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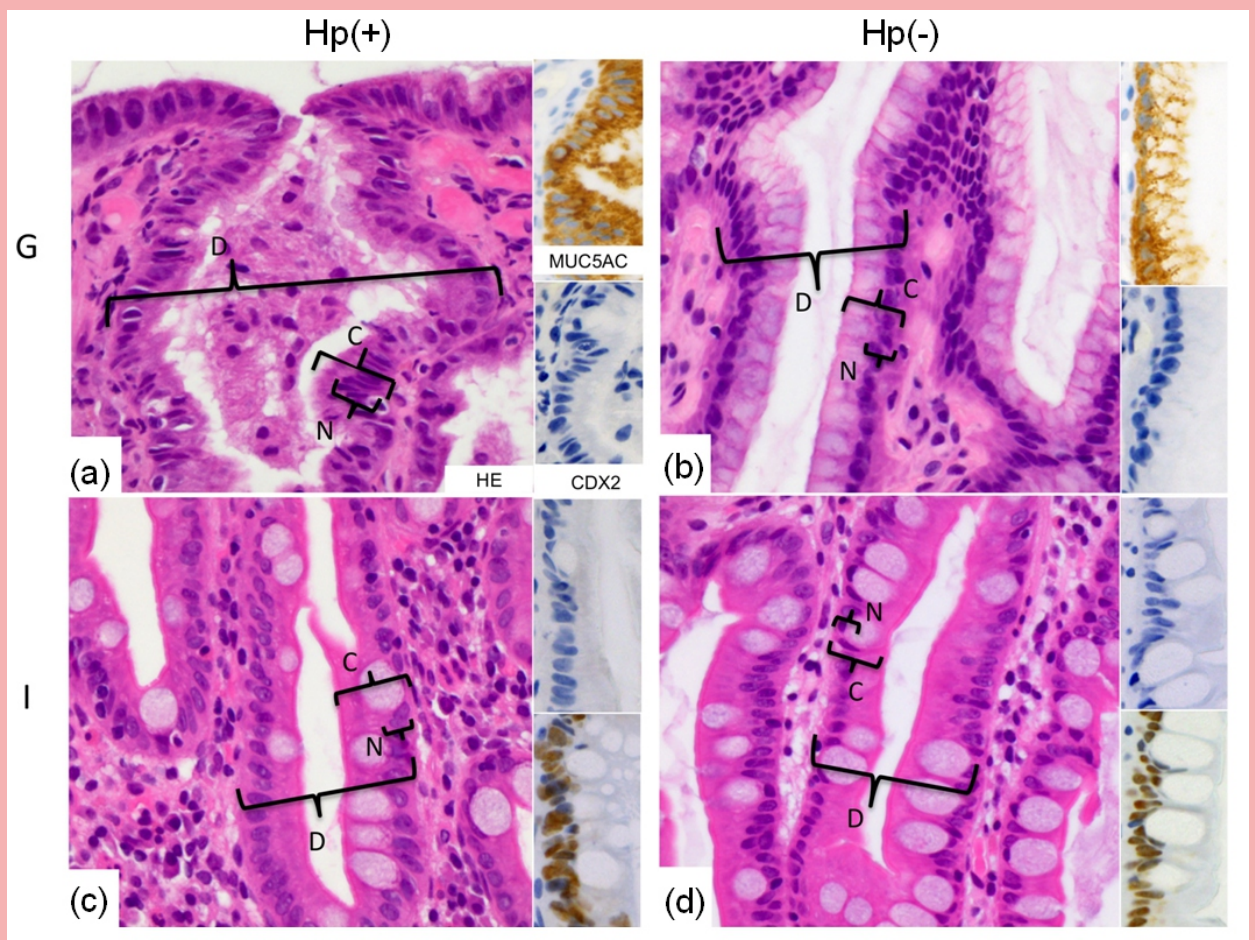

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The Relationship between p53 Expression and Human Papillomavirus in Premalignant and Malignant Uterine Cervical Lesions

Beyhan Varol Mollamehmetoglu[1*], Havva Erdem[2], Muzaffer Keles[3]

[1]Department of Pathology, Kanuni Training and Research Hospital, Trabzon, Turkey
[2]Department of Pathology, Faculty of Medicine, Ordu University, Ordu, Turkey
[3]Department of Pathology, Faculty of Medicine, Ataturk University, Erzurum, Turkey
Email: [*]bmollamehmetoglu@yahoo.com

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Abstract

Objective: We aimed to retrospectively examine a series of premalignant and malignant cervical tissues to study a high-risk HPV 16 infection that, among cervical tissue lesions, carries the greatest risk of conversion to cancer, and the presence of p53 protein immunoreactivity, a tumor suppressor gene product. Methods: Paraffin blocks were studied via immunohistochemical (IHC) method to explore the presence of HPV 16 in 59 premalignant and malignant cervical lesions as well as immunoreactivity of the p53 oncoprotein, the most common cellular tumor suppressor gene product in human cancers. Results: In our series, mutant p53 positivity rate was 35.3% for low-grade CIL, 40% for high-grade CIL, and 46.8% for invasive carcinoma cases. Immune staining for high-risk HPV 16 type yielded a positive staining rate of 47% in low-grade CIL, 80% in high-grade CIL, and 50% in invasive carcinoma. Conclusion: The results of our study indicate a progressive increase in p53 oncoprotein reactivity from cervical intraepithelial neoplasia to invasive carcinoma. This suggests the clinical importance of p53 immunoreactivity in dysplastic progression and neoplastic transformation. HPV is the most commonly encountered oncogenic type in cervical lesions, especially in high-grade CIL and invasive carcinomas. Results of the previous reports suggest that HPV-positive carcinomas release wild type p53 and HPV-negative ones release mutant type p53 were not confirmed by our results, which indicated a mutant type p53 reactivity in HPV-16 positive carcinoma cases.

Keywords

Cervical Intraepithelial Lesion, Cervix Carcinoma, HPV 16, p53, Immunohistochemistry

[*]Corresponding author.

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1. Introduction

Cervix carcinoma is the third most common cancers in women worldwide and most of cases occurring in low-to-medium-resource countries. The introduction of screening programs in many high resource countries has successfully decreased cervical cancer incidence and mortality. Nonetheless, stable or even higher trends have been observed in countries where cervical screening is either absent or of low quality and low coverage [1]-[3].

Infections by oncogenic high risk (HR) HPV are the most important factor in its etiology; it has been demonstrated that E6 and E7 oncoproteins of HPVs are bound to tumor suppressor genes p53 and Rb and eliminate their tumor suppressing properties, inducing malignancies. At least 13 genotypes of the alpha genus (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) have been found to be associated with the risk to develop cervical cancer and defined as "carcinogenic" viral types [1]-[5]. HPV typing has been shown to be a criterion determining which cervical intraepithelial neoplasia (CIN) lesions will turn into cancer. HPV16 is the most prevalent genotype in both squamous cell carcinoma (59.3%) and adenocarcinoma (36.3%) across the world. There is also a particularly strong correlation between HPV 16 and CIN2-3 lesions.

The likely primary site of HPV infection is transformation zone basal cells accessed via micro-wounds in the epithelial layer. A latent phase infection, in which low-copy number HPV episomers are maintained simply by cell division, shows minimal pathologic features [4]. The productive or permissive phase of infection may show koilocytic and CIN 1. A persistent infection may give rise to the transformative phase characterized by dysregulation of the regular HPV life-cycle and loss of cellular differentiation and maturation resulting in high-grade CIN morphology characterized by basaloid type cell involvement up to the middle to two thirds portion of the lesion (CIN 2) or the full thickness(CIN 3) By HPV typing of cervical biopsies, it is possible to differentiate reactive and neoplastic lesions, to grade morphologically defined CIN lesions, to determine high-grade cases and those who are at risk for the development of cervical carcinoma, and to detect occult and latent HPV infections. Contemporary opinion states that high-risk HPV test would be more efficient when incorporated into a cervical screening program [4] [5].

HR-HPV encodes two early proteins, E6 and E7, that target two major cellular tumor suppressor pathways. E6 targets the p53 tumor suppressor for degradation, result in loss of p53-dependent apoptosis and senescence. E7 binds to the pRb tumor suppressor, thereby disrupting G1/S transition control [1] [3]. Consequently, HR-HPV infection may lead to malignant transformation and tumor development.

Herein, we aimed to retrospectively examine a series of premalignant and malignant cervical tissues to study a high-risk HPV 16 infection that, among cervical tissue lesions, carries the greatest risk of conversion to cancer, and the presence of p53 protein immunoreactivity, a tumor suppressor gene product.

2. Methods

2.1. Human Uterine Cervix Samples

This study included 59 cervical tissue samples sent to Atatürk University Faculty of Medicine, Department of Pathology for examination. The tissue samples consisted of 42 punch biopsies, 14 radical hysterectomy materials, and 3 conization specimens. The histopathological diagnoses of the patients included 17 low-grade cervical intraepithelial lesions (LCIL), 10 high-grade intraepithelial lesions (HCIL), and 32 invasive squamous cell carcinomas. LCIL included CIN1 and koilocyticatypia; HCIL included all CIN2 and CIN3 cases.

2.2. Immunohistochemistry

All samples were stained with p53 and HPV 16 antibody using the immunohistochemical method. For staining, 3 - 4 micron thick sections taken from blocks fixated in 10% formalin and embedded in paraffin were mounted on positive charge microscopic slides. Anti-p53 DO7 antibody, Dako LSAB kit and HPV 16 Ab-2 clone CAM VIR-1 antibody, Biogen Universal kit were used as primary antibody.

2.3. Immunohistochemical Evaluation

Cytoplasmic staining was considered positive for HPV 16 whereas only nuclear staining was considered positive for p53. Nuclear staining frequency was graded as none (0), focal (1; staining in <10% of epithelial cells), re-

gional (2; staining in 10% - 50% of epithelial cells), and diffuse (3; staining in >50% of epithelial cells). These grades were then summated to categorize cervical lesions roughly into three groups with respect to p53: A-lesions with a score of 4 - 6 were graded as having a high level of p53 accumulation; B-lesions with a score of 2 or 3 were graded as having a low level of p53 accumulation; C-lesions with a score of 0 were grades as having no p53 accumulation.

2.4. Statistical Analysis

All comparisons were examined for statistical significance using Pearson's Chi-square test with Yates correction and Fischer's exact test (when the number in any category was 5 or less). The threshold for significant p values was established as $p < 0.05$.

2.5. Ethics

The ethics committee of medical faculty hospital approved this study (02.12.2014). As retrospective study, the Ethical Committee does not require the written informed consent from the patients.

3. Results

The mean age of the cases was 41 years for LCIL, 47 years for HCIL, and 58 years for invasive carcinoma. Of a total of 59 cases, 25 showed p53 protein expression. Six (35.3%) of 17 cases with LCIL, 4 (40%) of 10 cases with HCIL, and 15 (46.8%) of 32 cases with invasive squamous cell carcinoma showed a positive staining. When high-level p53 accumulation is taken into consideration, there was a significant difference between invasive cancers and high-grade and low-grade intraepithelial lesions with respect to staining ($x^2 = 6.44$, $p = 0.009$ and $x^2 = 7.98$, $p = 0.012$, respectively). However, there was no significant difference between invasive cancers and HCIL ($p = 0.44$) (**Table 1**).

Thirty-two of 59 cases were detected to have HPV 16 antibody. Eight (47%) of 17 cases with LCIL, 8 (80%) of 10 cases with HCIL, and 16 (50%) of 32 cases with invasive squamous carcinoma had positive staining. There was no significant differences between the groups with respect to HPV 16 positivity ($p = 0.094$, $p = 0.09$, $p = 0.92$) (**Table 2**).

Three of 17 cases (17.6%) with LCIL had p53 accumulation without having HPV infection. Two (20%) of 10 cases with HCIL had p53 accumulation. In invasive carcinomas, 7 (21.8%) of 32 cases had p53 accumulation, of which 5 were high-grade and 2 were low-grade (**Table 3**).

p53 accumulation with HPV infection was detected in 3 (17.6%) of 17 cases with LCIL. It was present in 2 (20%) of 10 cases with HCIL. Eight of 32 invasive cervical carcinoma cases had p53 accumulation (**Table 4**). No significant correlation was found between HPV infection and p53 positivity rate.

Table 1. p53 accumulation in cervical tissues with immunohistochemical staining.

Histological diagnosis	Number of cases (n)	Number of p53 positive cases		
		Total	Low-grade	High-grade
Low-grade CIL	17	6 (35.3%)	6 (35.3%)	0
High-grade CIL	10	4 (40%)	0	4 (40%)
Invasive carcinoma	32	15 (46.8%)	5 (15.6)	10 (31.2%)

Table 2. HPV 16 positivity and negativity rates of the cases.

	Number of cases (n)	HPV16 positive	HPV16 negative
LCIL	17	8 (47%)	9 (53%)
HICL	10	8 (80%)	2 (20%)
Invasive carcinoma	32	16 (50%)	16 (50%)

Table 3. p53 accumulation in the absence of HPV infection.

