bcl-2 expression is associated with better

OS. However, there was significant heterogeneity among the studies. Differences in treatment methods significantly contributed to the heterogeneity. We could not conclude any association between DFS and expression of bcl-2.

The expression of bcl-2 was correlated with survival in other solid malignancies, although most of the studies observed significant heterogeneity. Expression of bcl-2 was associated with better survival in lung cancer.[41] A recent lung cancer bcl-2 expression meta-analysis was associated with better overall survival (OS).[42] A meta-analysis of gastric cancer patients found no significant association between bcl-2 and OS. However, subgroup analysis indicated that bcl-2 was associated with better OS in the Asian population.[43] Breast cancer meta-analysis shows that bcl-2 is a predictive factor for chemotherapy sensitivity.[44] Significant prognostic association of bcl-2 in breast cancer was demonstrated in a meta-analysis done by Callagy *et al.*[45] A study by Silva *et al.* showed bcl-2 overexpression associated with the worst prognosis in head-and-neck cancer.[39] However, significant heterogeneity was reported in the study.

The prognostic significance of various biomarkers in cervical cancer has been reviewed in the literature.[2,46] There is a lack of agreement on evidence of the prognostic implications of apoptotic markers. This lacuna in the literature makes the present meta-analysis relevant, despite limitations related to the significant heterogeneity. Bcl-2 was not an independent predictor of response to neoadjuvant chemotherapy in cervical cancer.[47,48] Although there was a difference in bcl-2 staining in CIN and invasive cancer, no significant association with human papillomavirus 16/18 was observed.[49] Bcl-2 expression is increased with the severity of cervical lesions from cervicitis, CIN, and invasive carcinoma.[50]

At present, there is ongoing research on the development of various agents that can target the bcl-2 pathway.[51] These can broadly be classified as antisense oligonucleotide preparations, peptide inhibitors, and small molecule inhibitors of pro-survival BCL-2 proteins.[52] Venetoclax (Bcl-2 selective BH3 analog)–obinutuzumab with or without ibrutinib was superior to chemoimmunotherapy in first-line chronic lymphoid leukemia patients.[53] Therefore, these are promising agents for targeted therapy in the future.

The present study has several limitations. Most of the included studies were retrospective, which is a factor that affects the quality of the evidence. Secondly, in the absence of randomized controlled trials, there is significant heterogeneity as far as the treatment modality, selection of patients, assessment, and interpretation of IHC biomarkers are concerned. However, this result may be helpful in designing more extensive prospective studies encompassing the evaluation of biomarkers as a prognostic factor for disease outcomes.

CONCLUSIONS

bcl-2 can serve as a valuable prognostic factor in cervical cancer, especially for bulky, locally advanced extensive disease in developing countries where treatment outcomes are still poor. Using bcl-2 as a molecular marker may help to prognosticate the disease and intensify the treatment in selected populations. Higher bcl-2 expression was associated with better OS. The same trend was retained in the group of patients treated with surgery. We did not find any association with DFS. Treatment methods significantly contributed to the heterogeneity of the meta-analysis.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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