Histological diagnosis	Number of cases (n)	Number of HPV Negative cases (N)	p53 Immunoreactivity		
			None	Low-grade	High-grade
LCIL	17	9	6 (35.3%)	3 (17.6%)	0
HCIL	10	2	0	0	2 (20%)
Invasive carcinoma	32	16	9 (28.1%)	2 (6.3%)	5 (15.6%)

Table 4. p53 accumulation in the presence of HPV infection.

Histological diagnosis	Number of cases (n)	Number of HPV positive cases (N)	p53 accumulation		
			None	Low-grade	High-Grade
LCIL	17	8	5 (29.4%)	3 (17.6%)	0
HCIL	10	8	6 (60%)	0	2 (20%)
Invasive carcinoma	32	16	8 (25%)	3 (9.4%)	5 (15.6%)

Human papillomavirus infections were also studied in detail using the histological criteria. The typical morphological criteria for HPV infection were studied, which included koilocytosis, perinuclear halo, irregular hyperchromatic nucleus, and dyskeratosis and bi-or multinucleated squamous cells. The comparison of these two methods with each other was shown on **Table 5**.

4. Discussion

Since cervical cancer develops over time and many cervical malignancies originate from progressive malignant conversion of premalignant intraepithelial lesions, CIN lesions emerge at an earlier age than invasive cancer. The mean patient age in each of the three groups in our study support the literature findings [4]-[6]. The morphological criteria of HPV infection were present in 82% of low-grade CIL and 50% of high-grade CIL cases. Here, we used the specific and non-specific criteria for HPV infection recommended in the literature. The specific criteria include koilocytosis and dyskeratotic squamous cells. Nonspecific criteria are hypertrophy of squamous cells, bi-or multinucleation, and cytoplasmic amphophilia, clefting, and reddish granulation. Presence of at least three of these nonspecific criteria make the diagnosis of HPV. Koilocytosis has been observed at varying rates of 60%, 80%, and 98% in CIL lesions. Morphological criteria cannot differentiate high-risk HPV subtypes [6] [7]. According to our results, HPV was detected in 6% of low-grade CIL cases that had no histological criteria in IHC testing, while this rate went up to 40% in high-grade CIL. This difference may have originated from the presence of undifferentiated cells in superficial layers of epithelium and the absence of koilocytosis in high-grade lesions.

In a large-scale study in Greece, Labropaulou *et al.* [8] reported a HPV 16 positivity rate of 50% in cervical cancers, 41% in HCIL, and 13% in LCIL. Strand *et al.* [9], in a study on 2600 women with LCIL, demonstrated a HPV 16 positivity rate of 20%. It was reported that 22 of 26 LCIL cases that progressed to HCIL were HPV-16 positive. Bergenon *et al* [10]. detected HPV 16 in 21% of LCIL cases and 57% of HCIL cases. Our HPV 16 positivity rate was 50% in cervical cancer. Jacolien van der Marel *et al* [11]. demonstrate that HPV 16 is even more etiologically dominant than previously thought, based on various attribution models. Their data show that HPV 16 was the most predominant causal genotype, because in all cases positive for HPV 16 (77%) by cytology, this genotype was retrieved in whole tissue analysis of the biopsy. The unique role of HPV 16 as a carcinogenic agent has been widely recongnized. The attribution of HPV 16 to cervical cancers, where multiple type infection are far less common than CIN, is over 50% with a big distance to other carcinogenic types. A Yemelyanova *et al.* [12] reported 112 contained HPV16 DNA and 32 contained HPV18 DNA of their 150 samples by Q-PCR methods.

Our study found a p53 immunoreactivity rate of 35.3% in LCIL; 40% in HCIL; and a total of 46.8% in invasive carcinoma. Garzetti *et al.* [13] reported a p53 immunoreactivity rate of 78% in all CIN lesions studied. Bosari *et al.* [14] demonstrated a p53 immunoreactivity confined to basal layer in LCILs, while they demonstrated diffuse p53 reactivity at a rate of 25% in HCIL and 75% in invasive carcinoma. Akasofu *et al.* [15] observed no

Table 5. Histological and immunological detection of HPV infection.

	Histol/IHC				
	N	+ +	+ −	− +	− −
LCIL	17	7 (41%)	7 (41%)	1 (6%)	2 (12%)
HCIL	10	4 (40%)	1 (10%)	4 (40%)	1 (10%)

positivity in CIN 1 and CIN2, while they found a high grade staining of 27% in CIN3 and 43.5% in invasive cancer. Holm *et al.* [16] observed no p53 staining in condyloma, normal and dysplastic tissues, while they showed a p53 imunoreactivity in 7% of insitu carcinomas and 62% of invasive squamous cell carcinomas. Li-Di Xu *et al.* [1] have examined in their study WIG-1 gene in cervical carcinoma cell lines and Wig-1 expression in both cervical carcinoma cell lines and tumor samples. WIG-1 is a bona fide p53 target gene. Since WIG-1 is a p53 target gene, the question arises as to whether Wig-1 expression levels correlate with p53 status of the and/or presence of HPV, which encodes the E6 protein that targets p53 for degradation. The TP53 gene is more frequently mutated in HPV-negative than HPV-positive cervical cancer cell lines and tumors. Their demonstration that Wig-1 expession levels are higher in HPV-negative cervical carcinoma suggests a possible role of Wig-1 in HPV-negative cervical carcinogenesis.

Many researchers reported that HPV-positive carcinomas release wild-type p53 whereas HPV-negative carcinomas release mutant p53. They showed that E6 protein of oncogenic HPVs have the ability to be bound to p53, a cellular suppressor, and the complex results in inactivation of the suppressor function, causing a decrease in p53 protein. The selective decrease in tumor suppressor protein has been held responsible for cervical carcinogenesis. However, allelic losses and mutations in p53 gene inactivate the tumor suppressing activity and directly contribute to the progression of cellular transformation in HPV-negative cancers. Nevertheless, subsequent studies showed p53 gene mutations also in HPV-positive lesions. The presence of p53 immunoreactivity in HPV 16 positive cases is one of the important findings of our study.

5. Conclusions

In conclusion, HPV transmission is increasingly common in young women wordwide and several biological factors may help us to develop more effective preventive screening strategies for detection and treatment of women with cervical carcinomas.

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LMP1 Immunohistochemistry in Non-Hodgkin's Lymphoma of Sudanese Cases

Amal Ismail[1*], Ihsan Osman[2], Nazik Elmalaika Husain[3]

[1]Medical Doctorate Student, Pathology Department, Khartoum University, Khartoum, Sudan
[2]Pathology Department, Alzaiem Alazhari University, Khartoum, Sudan
[3]Pathology Department, Omdurman Islamic University, Omdurman, Sudan
Email: *amal.zumrawy2012@gmail.com, Ihsanosman@hotmail.com, nazikhusain@gmail.com

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Abstract

Back ground: Non-Hodgkin's lymphoma (NHL) with its different subtypes is strongly related to Epstein Bar virus (EBV) infection mainly Burkitt's lymphoma in Africa. Studies proved the role of EBV in tumor-genesis and linked it to prognosis and therapy of patients. Objectives: To determine the frequency of EBV in non-Hodgkin lymphomas using EBV latent membrane protein 1 (EBV-LMP1) immunohistochemical stain. Methods: This cross-sectional study was conducted at radio-isotope centre of Khartoum (2012-2014). A total of 75 cases of non-Hodgkin lymphoma paraffin embedded sections were stained for EBV LMP1 antibody. Data were analyzed by SPSS 16 and statistical cross linking of the results of immune staining with other data was done. Results: Out of 75 patients of non Hodgkin's lymphoma (74.7%) were males. EBV-LMP1 Immune-staining was positive in (17.3%) with predominance of Burkitt's lymphoma (33.3%), followed by diffuse large B cell lymphoma (17.9%). Conclusion: Burkitt's lymphoma expressed the highest percentage of non-Hodgkin's lymphoma positive cases (46.2%) out of the total (17.3%) positive cases. Different methods need to be used in studying Burkitt's lymphoma expression of EBV and its effects on the treatment and prognosis of cases.

Keywords

Epstein Bar Virus, Latent Membrane Protein 1 LMP1, Non-Hodgkin's Lymphoma, Immunohistochemistry

*Corresponding author.

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1. Introduction

Epstein Bar virus (EBV) is a member of the G herpes virus family with demonstrated B-cell lympho-tropism. Many studies relate it to carcinogenesis in lymphoma mainly Burkitt's lymphoma cell and HIV related lymphoma. Furthermore, it has been associated with epithelial malignancies arising in the gastric region, the breast and nasopharyngeal carcinoma in immune-compromised individuals [1].

EBV approximately infects most of the adult population in the world. It encodes series of products interacting with or similar to many of antiapoptotic molecules, cytokines, and signal transducers, so enhancing EBV infection, defected apoptosis and malignant transformation [2]-[5].

Different molecules are involved in EBV latent infection, including EBV-encoded nuclear antigens (EBNAs); EBNA leader protein (EBNA-LP); latent membrane protein (LMP) 1, LMP2A, and LMP2B; and EBV encoded RNAs (EBERs) EBER1 and EBER2. Among these, LMP1 is essential for EBV-mediated oncogenic effects. The C-terminal region ofLMP1 protein can regulate different cellular signaling pathways such as TNF receptor, NF-κB and JAK/STAT in order to regulate proliferation, immortalization, and invasion process [1] [6] [7].

Non-Hodgkin lymphomas (NHL) represent a heterogeneous group of lymphoid malignancies with distinct molecular pathogenesis leading to activation of proto-oncogenes (e.g. BCL-1, BCL-2, BCL-6, c-MYC) or disruption of tumor suppressor genes (e.g. p. 53). These lesions combine into multiple molecular pathways [8]. The number of non-Hodgkin's lymphoma (NHL) cases has increased rapidly through the last few years. There were an estimated 356 000 new cases of NHL and 192 000 deaths from NHL worldwide in 2008. The disease accounts for ~5.1% of all cancer cases and 2.7% of all cancer deaths. Areas with highest incidence of NHL include North America, Europe, Oceania, as well as several African countries [9]. At the current time, few data are given about the reasons for this increase or about exactly what causes non-Hodgkin's lymphoma; we only know some of the risk factors of non-Hodgkin's lymphoma. Studying patterns of cancer in the population has identified certain risk factors which are more common in people who get non-Hodgkin's lymphoma than in those who do not. However, most people with these risk factors do not get non-Hodgkin's lymphoma, and vice versa. Getting non-Hodgkin's lymphoma increases with age and is more common in men than in women. It is more common among people with immunodeficiency and those infected with Human T-lymphotropic virus type I (HTLV-1), helicobacter pylori and HIV. People exposed to certain chemicals, such as fertilizers pesticides, or solvents are more prone to develop non-Hodgkin's lymphoma [10].

Burkitt's lymphoma is a common type of non Hodgkin's lymphoma in Sudanese population specially the pediatric group either in the endemic form in the jaw or as sporadic cases. It's classification as high grade lymphoma and its impact on young age group are deserving more digging in etiological factors and more searching for causes. Different studies are suggesting EBV not only as etiological factor but also as prognostic and therapy affecting factor [2] [6] [11] [12]. Diagnosis of cases of non Hodgkin's lymphoma needs to be screened and reclassified according to its association with EBV. In this study we tried to find out the frequency of EBV in non Hodgkin's lymphoma subclasses depending on the positivity of LMP immunohistochemical staining and relate it to age, gender and site.

2. Methods

This cross sectional and descriptive, hospital-based study was conducted at Radioisotope Centre of Khartoum (RICK). All cases of non-Hodgkin's lymphoma diagnosed at histopathology and immunohistochemistry department in RICK within the years (2012-2014) were reviewed. Poorly fixed or inadequately processed tissue blocks were excluded. Seventy five cases of non Hodgkin's lymphoma were included in this study. Four histological sections were obtained from each block one in ordinary frosted slide for (hematoxylin and eosin stain-H/E-stain) and three in positively charged slides (for immunohistochemical stain. Briefly the first section wasde-paraffinized and dehydrated. Mayer's hematoxylin applied for 15 minutes then washed in running tap water for 20 minutes, counter stained with eosin for 2 minutes, dehydrated in 95% absolute alcohol until excess eosin was removed then cleared in xyleme and checked under microscope the other three sections were incubated overnight in a hot air oven (LABTECH-INDIA) at 60˚C c temperature. Properly fixed samples as much as possible were selected and satisfactory overnight incubation not to lose the tissue in immune staining was guaranteed. Antigen retrieval was done by (Dako-USA) citrate buffer in PH 9 in 95c for 30 minutes, endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol (Sigma Aldrich) for 10 minutes, Then slides were incubated with 100 - 200 µl of primary antibody for 30 minutes at room temperature followed

by adding antibody enhancer for 10 minutes. The primary antibody for EBV LMP1 (Dako-USA) was used. The negative control slides were incubated with Bovine serum albumin instead of the primary antibody. After washing with Phosphate buffer saline for 3 minutes, binding of antibody was detected by incubation for 20 minutes with dextran labeled polymer Dako envision TM flex kit (secondary antibody). Diaminobenzidine tetra hydrochloride DAB chromogen (prepared by adding 1drop in 1 ml) was added for up to 10 minutes. Counter staining with hematoxylin was done and lastly slides were prepared. H/E slides were revised in order to select the tissue representative for tumor cells in a trial to avoid non specific staining and necrotic areas and compared with immunohistochemistry slides. Instructions of manufacturer of the antibody were followed and detection system was freshly prepared to avoid contamination problems. Interpretation of positive slides with the supervisor (heamato-pathologist) was accomplished by light microscopy (Olymbus BX51TF Tokyo, Japan). Positive results are considered if there is cytoplasmic staining. Scoring was done on the basis of the percentage of positive tumor cells and the relative immune staining intensity. Sections grading system was used to score the number of positive tumor cells: 0, none seen in the section; 1, presence of positive cells even rare but not exceeding 25%; 2, 26% to 50% positive cells; 3, 51% to 75%; and 4, 76% to 100%. Immune-staining intensity was rated as follows: 0, none; 1, weak; 2, moderate; and 3, intense. When the staining intensity was heterogeneous, each component of the tumor was scored independently and the results were averaged. For example, when a specimen contained 50% of the tumor cells with moderate intensity ($2 \times 2 = 4$), 25% of tumor cells with intense immune staining ($1 \times 3 = 3$), and 25% of cells with weak intensity ($1 \times 1 = 1$), the score were 4 +3 +1 = 8. The maximum possible score was twelve [13]. False positive interpretation especially of the edge artifacts was avoided. Photographing of positive samples with different staining intensity lastly was done using Olympus DP 70 controller software and was processed using Olympus DP manager software. Data acquisition sheet filled with data and results of LMP1 staining. Data were analyzed electronically using computer program (SPSS version 17). T test and Chi-Square tests were calculated to compare the association.

The study was approved by the ethical committee of Medical Doctorate of Pathology University of Khartoum. Permission for conduction of the study was achieved from RICK director and patient's written consent was obtained.

3. Results

The included 75 cases of Non Hodgkin lymphoma were 56 males and 19 females. The minimum age was three years and the maximum age was ninety-five with mode of four years, 49 years was the median and the standard deviation was 24, 5. Cases were stained for EBV LMP1 immunostain and 17% cases of non Hodgkin lymphoma were positive. About (23.1%) of patients expressed positive results below the age of 16 years while (23.8%) of the twenty three of the range group (46 - 60) showed positive results (**Figure 1**). There was no significant difference between age groups with positive and negative LMP1 stained cases.

Regarding the gender distribution from the Fifty six male patients (84.6) % expressed positive results versus (15.4%) of the nineteen female patient's (**Figure 2**).

No statistical difference between male and female positive cases.

Site of the lymphoma: There is nodal distribution of sixty seven (89.3%) of the cases in different lymph node groups (**Table 1**). Extra nodal distribution of non Hodgkin lymphoma cases were as follow: Two cases (2.6%) from gastric tissue, eyelid, thyroid, ovary, oro-pharynx, testis and bowel. The total number of extra nodal cases is eight (10.7%).

Thirty three (44%) of the cases were diffuse large B cell lymphoma while eighteen (24%) of Burkett's lymphoma (**Figure 3**).

LMP1 stain result: Thirteen (17%) cases of non Hodgkin lymphoma were positive with three (23.1%) exhibiting weak positivity and six (46.2%) intermediate positivity and only four 30.8% showed strong positivity (**Figure 4** and **Figure 5**).

4. Discussion

EBV role in tumor-genesis of non Hodgkin's lymphoma is becoming more obvious, while still the management remains unsatisfactory. Exploration of antiviral drugs and therapies based on specific monoclonal antibodies has encouraging results [14] [15]. Immunohistochemistry has developed as an efficient tool to demonstrate the presence of EBV by detecting latent viral antigens. In this study non Hodgkin's lymphoma cases showed 17.3%

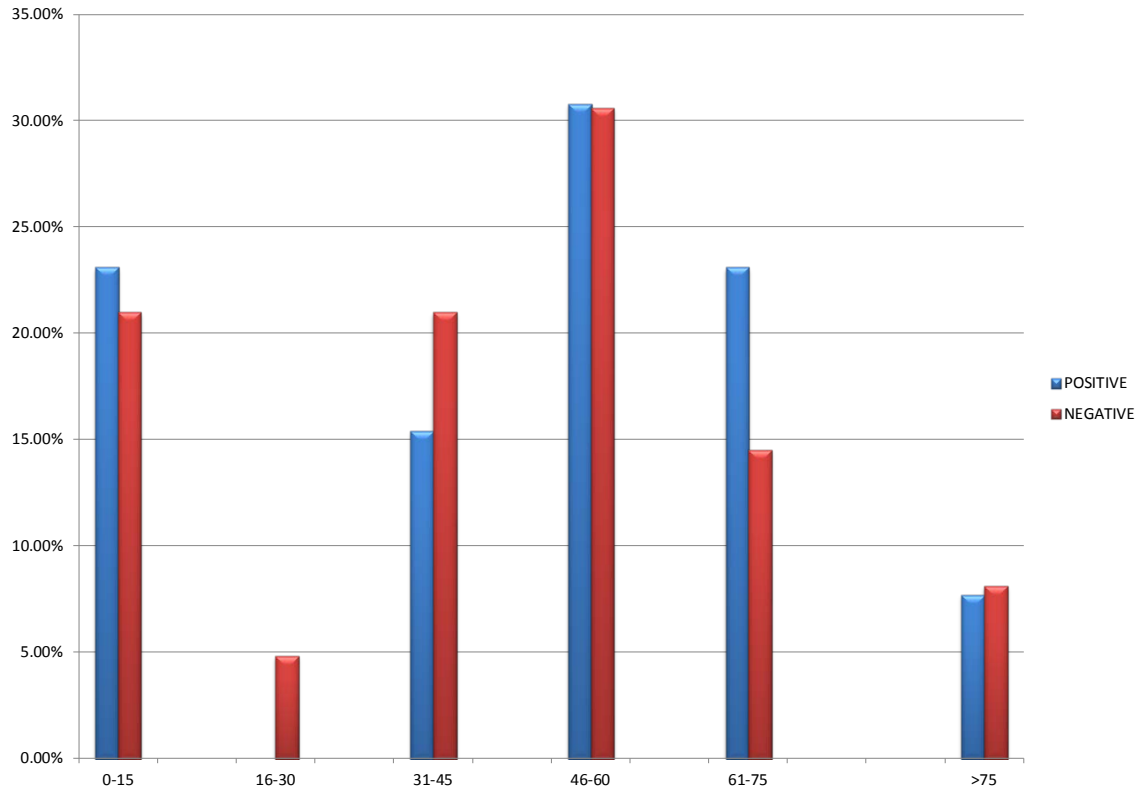

Figure 1. Age groups distribution with LMP1 results among the studied patients.

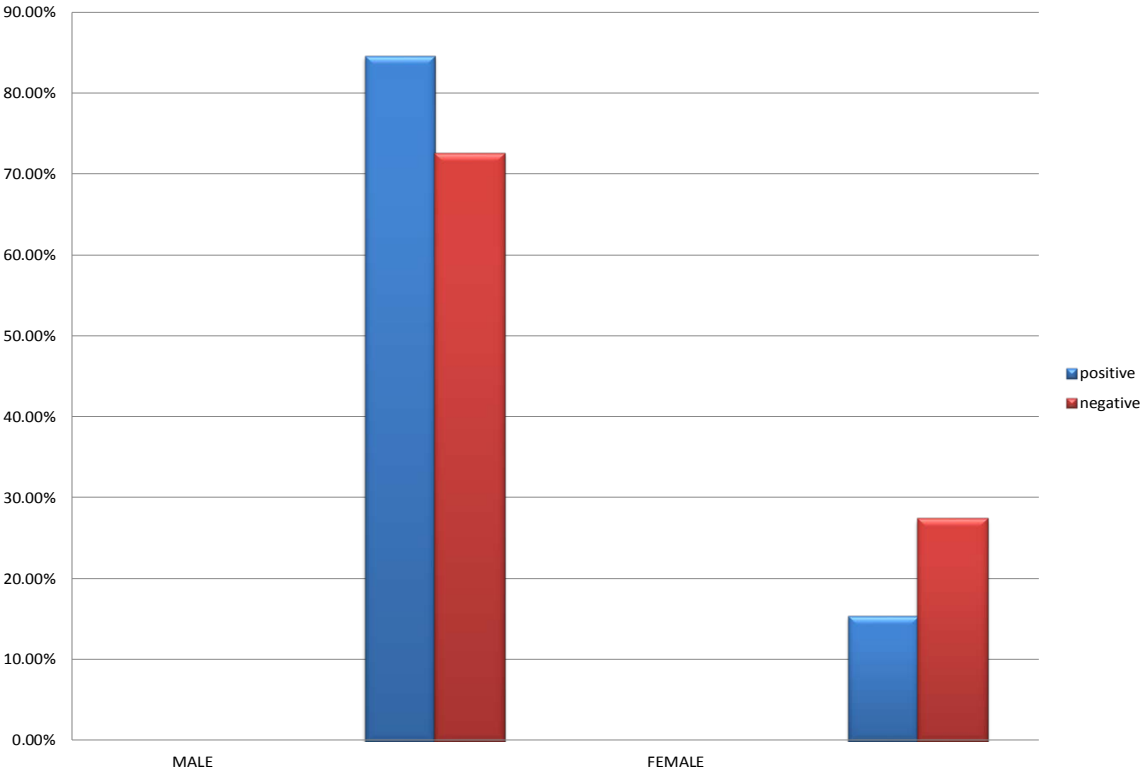

Figure 2. Distribution of the positive LMP1 according to gender among the studied group.

Table 1. Nodal distribution and LMP1 results among the studied group.

		LMP1 RESULT		TOTAL
		POSITIVE	NEGATIVE	
MESENTRIC	Count	5	13	18
	% within LMP1 RESULT	45.5%	23.2%	26.9%
AXIILLARY	Count	1	15	16
	% within LMP1 RESULT	9.1%	26.8%	23.9%
CERVICAL	Count	5	21	26
	% within LMP1 RESULT	45.5%	37.8%	26.9%
INGUINAL	Count	0	5	5
	% within LMP1 RESULT	.0%	10.7%	9.0%
PARAAORTIC	Count	0	2	2
	% within LMP1 RESULT	.0%	3.6%	3.0
TOTAL	Count	11	56	67
	% within LMP1 RESULT	100.0%	100.0%	100.0%

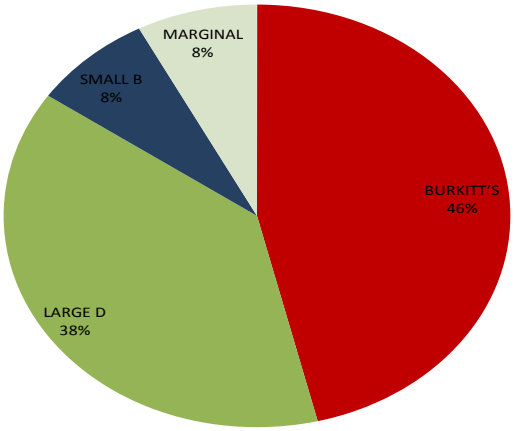

Figure 3. Non-Hodgkin's lymphoma subtypes among the studied group.

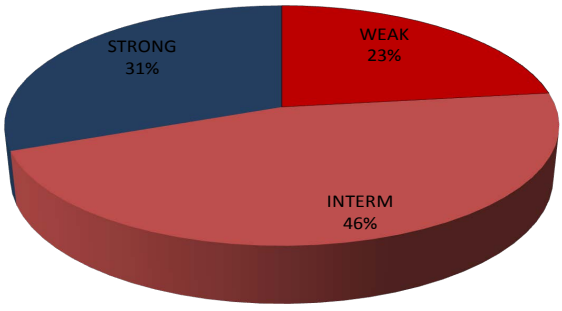

Figure 4. LMP1 intensity of staining in the studied group.

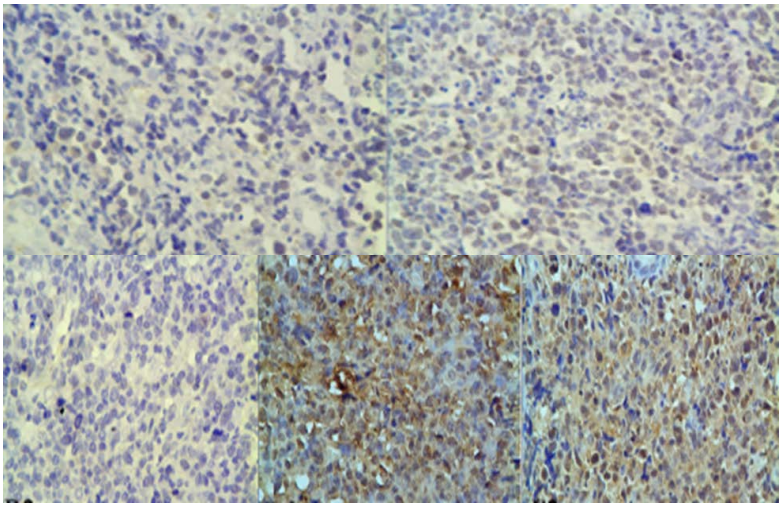

Figure 5. Different staining among the studied group.

positivity of latent membrane antigen antibodies compared with 30% and 12.7% expression of positivity of NHL cases in other similar studies [16] [17]. Other studies were done only on B cell type of lymphoma mostly Burkitt's lymphoma. Gonin J. and Larousserie F expressed positivity in 34.7% [17], while Oyama [18], Morales [19] and Park studies [20] showed positivity ranging from 9% to 15%.

EBV positive NHL can occur in different age groups. In this study it was found to be more common in age group 46 - 60 years (30.7%) followed by the group of ages from 31 - 46 years forming 20% of the total with a mean age (48.7). The low mean in this study is resulting from more pediatric group range (1 - 15 years) (21.3%) with 4 years old as the more repeated mode. The youngest patient with Burkitt's lymphomain this study is 3 years old. Most of the patients were in the 6th decade followed by the 1st decade. LMP1 positivity was seen more among the age group (46 - 60) years (30.8%). This may be related to immunity deterioration with increasing age [21]. No significant difference among age groups expressing positivity in comparison with significant variation in another study done by Park and his colleagues [20].

It was found that NHL was more common among males (74.7%). expressing (84.6%) of the positive LMP1 cases in the current study. This is in concordance to Tumwine LK and his colleagues study with similar male cases predominance but with majority of female positive cases comprising 86.5% [22].

LMP1 positivity among the different morphological subtypes showed that nearly half (46.2%) of the positive cases were Burkitt's lymphoma this supports the theory of latency type transformation and the relationship between Burkitt's lymphoma and EBV infection [22]. It was followed by Diffuse large B cell lymphoma (38.5%) similar to the results of Lynnette K Tumwine and his group study in Uganda [22] and this may be due to geographical distribution of Burkitt lymphoma in African malaria endemic area. It also agrees with the study of Osman. IM concluding that sporadic Burkitt's lymphoma of the gastrointestinal tract is the predominant pediatric lymphoma while diffuse large B cell lymphoma is the commonest in adult [23].

Regarding marginal zone lymphoma a study of 17 cases of inflammatory bowel disease (IBD) associated colorectal cancer and 9 of NHL were considered for EBV expression of latent membrane proteins and results suggested that EBV to be involved in the pathogenesis of proportion of cases of marginal zone lymphoma [22] and this may explain our positive case result.

No studies found in the literature on small cell lymphoma and mantle cell lymphoma relating it with EBV latent proteins expression but theory of malignancy transformation is not proofed.

A study on T cell lymphoma represented by Yuan Mao and colleagues using LMP1 on 16 cases yielded (56.25%) positivity [24]. In the current study T cell lymphoma cases were few and no case showed positivity to be compared with the B cell lymphoma positivity.

In this study grading system was applied on positive results. It is found that 77% were of intermediate and strong positivity. Some studies relate the concentration of LMP1 to the prognosis of patients and potentiality of metastasis [25]. Further study is needed to follow up these patients and relate grade of LMP1 with outcome and risk of metastasis.

5. Conclusions

LMP1 positive expression was in 17.3% non Hodgkin's lymphoma cases. The majority were within the age group (46 - 60 years) and pediatric age group below fifteen years old with none statistically significant male predominance. About one third of the LMP1 positive cases were sporadic Burkitt's lymphoma with the commonest site in mesenteric lymph nodes. Only two cases of endemic Burkitt's lymphoma were identified, one of them was LMP1 positive. Diffuse large B cell lymphoma following Burkitt's lymphoma in frequency of positive cases, with small B cell and marginal zone lymphoma coming next. Most of the findings of this study correlate with the published English literature.

It is recommended that similar study to be carried out in larger sample size and combined with additional methods like PCR and ERB1 *in situ* hybridization or EBNA1 immune stain to detect EBV antigen and make interpretation comparable with the co-method used.

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Abbreviations

BCL2	B cell lymphoma 2
EBERs	EBV encoded RNAs
EBNA-LP	EBNA leader protein
EBNAs	Encoded nuclear antigens
EBV	Epstein Bar virus
HTLV-1	Human T-lymphotropic virus type I
IBD	Inflammatory bowel disease
JAK/STAT	Janus kinase and signal transducer and activator of transcription
LMP	latent membrane protein
MYC	Myelocytomatosis oncogen homolog
NF-κB	nuclear factor kappa-light chain-enhancer of activated B cells
PCR	Polymerase chain reaction
RICK	Radioisotope Centre of Khartoum
SERS	surface enhanced Raman scatter
TNF	Tumor necrosis factor

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Solid Aneurysmal Bone Cyst of the Distal Metatarsus in a Horse

Nadja Herbach[1*], Christina Terboven[2], Peter Lambrecht[3]

[1]Center for Clinical Veterinary Medicine, Institute of Veterinary Pathology, LMU Munich, Munich, Germany
[2]Equine Veterinary Practice, Warngau, Germany
[3]Equine Veterinary Clinic, Zorneding, Germany
Email: *n.herbach@lmu.de

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Abstract

A horse was presented with a slowly growing mass of the distal metatarsal region of the right hind leg. Radiographic examination revealed an eccentric cyst-like lesion with distortion of the lateral margin of the distal metatarsus. The lesion involved approximately half of the metatarsal width, exhibited fine septa, a sclerosed margin towards unaffected bone and a smooth rim of thin compact bone at the periphery. Longitudinal sectioning of the distal metatarsus revealed a solid pale yellow mass with reddish foci and margin. Histologically, the mass consisted of a cell-rich, well vascularized fibrous stroma, containing numerous irregularly shaped trabeculae of woven bone with regular osteocytes. Osteoblasts were observed around the osteoid, and there were foci of numerous osteoclast-like giant cells. The lesion was diagnosed solid variant of an aneurismal bone cyst.

Keywords

Bone Tumor, Aneurysmal Bone Cyst, Horse

1. Introduction

According to the WHO, aneurismal bone cysts (ABC) are benign cystic bone lesions, containing blood filled cavities and fibrous septa composed of fibroblasts, reactive woven bone and osteoclast-like giant cells [1]. ABC in man usually arise in the metaphysis of long bones and clinically lead to swelling and pain of the affected area. Radiographically, ABC present as lytic, eccentric, expansile mass with well defined margins and most ABCs are surrounded by a thin shell of subperiosteal reactive bone. Septa within the cysts give the lesion a "soap-bubble-

*Corresponding author.

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like" appearance in radiographic images. ABCs are rarely reported in domestic animals, including dogs, cats, horses, and cattle [2], and appear similar to the lesion in humans [1]. The aetiology of the disease is unclear, altered blood flow, trauma and other bone diseases have been suggested to play a role in ABC development of animals [2]. In humans, a genetic component was identified [1]. ABC may arise *de novo* (primary ABC) or complicate other benign or malignant bone tumours (secondary ABC) in humans and animals, e.g. giant cell tumours, osteoblastoma and fibrous dysplasia [1] [2]. In animals, no age-, sex-, breed- or site-predilection can be assigned due to the sparsity of cases. In humans, ABCs develop in the first two decades of life and have no sex predilection.

The present report describes a solid aneurismal bone cyst of the distal metatarsus in a horse.

2. Case Presentation

An eight-year-old Quarter horse gelding was first presented in 2007, exhibiting a small (3 cm in diameter) non-painful mass at the lateral aspect of the right distal metatarsus. Since lameness was not observed, no further diagnostic or treatment efforts were made.

In 2009, the mass had increased in size and caused grade 1 - 2 lameness which was progressive and did not respond to treatment. When the gelding was presented to the Equine Veterinary Practice Gmund, the animal showed grade 2 lameness, and a mass was evident at the lateral aspect of the distal end of the right metatarsus, measuring approximately 5 × 5 × 3 cm.

The lesion involved the lateral aspect of the distal metatarsus and occupied approximately half of the metatarsal width. The lateral margin of the metatarsus was distorted, and the lesion exhibited a rim of thin compact bone at the periphery and showed well defined margins. Fine septa could be observed within the lesion, giving it a soap-bubble-like appearance (**Figure 1**). Due to the size of the process and the risk of a pathological fracture, the horse was euthanized and the right lower hind leg was submitted for post mortem examination. The horse was treated following the national principles of animal care.

Longitudinal sectioning of the metatarsus revealed a solid, firm, pale yellow mass with reddish areas and margins (**Figure 2**) that could be cut with a knife. Tissue samples were fixed in neutral buffered 4% formaldehyde and embedded in paraffin. Sections were routinely stained with H & E, Giemsa, Turnbull and Masson trichrome stain. Histologically, the mass consisted of a cell-rich, well vascularized fibro-osseous stroma with small whorls and interlacing strands of fibrous tissue (**Figure 3**). The cellular component of this fibrous stroma consisted of well differentiated spindle cells with elongated nuclei, and uniform chromatin. Mitotic figures were rarely observed. Within the fibrous stroma, there were numerous irregularly shaped trabeculae of woven bone with varying degrees of mineralization. The bone spicules exhibited regular osteocytes, and were radiated by collagen fibres. Osteoblasts were observed around the osteoid, and there were foci of numerous osteoclast-like giant cells (**Figure 4**). In addition, perivascular aggregates of siderophages were observed. The lesion was diagnosed solid aneurismal bone cyst.

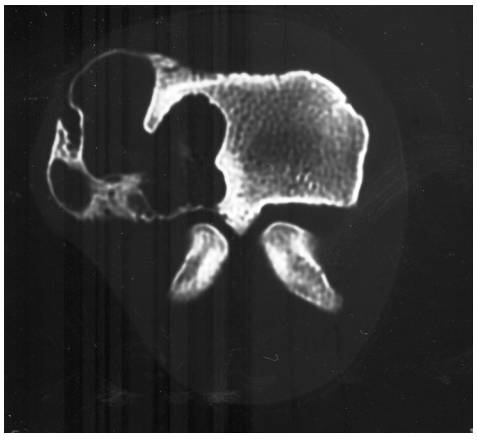

Figure 1. Computed tomography of the metatarsal lesion.

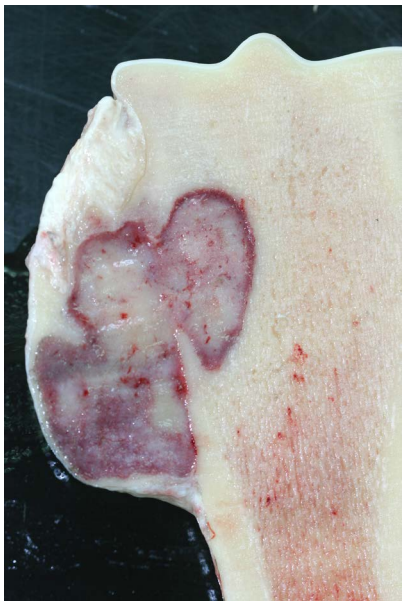

Figure 2. Cross-section of the mass of the distal metatarsus.

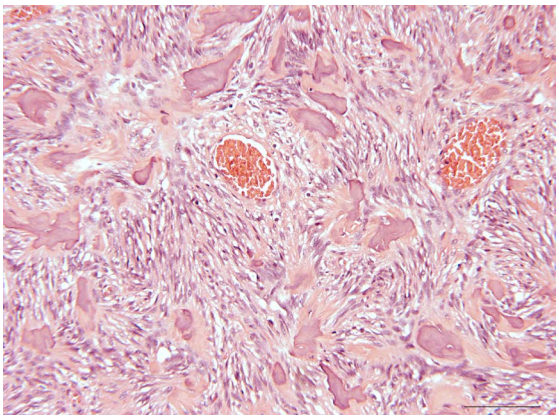

Figure 3. Overview showing the well vascularised fibro-osseous tissue with irregularly arranged mineralized trabeculae in a spindle cell-rich stroma; H & E stain, bar 100 μm.

3. Discussion

Aneurysmal bone cysts are a well-known benign cystic lesions of bone in humans, most frequently involving long bones, and have occasionally been described in animals [2].

ABC have rarely been observed in horses, and were described in long bones, including two cases reporting on ABC of the third metatarsal bone of foals [3] [4], another foal exhibited an ABC in the third metacarpal bone and one in the tibia [5] and a 9 year old horse showed an ABC with pathological fracture in the radius [6]. Four reports are on ABC of the mandible, including one foal and an adult horse [7] [8]. To our knowledge, this is the first report of a solid aneurismal bone cyst in horses.

According to the radiographic finding, the diagnosis *aneurysmatic bone cyst* was suspected. Radiographically, ABC have to be differentiated from osteosarcoma, hemangiosarcoma, fibrosarcoma and plasma cell myeloma [2]. A malignant bone tumour, such as osteosarcoma, may be excluded by radiographic findings, since the lesion described showed a sclerosed rim around a well circumscribed process, as well as a thin rim of bone in the periphery and a smooth surface [9].

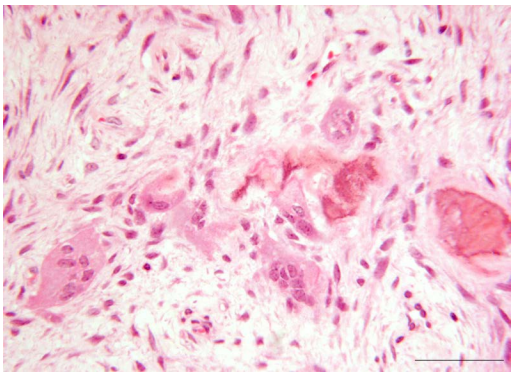

Figure 4. Mineralized bone trabeculae in a spindle cell-rich stroma with occasional osteoclast-like giant cells, H & E stain; bar 50 µm.

Macroscopically, aneurysmatic bone cysts are dark-red to black in colour, due to multiple blood-filled cysts with tan white gritty septa. Histologically, cavernous blood-filled spaces predominate, separated by septa of loosely arranged spindle cells, multinucleated giant cells and haemosiderin containing macrophages [2] [3] [10]. Solid ABC lack the blood filled cystic spaces and histologically appear like the septa described above [1]. Therefore, the macroscopic and histologic appearance of the presented bone tumour fills the criteria for an aneurysmatic bone cyst.

Histologically, other fibro-osseous lesions and bone tumours described in horses have to be ruled out, including ossifying fibroma, fibrous dysplasia, osteoma, and osteosarcoma [11].

Ossifying fibroma is a benign lesion, characterized by the presence of fibrous connective tissue and trabeculae of immature woven bone [12]. The surface of bone trabeculae of ossifying fibroma show prominent osteoblast rimming [13]. Ossifying fibroma is almost exclusively located at the horse's head, with the exception of one report on ossifying fibroma/osteoma occurring in the proximal tibia [14]. In humans, ossifying fibroma also occurs mainly in bones of the head. *Ossifying fibroma* and fibrous dysplasia may represent different phases of the same process [11].

Fibrous dysplasia comprises a fibrous and an osseous component. The fibrous component is composed of bland spindle cells, the osseous component of trabecular or woven bone [15]. Osteoblast rimming of trabeculae may only be observed in the periphery near the corticalis [11]. Fibrous dysplasia has rarely been observed in horses, including one case reporting fibrous dysplasia of the accessory carpal bone [16], one of the maxillary sinus [17], and another involving the nasal cavity [13].

Osteoma of humans is defined a benign, slowly growing lesion, consisting of well differentiated mature bone [12]. In osteoma, lamellar bone is formed and the spaces between trabeculae may contain marrow rather than fibrous connective tissue. In domestic animals, osteomas are most often diagnosed in bones of the head [2].

Despite some clear characteristics of the different disease entities, there is a smooth transition between ossifying fibroma, osteoma and fibrous dysplasia and therefore, distinction between the different lesions is not always possible [11].

Osteosarcoma may be ruled out due to the clinical course, non-invasive growth, low mitotic index, lack of pleomorphism and atypia of cells and their nuclei, as well as uniform chromatin of the tumour cells. Osteosarcomas are rare in horses, but have been described, mainly involving bones of the head [2] [17].

Treatment of choice in man is surgical excision but recurrence is possible [18] [19].

4. Conclusion

Taking the clinical course, as well as radiographic and histologic features into account, we were able to diagnose a solid aneurysmal bone cyst of the distal metatarsus in this horse. ABC is a rare disease of horses; therefore an age- or sex-predilection is not known. Three cases of ABC in long bones of horses were diagnosed in two colts and one filly under one year of age [3]-[5] and there is one report on ABC in the radius of a 9-year-old thoroughbred gelding [6]. This is the first case of solid ABC in the metatarsus of an adult gelding and shows that this entity has to be taken into account when proliferative bone lesions of horses are examined.

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Gastric-and-Intestinal Mixed Intestinal Metaplasia Is Irreversible Point with Eradication of *Helicobacter pylori*

Yuka Kiriyama[1], Tomomitsu Tahara[2], Tomoyuki Shibata[2], Masaaki Okubo[2], Mitsuru Nakagawa[1], Asako Okabe[1], Naoki Ohmiya[2], Makoto Kuroda[1], Atsushi Sugioka[3], Masao Ichinose[4], Masae Tatematsu[5], Tetsuya Tsukamoto[1*]

[1]Department of Diagnostic Pathology, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan
[2]Department of Gastroenterology, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan
[3]Department of Hepatobiliary and Liver Transplant Surgery, School of Medicine, Fujita Health University, Toyoake, Japan
[4]Second Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan
[5]Japan Bioassay Research Center, Hadano, Japan
Email: *ttsukamt@fujita-hu.ac.jp

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Abstract

***Helicobacter pylori* (*H. pylori*) represents an important factor in the development of atrophic gastritis, intestinal metaplasia (IM), and gastric cancer. Eradication of *H. pylori* has been reported to prevent gastric cancer only in cases without atrophy or IM. However, histological changes with eradication have yet to be fully clarified. We evaluated 38 *H. pylori*-positive cases before and after eradication at the gland level; pyloric glands were classified as showing gastric proper (G) and IM gland types, with the latter including gastric-and-intestinal mixed IM (GI-IM) and solely intestinal IM (I-IM), depending on the remaining gastric phenotypes. On eradication, acute and chronic inflammation attenuated rapidly and gradually, respectively, whereas levels of MUC5AC and MUC6 expression were not markedly altered. Gland width, size of nuclei and cytoplasm and their ratio in surface foveolar epithelium, the number of Ki-67-positive cells and the length of the proliferating zone in each gland were significantly decreased in G glands after eradication compared with those in GI-IM and I-IM. The number of mitotic phase cells, positive for phosphorylated histone H3 at serine 28, was increased in both types of IM compared to that in G glands in the *H. pylori*-infected state, but unexpectedly remained unchanged with eradication. These results suggest that GI-IM, as the beginning of IM, could represent a histological irreversible point with eradication and be considered as a "histological point of no return".**

*Corresponding author.

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Keywords

***Helicobacter pylori*, Chronic Atrophic Gastritis, Intestinal Metaplasia, Eradication, Stomach**

1. Introduction

Gastric cancer remains the most common type of cancer and the second leading cause of cancer-related deaths in Japan, despite recent decreasing trends. Worldwide, this malignancy remains the fourth most frequent cause of morbidity and the second-most widespread cause of cancer death especially in East Asian countries, 41% from China and 11% from Japan in 2002 [1]-[3]. *Helicobacter pylori* (*H. pylori*) was discovered by Warren and Marshall in 1983 and suggested as a causative factor for gastric disorders [4]. *H. pylori* infection has been illustrated to be a consequential risk factor for the development of chronic atrophic gastritis and intestinal metaplasia (IM) [5]. In humans, IM has been extensively analyzed and attracted attention of pathologists as a precancerous lesion. We have proposed a novel classification depending on emerging intestinal properties together with remaining gastric phenotypes. With this classification, IM could be divided into two main types, gastric-and-intestinal mixed IM (GI-IM) and solely intestinal IM (I-IM); the former still possess gastric components including MUC5AC-positive foveolar epithelium and/or MUC6-positive atrophied pyloric gland cells and the latter consists only of intestinal epithelium characterized by MUC2 possessing goblet cells or CD10-positive absorptive cell with nuclear CDX2 expression with or without Paneth cells [6] [7]. The Mongolian gerbil (*Meriones unguiculatus*) model was established to show successful *H. pylori* infection and subsequent chronic active gastritis and emergence of IM [8]. Regenerative glands often developed and proliferated beyond the muscularis mucosae to form cystic dilated glands in the submucosa, designated as heterotopic proliferative glands (HPGs). HPGs initially consisted of only gastric epithelial cells but gradually possessed intestinal epithelial cells 25 weeks after infection to form GI-IM, and then finally containing Paneth cells to compose I-IM. These results explain *H. pylori* infection as initially causing chronic gastritis, then induce GI-IM, and finally progress to I-IM dependent on the duration of *H. pylori*-induced inflammation [9]. Taking into account both human and animal data, IM was considered to represent a serial and simultaneous progression of atrophy and intestinalization via GI-IM toward finally I-IM with *H. pylori* infection.

Several case-control studies have revealed a positive correlation between *H. pylori* infection and gastric carcinogenesis [10]-[13]. Based on these epidemiological findings, the World Health Organization (WHO)/International Agency for Research on Cancer (IARC) defined *H. pylori* as a “definite carcinogen” in 1994 [14]. In Mongolian gerbil models, *H. pylori* infection strongly promoted chemical carcinogen-induced gastric carcinogenesis [15] [16]. In turn, eradication of the bacteria proved effective in preventing carcinogenesis in *H. pylori*-infected carcinogen-treated gerbils; the earlier, the more effectively [17] [18]. In human trials, Fukase *et al.* [19] reported that eradication of *H. pylori* was effective for preventing metachronous gastric carcinoma. However, Wong *et al.* [20] and Yanaoka *et al.* [21] indicated that eradication was not practically effective in preventing gastric carcinogenesis for subjects who had already passed the irreversible point and suffered from severe atrophic and metaplastic gastritis. In this regard, the pathological findings that really represent risk factors and the point beyond which recovery cannot be achieved with eradication of *H. pylori* remain unclear.

In this study, we classified pyloric glands as gastric proper (G), GI-IM, and I-IM, then analyzed the reversibility of each gland type after eradicating *H. pylori* in an attempt to identify histological points of irreversibility.

2. Materials and Methods

2.1. Study Subjects

The subjects had been analyzed for *H. pylori* infection using the ^{13}C-urea breath test (using UBIT tablets, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Briefly, the patients were judged “*H. pylori* infected” if ^{13}C-carbon dioxide was detected in their exhaled breath when ^{13}C-urea was taken, which should be decomposed into ^{13}C-carbon dioxide (CO_2) and ammonia with *H. pylori*-derived urease in the stomach. The patients underwent eradication of the bacteria with 30 mg of lansoprazole b.d. (bis die, twice a day), 200 mg of clarithromycin b.d, and 750 mg of amoxicillin b.d. for 1 week as described [22] in the Endoscopy Center at Fujita Health University Hospital between 2003 and 2013. This study using human tissue was conducted with approval from the Institu-

tional Review Board of Fujita Health University. Thirty-eight patients, who have undergone gastric biopsies before and after eradication, were selected, from whom informed consents had already been obtained. Biopsy specimens were classified into *H. pylori*-positive [Hp(+)] and successfully eradicated [Hp(−)] groups. Thirty-one patients underwent stomach biopsies both before and after eradication once each. Seven cases were biopsied at least twice after eradication. Five cases were failed for eradication of the bacteria and were included in an Hp(+) group. Total of 43 Hp(+) and 45 Hp(−) samples were analyzed. Mean age was 59.2 ± 14.5 years (median, 63.5 years). Male/female ratio was 28/10. In the Hp(−) group, biopsies were sampled after 14.7 ± 17.1 months (median, 4 months). Among these, 24 biopsies were taken between 2 and 6 months after eradication [Hp(−) < 6 M], and 21 were between 7 and 69 months [Hp(−) > 7 M].

2.2. Inflammatory Score

Degree of inflammation was scored according to the updated Sydney system [23] including *H. pylori*, neutrophils, mononuclear cells, atrophy of antrum and corpus region, and IM into scores of: 0, normal; 1, mild; 2, moderate; and 3, marked. If immunohistochemical analysis could not detect *H. pylori*, final judgment of the infection was determined according to the ^{13}C-urea breath test and was scored as 1 in case of the infection-positive.

2.3. Immunohistochemistry and Classification of Each Gland with Gastric and Intestinal Phenotypes

Tissue samples embedded in paraffin blocks were utilized for hematoxylin (Merck KGaA, Darmstadt, Germany) and eosin (Muto Pure Chemicals, Co., Ltd., Tokyo, Japan) (HE) staining and immunohistochemical analyses. For the immunohistochemical detection of gastrointestinal phenotypic markers, antibodies against MUC5AC (clone CLH2; Novocastra, Newcastle-upon-Tyne, UK), MUC6 (clone CLH5; Novocastra), and CDX2 (CDX2-88; BioGenex, San Ramon, CA) were applied. Ki-67 antibody (clone MIB-1; DAKO Japan, Tokyo, Japan) was used to detect proliferative cells and anti-phosphorylated histone H3 at serine 28 (H3S28ph, clone HTA28; generously providedby Dr. Masaaki Inagaki, Aichi Cancer Center Research Institute, Nagoya, Japan) was utilized for visualization of mitotic phase nuclei [24]. *H. pylori*was immunohistochemically detected using a polyclonal antibody (DAKO). All immunohistochemical procedures were performed using iView DAB universal kits with Ventana Benchmark Ultra apparatus according to the instructions from the manufacturer (Roche Diagnostics, Tokyo, Japan). Briefly, sections were deparaffinized, treated with CC1 antigen retrieval buffer (5 mM ethylenediaminetetraacetic acid, pH 8.0), and incubated with the primary antibodies described above. Then, the sections were treated with the universal secondary antibody (mixture of anti-mouse and anti-rabbit antibodies), visualized with 3, 3'-diaminobenzidine, and counter-stained with hematoxylin. Glands were classified into G and IMglands, with the latter including GI-IM and I-IM according to the morphology and immunoexpression of gastric and intestinal markers, as previously described [7].

2.4. Mucin Core Protein Expression

Expression of MUC5AC was analyzed in the whole area of mucosa, whereas MUC6 was judged in the antrum and corpus separately. Evaluations were made semiquantitatively according to the staining intensity into scores of: 0, none; 1, weak; 2, moderate; and 3, strong.

2.5. Histological Analyses

Antral region was chosen to evaluate progression of IM, since corpus region rarely possessed GI-IM. Diameter of the glands at the widest part was measured in the shallow region of the foveolar epithelium in G, GI-IM, and I-IM glands to monitor hyperplastic or hypertrophic responses to *H. pylori* infection. Height of the cytoplasm (C), nucleus (N), and nucleus-to-cytoplasm (N/C) ratio were also measured at the same points to assess relative nuclear enlargement and disturbance of polarity at the cellular level. In the relatively deeper zone, length of the Ki-67-positive proliferative region was measured in each gland. Numbers of Ki-67- and H3S28ph-positive cells per gland were also counted.

2.6. Statistical Analysis

Quantitative values are expressed as means ± standard deviation (SD) and medians, and differences between

means were statistically analyzed using the Kruskal-Wallis test followed by Dunn's multiple comparisons using Prism 6 software (GraphPad Software, La Jolla, CA). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Gastritis, Atrophy, and Intestinal Metaplasia

The amount of *H. pylori*, degree of inflammatory cell infiltrates, atrophy of mucosa, and IM are described in **Table 1** for Hp(+) and Hp(−) groups. *H. pylori* disappeared in the Hp(−) group. Neutrophils were drastically decreased after successful eradication. Mononuclear cells gradually showed a relative decrease after eradication. In contrast, atrophy in both the antrum and corpus and IM were not significantly altered.

3.2. Mucin Expression

Expression of MUC5AC in the surface foveolar epithelium and those of MUC6 in the antrum (pyloric mucosa) and corpus (fundic mucosa) were evaluated, but no significant alteration was found (**Table 1**).

3.3. Analysis of Surface Foveolar Epithelium

In the *H. pylori*-infected condition [Hp(+)], gastric-type foveolar glands show hyperplastic and hypertrophic proliferation, resulting in enlargement of the diameter of the glands, lengthening of the foveolar cytoplasm, increasing size of nuclei, and increased N/C ratio (**Figure 1**).

Diameters of foveolar glands were 125.0 ± 43.9 μm, 108.6 ± 22.1 μm, and 116.7 ± 27.8 μm in G, GI-IM, and I-IM glands in the Hp(+) group, compared to 74.8 ± 22.6 μm, 100.8 ± 21.2 μm, and 102.6 ± 27.8 μm in the Hp(−) group, respectively. The numbers of the glands analyzed are 26, 14, and 19 in G, GI, and I in the Hp(+) groups, and 22, 10, and 18 in the Hp(−) group, respectively. Diameter of G glands was significantly narrowed after eradication of *H. pylori* [Hp(−)/G] compared to Hp(+)/G, and also became significantly slimmer than the Hp(−)/I group (**Figure 2(a)**).

Heights of foveolar cell (C) were 31.3 ± 12.4 μm, 34.5 ± 10.5 μm, 36.4 ± 10.4 μm, 25.2 ± 6.5 μm, 35.2 ± 9.9 μm, and 34.1 ± 11.7 μm in Hp(+)/G, Hp(+)/GI, Hp(+)/I, Hp(−)/G, Hp(−)/GI, and Hp(−)/I groups, respectively. The numbers of the glands analyzed are 45, 28, 39, 44, 19, and 36, respectively. A decrease was seen in Hp(−)/G compared with Hp(+)/G, indicating significant alleviation with eradication. However, height of the IM epithelium, including both GI-IM and I-IM, was not altered by eradication of the bacteria (**Figure 2(b)**).

Heights of N from the basal layer were 16.6 ± 7.7 μm, 16.9 ± 5.8 μm, 13.3 ± 4.7 μm, 8.5 ± 3.0 μm, 15.2 ± 4.3 μm, and 15.0 ± 6.6 μm in Hp(+)/G, Hp(+)/GI, Hp(+)/I, Hp(−)/G, Hp(−)/GI, and Hp(−)/I groups, respectively. The analyzed numbers are 44, 28, 39, 44, 19, and 36, respectively. N moved to the luminal side from the surrounding basal region in the *H. pylori*-infected state, but lay above the basal layer after eradication of the bacteria in gastric foveolar epithelium [Hp(+)/G vs. Hp(−)/G]. In contrast, N localization was not significantly changed between Hp(+) and Hp(−) conditions in IM (**Figure 2(c)**).

N/C ratio was further evaluated to assess the effect of eradication, and was 51.9% ± 11.3%, 49.5% ± 12.4%, 37.2% ± 8.7%, 33.9% ± 8.0%, 45.6% ± 14.1%, and 44.7% ± 13.4%, in the Hp(+)/G, Hp(+)/GI, Hp(+)/I, Hp(−)/G, Hp(−)/GI, and Hp(−)/I groups, respectively. The analyzed numbers are 44, 28, 39, 44, 19, and 36, respectively. In the *H. pylori*-positive condition, N/C ratio was highest in G glands. With eradication, the ratio in

Table 1. Summary of updated Sydney system and mucin core protein expression.

	No. of biopsies	*H. pylori*	Neutrophils	Mononuclear cells	Atrophy (antrum)	Atrophy (corpus)	Intestinal metaplasia	MUC5AC	MUC6 (antrum)	MUC6 (corpus)
Hp(+)	43	1.09 ± 0.29	1.79 ± 1.01	2.00 ± 0.65	1.61 ± 0.93	1.33 ± 0.87	0.81 ± 1.16	2.51 ± 0.77	2.24 ± 0.71	1.82 ± 0.98
Hp(−) <6M	24	0.00 ± 0.00****	0.29 ± 0.62****	1.54 ± 0.51*	1.62 ± 0.80	1.00 ± 1.41	0.67 ± 1.09	2.54 ± 0.59	2.25 ± 0.72	2.00 ± 0.82
Hp(−) >7M	21	0.00 ± 0.00****	0.38 ± 0.50****	1.48 ± 0.51**	2.15 ± 0.55	0.75 ± 1.04	1.43 ± 1.25	2.52 ± 0.68	2.54 ± 0.90	2.00 ± 1.07

*$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ compared with Hp(+) groups.

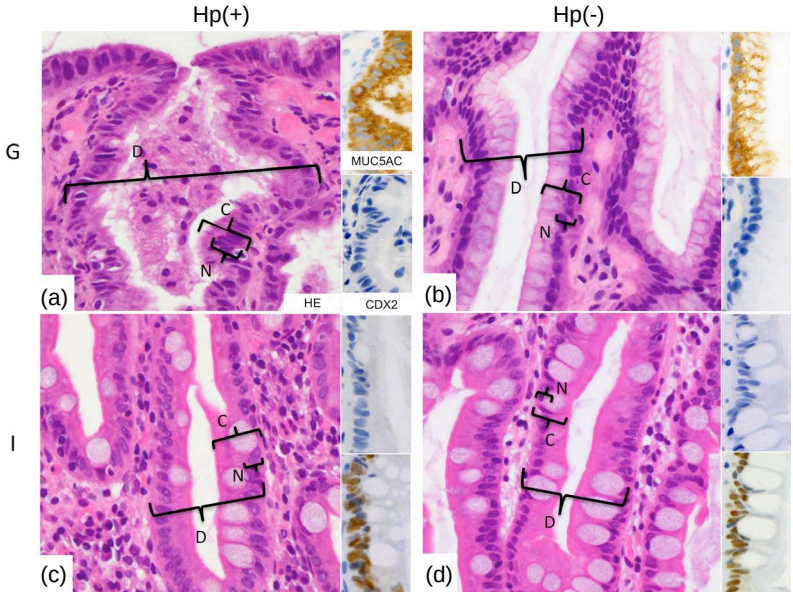

Figure 1. Histological change of foveolar epithelium with or without intestinal metaplasia with eradication. (a) and (b) G type glands with *H. pylori* infection [Hp(+)] and after eradication [Hp(−)] with expression of gastric marker, MUC5AC. (c) and (d) Hp(+) and Hp(−)I type glands with nuclear CDX2 staining as an intestinal marker. D, diameter of foveolar glands; C, height of the foveolar cells; N, size of the nuclei. HE staining, Inset, top and bottom each, MUC5AC and CDX2 immunostaining. Original magnification, 400×.

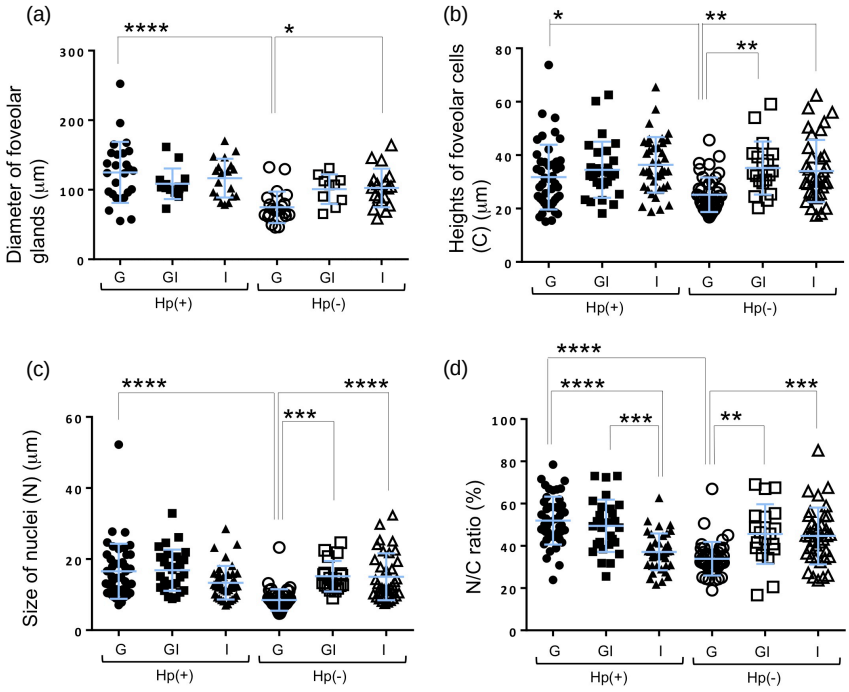

Figure 2. Analysis of surface foveolar epithelium in G, GI-IM, and I-IM glands between Hp(+) and Hp(−) groups. (a) Diameter of foveolar glands; (b) height of cytoplasm of foveolar cells; (c) size of nuclei. (d) N/C ratio of foveolar cells. In all of four factors, significant decrease was observed in Hp(−)/G glands after eradication compared with those in Hp(+)/G. Since IM glands were not recovered, Hp(−)/G shows significant lower values after eradication. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

G gland [Hp(−)/G] decreased dramatically and was the lowest compared with Hp(−)/GI and Hp(−)/I (**Figure 2(d)**).

3.4. Analysis of Proliferative Zone

The proliferative zone was localized at the bottom of foveolar epithelium above pyloric glands in G and GI-IM. In I-IM, however, the proliferative zone was localized at the bottom of the gland, since pyloric glands showed almost complete atrophic change. Gastric glands showed regenerative proliferation with *H. pylori* infection, and this proliferation was drastically reduced with eradication, whereas IM glands (both GI-IM and I-IM) did not show alleviation in terms of Ki-67-positive cells. Surprisingly, the number of H3S28ph-positive mitotic cells was unchanged with eradication in G glands, and was much higher in IM glands irrespective of *H. pylori* infection (**Figure 3**).

Lengths of the proliferative zone were determined as the region of Ki-67-positive cells in each gland and were 276.2 ± 98.8 μm, 356.5 ± 111.3 μm, 297.7 ± 77.0 μm, 169.8 ± 43.3 μm, 370.6 ± 98.4 μm, and 358.5 ± 81.5 μm, respectively, in the Hp(+)/G, Hp(+)/GI, Hp(+)/I, Hp(−)/G, Hp(−)/GI, and Hp(−)/I groups. The numbers of glands analyzed are 25, 14, 18, 17, 14, and 10, respectively. The proliferative zone became smaller only in G-type glands after eradication of *H. pylori* in contrast to IM glands (including GI-IM and I-IM). After eradication, the proliferative zone was significantly shorter in G glands compared with GI-IM and I-IM (**Figure 4(a)**).

Numbers of Ki-67-positive cells per gland were 92.4 ± 40.1, 132.4 ± 55.5, 101.6 ± 44.6, 30.6 ± 17.9, 150.2 ± 51.9, and 141.6 ± 39.5, respectively. The analyzed numbers are 25, 14, 18, 17, 14, and 10, respectively. The number in Hp(−)/G was significantly reduced after eradication, whereas those in Hp(−)/GI and Hp(−)/I were not (**Figure 4(b)**).

Mean numbers of H3S28ph-positive mitotic cells per gland were 1.04 ± 0.87, 1.70 ± 0.97, 2.42 ± 1.32, 0.70 ± 0.76, 2.36 ± 1.52, and 2.34 ± 2.41, respectively in Hp(+)/G, Hp(+)/GI, Hp(+)/I, Hp(−)/G, Hp(−)/GI, and Hp(−)/I groups. The numbers analyzed are 36, 29, 21, 26, 28, and 8, respectively. The number of H3S28ph-positive cells

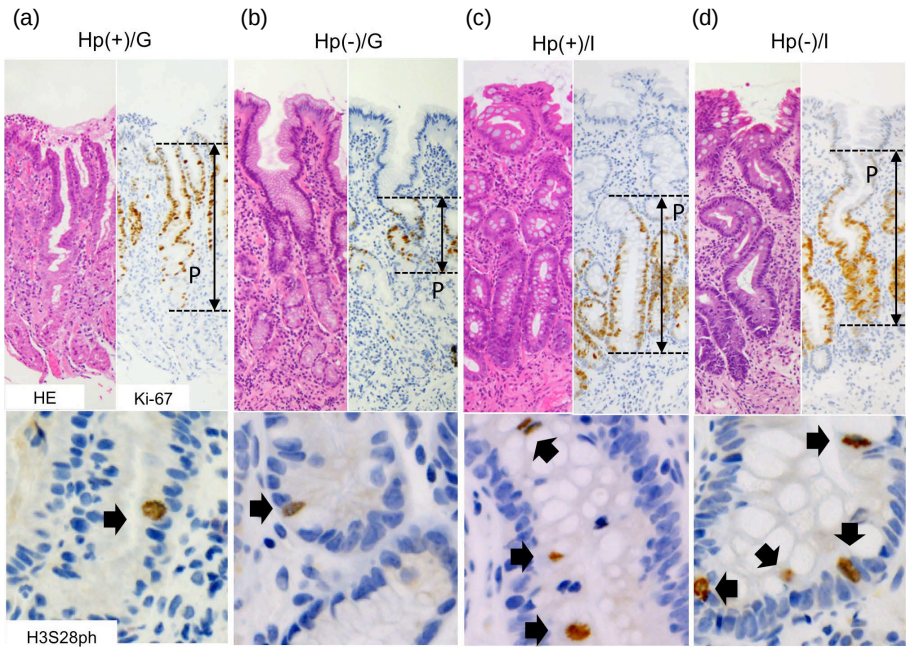

Figure 3. Histological change of proliferative zones with or without IM before and after successful eradication. (a) and (b) G type glands with *H. pylori* infection [Hp(+)] and after eradication [Hp(−)]. Proliferative zone localizes in the lower part of foveolar epithelium above partially atrophied pyloric glands. (c) and (d) Hp(+) and Hp(−) I type glands. Proliferative zone stays at the bottom of the gland, since pyloric gland has been already completely atrophied. P, length of the proliferative zones. Arrow, H3S-28ph positive mitotic cells. Top left, HE staining, top right, Ki-67 immunostaining, Original magnification, 100×. Bottom, H3S28ph immunostaining. Original magnification, 400×.

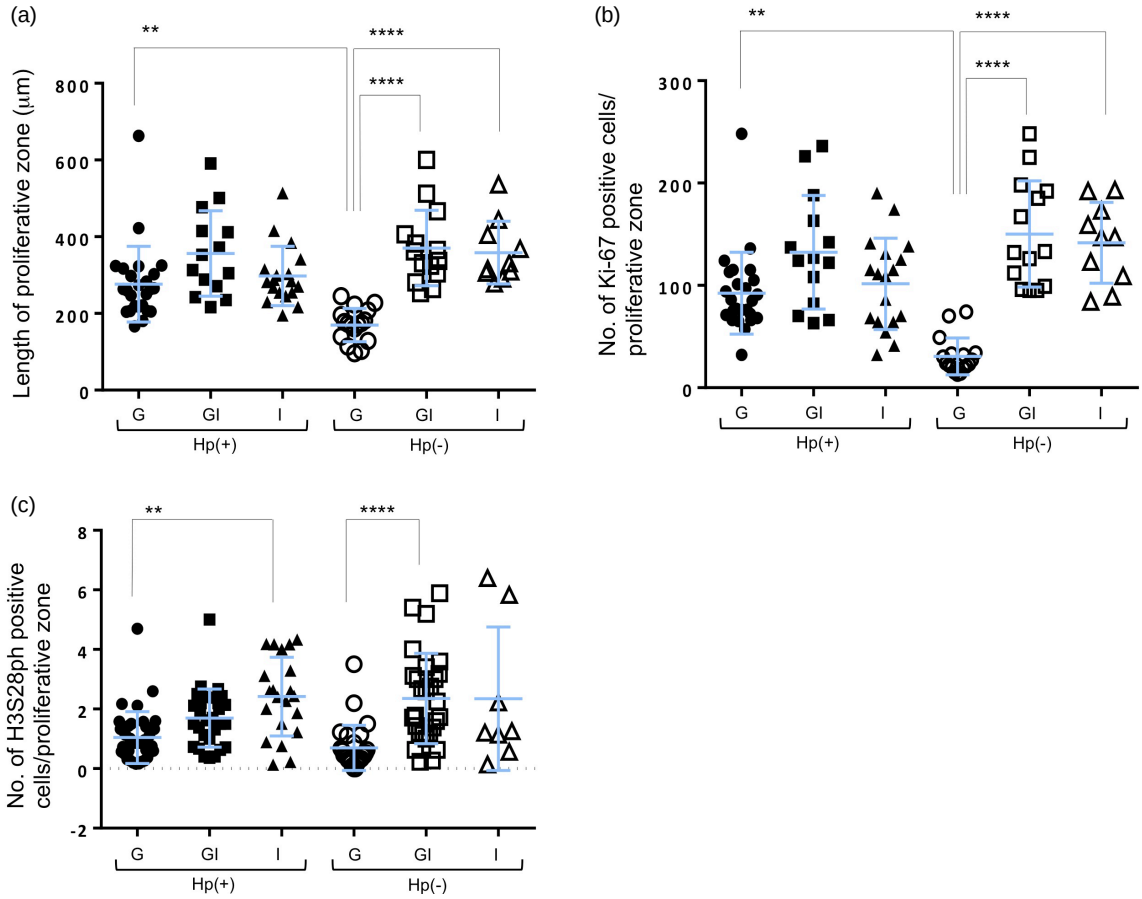

Figure 4. Analysis of proliferative zone in G, GI-IM, and I-IM glands between Hp(+) and Hp(−) groups. (a) Length of the proliferative zone; (b) the number of Ki-67 positive cells/proliferative zone. In (a) and (b), significant decrease was observed in Hp(−)/G glands after eradication compared with those in Hp(+)/G. IM glands were not recovered upon eradication. Hp(−)/G shows significant lower values compared with Hp(−)/GI and Hp(−)/I after eradication; (c) the number of H3S29ph positive cells/proliferative zone. Significant lower values in Hp(+)/G and Hp(−)/ G compared with IM counterparts, not being affected with eradication. $^{**}P < 0.01$, $^{****}P < 0.0001$.

in Hp(+)/I glands was increased significantly compared to that in Hp(+)/G glands. After eradication, numbers of mitotic phase cells in each gland were not significantly changed, with the number in Hp(−)/G glands still significantly lower than that in Hp(−)/GI glands (**Figure 4(c)**).

4. Discussion

Since the discovery of *H. pylori*, this organism has been clarified to play a major role in the induction of chronic atrophic gastritis and development of IM [25] [26]. In the current analysis, we analyzed whether and how far this serial process of atrophy and intestinalization could be recovered to normal gastric mucosa with eradication of *H. pylori*.

In previous trials, several reports did not always show the improvement of gastric atrophy and IM with eradication of *H. pylori* [27]-[29] as in our analysis. In contrast, other publications have shown the effectiveness of eradication for improving gastric lesions in the antrum or corpus, at least in part [30]-[33]. Although recent findings tend to show recovery of atrophy and controversial results for IM, it is difficult to precisely compare the degree of factors for the updated Sydney System in small biopsy samples. Exacerbation of atrophic gastritis is often coupled with development and progression of IM. However, since the schematic view of the updated Sydney System [23] describes atrophy and IM separately, only mild atrophy without IM might be acknowledged as “atrophy”. Since the observation period after eradication in our analysis was relatively short, for 14.7 ± 17.1 months (median, 4 months), finding of histological changes might have been hindered.

We have analyzed the expression of mucin core protein before and after *H. pylori* eradication. However, no significant changes in expression of mucin core protein were seen in our scoring system, despite alleviation of foveolar epithelium in G glands in this analysis. A large discrepancy was found in the expression of mucin core proteins among our and previous reports. Matsuzwa *et al*. [34] reported that MUC6 in pyloric gland cells were increased in *H. pylori*-associated gastritis and decreased to almost normal levels after eradication. In contrast, Fichman and Niv [35] and Kang *et al*. [36] analyzed histological changes to reveal up-regulation of MUC6 mucin core protein with eradication of *H. pylori*. These two reports, however, showed controversial results for MUC5AC. This might have been caused by sampling deviation of biopsy specimens and evaluation methods.

Eradication of *H. pylori* alleviated hyperplastic and hypertrophic enlargement of foveolar epithelium only in G-type, but not in GI-IM and I-IM. Consistent with our results, endoscopic observation showed that enlarged and elongated gastric pits improved to small oval or round pits, whereas no such change was observed in subjects with severe atrophy and IM [22].

In the antrum, the proliferative zone is localized between the surface foveolar epithelium and pyloric glands, and is characterized by Ki-67 immunopositivity. Our results showed that proliferative zones widened and numbers of Ki-67-positive cells per gland were increased in all G, GI-IM, and I-IM glands in *H. pylori*-positive cases. Nonetheless, upon eradication of *H. pylori*, the proliferative region and Ki-67-positive cells were significantly decreased only in G-type glands. Murakami *et al*. [37] observed mucosal cell proliferation in *H. pylori*-associated gastritis and showed that eradication of the bacteria markedly reduced proliferation in both the antrum and corpus. In contrast, El-Zimaity *et al*. [38] documented that antral mucosal proliferation was sustained despite successful eradication of *H. pylori*. However, the presence of IM was not clearly described in their reports. Erkan *et al*. [39] compared Ki-67-positive proliferative index in IM and chronic gastritis and showed a significantly higher index in IM.

Histone H3 is phosphorylated at serine 28 during the M phase in the cell cycle, being detected with HTA28 antibody [24]. In G-type glands harboring no IM, H3S28ph-positive cells were not increased with *H. pylori* infection, which was unexpectedly not altered by eradication in contrast to the proliferative zone characterized as the Ki-67-positive region. On the other hand, in IM glands including both GI-IM and I-IM, the number of M-phase cells increased in the *H. pylori*-infected state and did not recover with eradication. In contrast to our results, Hibi *et al*. [40] reported that mitotic index was elevated in the non-eradicated group, but was significantly decreased in the eradicated group. Since this report does not precisely identify what types of gland we reevaluated, mixtures of G, GI-IM, and I-IM glands could have been used for evaluation. Taking into account all the data, the beginning of IM could represent a point of irreversible change, despite eradication of *H. pylori* (**Figure 5**).

To prevent gastric lesions, eradication of *H. pylori* has been approved not only for gastric cancer or peptic ulcer patients but also for cases of *H. pylori*-induced chronic gastritis. Several reports suggested that successful eradication might reduce the occurrence of metachronous gastric cancer after endoscopic resection of early gastric cancer in prospective studies up to 3-year follow up [19] [41] or in a retrospective study [42]. Contrasting with the above favorable analyses, other reports have not always shown welcome consequences with the eradication of *H. pylori*. Wong *et al*. [20] conducted a 7.5-year randomized controlled trial in China and revealed that eradication of *H. pylori* was clarified to significantly decrease the subsequent development of gastric cancer only in the subgroup without gastric atrophy, IM, or dysplasia but the overall incidence of gastric cancer did not differ significantly between participants receiving *H. pylori* eradication and those receiving placebo. Yanaoka *et al*. [21] performed a prospective study to clarify the risk of gastric carcinogenesis with simultaneously monitoring degree of chronic gastritis using serum pepsinogen level and pepsinogen I/II ratio and observed significant reduction in cancer incidence in pepsinogen test-negative subjects with mild gastritis after *H. pylori* eradication over a mean period of 9.3 ± 0.7 years. Maehata *et al*. [43] performed a retrospective multicenter study including 268 *H. pylori*-positive patients who had undergone endoscopic resection of gastric cancer and revealed severe mucosal atrophy as an independent risk factor for the development of metachronous cancers despite unimproved overall risk. Another meta-analysis [44] supports the idea that eradication of *H. pylori* is effective only in the subgroup without IM or dysplasia. Animal models support the idea that *H. pylori* eradication was useful to prevent gastric carcinogenesis especially when performed in an earlier period [15]-[18] [25] [26].

5. Conclusion

In conclusion, as mentioned above, mucosal damage with IM may not recover to gastric-type mucosa, so the

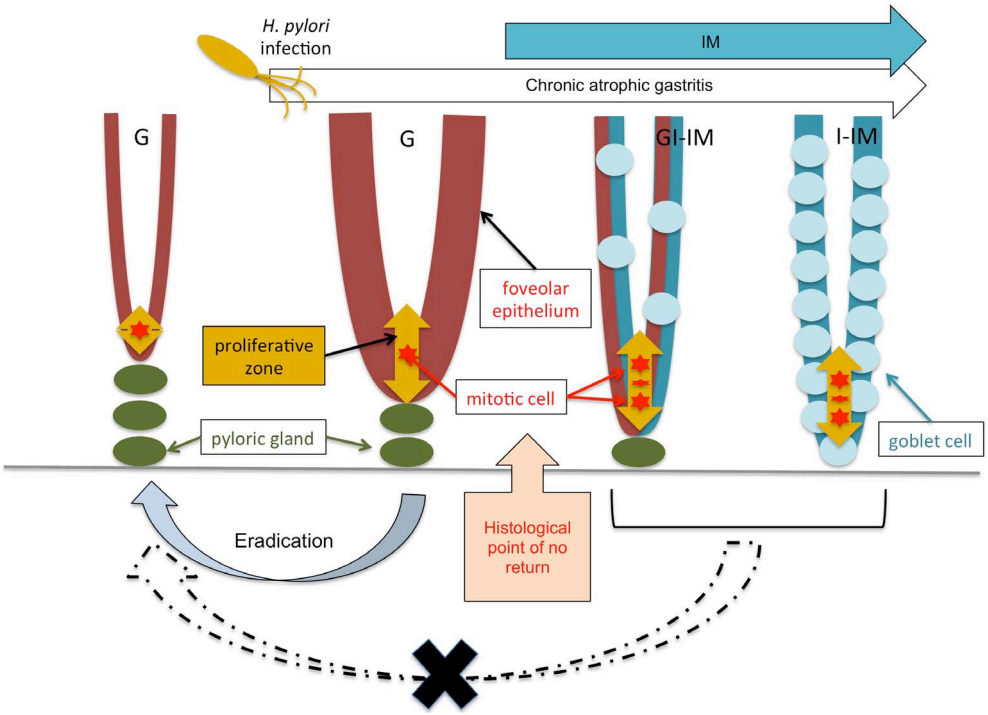

Figure 5. Progression of chronic atrophic gastritis and IM and reversibility of histological change with eradication of *H. pylori*. Chronic atrophic gastritis progress with process of IM. "Histological point of no return" indicates irreversible point. G, gastric-type gland; GI-IM, gastric-and-intestinal mixed intestinal metaplasia; I-IM, solely intestinal-intestinal metaplasia.

shift from G to GI-IM would represent a candidate for a "histological point of no return" with eradication of *H. pylori*. As a result, it is advised that *H. pylori* would better be eradicated at a younger age before the development of IM, to most effectively prevent gastric carcinogenesis.

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Conflict of Interests

The authors have no conflict of interests to disclose.

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Multiple Spinal Intradural-Intramedullary Involvement by Metastatic Carcinoma with Neuroendocrine Differentiation with Occult Primary—An Unusual Case Report and Review of Literature

Anshu Gupta[1*], Sachin Sinha[2]

[1]Pathology, Institute of Human Behavior and Allied Sciences (IHBAS), Delhi, India
[2]Oral Pathology, Narain Sewa Sansthan, Bengaluru, India
Email: *dransh2002@yahoo.co.in, drsachinsinha@rediffmail.com

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Abstract

Although vertebral column is recognized as the most common site for bony metastasis in patient with systemic malignancy, intramedullary metastases to the spinal cord is infrequent. Between 5% - 10% of cancer patients develop spinal metastasis during the course of their diseases. Intramedullary tumors are rare, comprising 3.5% of spinal metastasis. Most metastatic spinal lesions (70%) are found at the thoracic level, 20% in lumbar region and 10% in the cervical region. We report a rare biopsy proven case of intramedullary spinal metastatic carcinoma with neuroendocrine differentiation because of its unusual presentation, involving spine at multiple noncontiguous levels, which appeared as irregular small nodules on MRI. The primary tumor was most likely from occult primary in lung. Biopsy from the spinal lesion established the diagnosis of metastatic carcinoma with neuroendocrine differentiation. Therefore, in patients with spinal metastasis, a thorough work up is advised to evaluate primary site. This would help to delineate the nature and the extent of the systemic disease. We highlighted herein the clinical presentation, radiological findings particularly MRI and role of biopsy in the diagnosis and treatment of intramedullary spinal metastasis.

Keywords

Spinal Cord, Metastasis, Carcinoma, Neuroendocrine

*Corresponding author.

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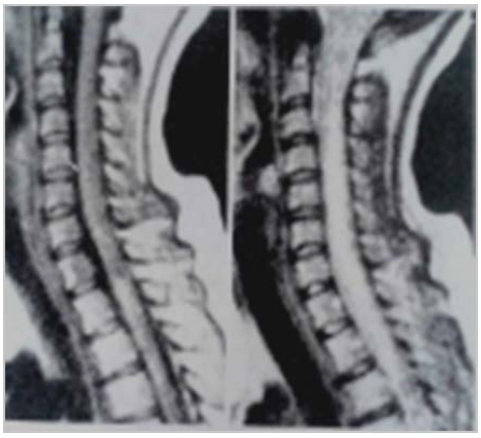
1. Introduction

Intramedullary spinal cord metastases (ISCMs) are an unusual complication of malignancies outside the central nervous system [1]. Intramedullary tumors arise within the substance of the spinal cord whereas extramedullary tumors are extrinsic to the cord [2]. Metastatic lesions occur at multiple noncontiguous levels in 10% to 38% of cases. As many as 10% of the patients with symptomatic spinal metastases present with no known primary lesion [3]. Intramedullary metastasis arises hematogenously from tumor emboli in para-vertebral venous plexus, which is characterized by absence of valves or from direct extension from the nerve roots or CSF. The clinical presentation of the metastatic spinal disease is predominantly pain, neurological deficit, progressive deformity, general weakness and bladder and bowel dysfunction. MRI is now the method of choice to detect the presence and extent of spinal metastasis. It provides excellent visualization of spinal cord involvment, ligamentous involvment, spinal cord edema, and degree of canal compromise and cord compression. Biopsy from the affected site proves to be useful in the confirmative diagnosis, treatment and prognosis of metastatic lesion. Prognosis is based on degree of deficit, duration of symptoms, type and location of tumor and degree of advancement of disease. Management of metastatic spinal tumor is focused on pain relief, preservation of neurologic function, prevention of pathologic fracture and correction of spinal instability for improving quality of life. Palliation is the real treatment goal. Treatment includes conservative and operative treatment. Conservative treatment methods are analgesics and braces, chemotherapy, radiotherapy and steroid therapy.

2. Case Presentation

A 38 year old male presented with progressive weakness in bilateral lower limbs from 2 - 3 months. Patient also develops urinary incontinence in the due course of time and finally patient became bed-ridden. Patient had no history of cough or respiratory distress or pain abdomen. On examination, no thyroid swelling was palpable on deglutination. Per abdomen and pelvic examination were normal. Routine investigations that included hematological and biochemical (T_3, T_4, TSH, urea, creatinine etc.) investigations were normal. X-ray and CT chest were also normal. Ultrasound abdomen and pelvis showed no mass or any other abnormal finding. MRI of whole spine done with following findings: *MRI dorsolumbar spine* revealed nodular intradural and intramedullary lesions in dorsal aspect of cord at T1, T5 and T10 level producing intramedullary T2 hyperintense signal intensity from C7 to T2 and T9 to conus. Mild diffuse disc bulge is seen at the level of C3/C4, C4/C5 and C6/C7 (**Figure 1**). *MRI Lumbosacral spine* revealed intramedullary T1 hypointense signal intensity in the conus associated with T1/T2 hypointense loculated cystic lesion in the spinal canal at S1 & S2 level (**Figure 2**). A tiny nodular intermediate signal intensity lesion was seen with cauda equine nerve root at L4 nerve root. Cerebrum, cerebellum and brain stem showed no significant pathology. The lesions in lumbo-sacral region resulted in clinical symptoms of weakness in bilateral lower limbs and urinary incontinence.



(a) (b)

Figure 1. (a) & (b) MRI cervico-thoracic region showing intramedullary lesions producing T_1 hypointense/T_2 hyperintense signal intensity from C7 to D2 respectively.

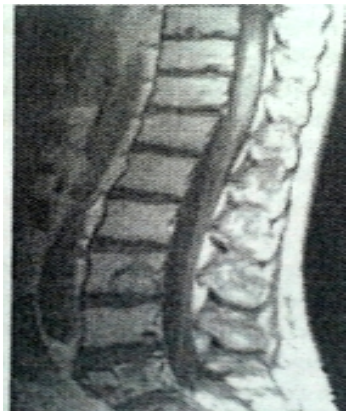


Figure 2. MRI lumbo-sacral region showing intramedullary heterogeneously enhancing masses in the lumbar & sacral region in T_1.

Intra-operative biopsy from nodular lesion in dorsolumbar spine was performed and sent to pathology department for frozen section examination. A provisional diagnosis of malignant small round cell tumor was made on frozen section by cryostat. Based on the provisional diagnosis, further excision biopsy done to relieve compression symptoms and sent for microscopic examination. The biopsy specimen showed multiple grayish brown soft tissue pieces that together measured 2.8 × 2.5 × 0.5 cms grossly. Cut surface was gray white with few hemorrhagic areas. Paraffin sections were prepared and stained with routine Haematoxylin and Eosin (H & E) stain, special stains such as reticulin and immunohistochemical stains for cytokeratin, synaptophysin and chromogranin.

2.1. Procedure for Haematoxylin and Eosin (H & E) Stain

1). Take sections to water.
2). Place sections in haematoxylin for 5 minutes.
3). Wash in tap water.
4). "Blue" sections in Scott's tap water.
5). Wash in tap water.
6). Place sections in 0.3% acid alcohol for a few seconds.
7). Wash in tap water.
8). Place sections in eosin for 2 minutes.
9). Wash in tap water.
10). Dehydrate, clear and mount sections in DPX.

Results

Nuclei-blue-black, cytoplasm-varying shades of pink, muscle fibres-deep pinky red, fibrin-deep pink, red blood cells-orange/red.

2.2. Immunohistochemical (IHC) Staining Procedure

1). Paraffin embedded 4 micron-thick sections used.
2). Dewax in xylene, 3 - 5 min
3). Place the slides into glass slide chamber and fill them with the processing buffer (citric acid buffer, ph 6.0 4.
4). Antigen retrieval by autoclave (121˚C, 15 min).
5). Take out the glass slides in chamber, wait for 40 min, and rinse the slides 3 times with distilled water or Phosphate Buffer Solution (PBS) by emptying and refilling the chambers.
6). Block endogenous peroxidase activity with freshly made 0.3% H_2O_2 in methanol, 20 min.
7). Three changes of PBS for five minutes.
8). Incubate with monoclonal primary antibody, 4˚C, overnight.
9). Wash with PBS, 3 × 5 min.

10). Incubate with secondary antibody, 60 - 120 min. at room temperature.
11). Three changes of PBS for five minutes.
12). Stain with diaminobenzidin (DAB) solution, 10 min. at room temperature. 0.01% DAB in 0.5 M Tris/HCI (pH 7.4) solution should be filtrated. H_2O_2 must be added to a final concentration of 0.01%.
13). Wash with running tap water, 3 min.
14). Counterstain with Mayer's hematoxylin, 30 sec.
15). Wash with running tap water.
16). Dehydrate with increasing solutions of ethanol: 50%, 70%, 96%, absolute, 3 min. each.
17). Clear with xylene, 3 × 3 min.
18). Mount with mounting medium.

Microscopic examination: Biopsy showed a cellular tumor in which cells were arranged in sheets, well defined clusters, trabeculae, around blood vessels forming pseudo-rosettes and scattered diffusely in highly vascularised fibrocollagenous stroma. Tumor cells were small, monomorphic round to oval to elongated with moderate atypia, stippled chromatin and inconspicous nucleolus. Cytoplasm is scanty and barely visible. Nuclear molding was seen in occasional cells. Some of the cells were slightly larger and showed moderate amount of eosinophilic cytoplasm. Mitosis was infrequent (**Figure 3**). Reticulin stain showed reticular fibers around group of cells. On immunohistochemistry, tumor cells showed diffuse positivity for cytokeratin, synaptophysin and chromogranin (**Figure 4**). S-100 was focally positive. Vimentin positivity is seen in fibro connective stroma. Findings were that of metastatic carcinoma with neuroendocrine differentiation. Small cell carcinoma lung was considered as the first possibility but chest X-ray and CT lung was normal.

Postoperatively, patient was given external beam radiotherapy but continued to experience pain and paraesthesias. Patient showed no improvement and his general condition gradually deteriorated in due course of time. Ultimately, he died within six months.

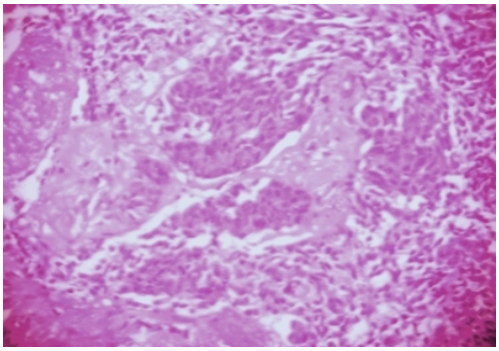


Figure 3. Tumor cells in clusters and scattered diffusely in loosely vascularised fibrocollagenous stroma. (Haematoxylin & Eosin stain, 10×).

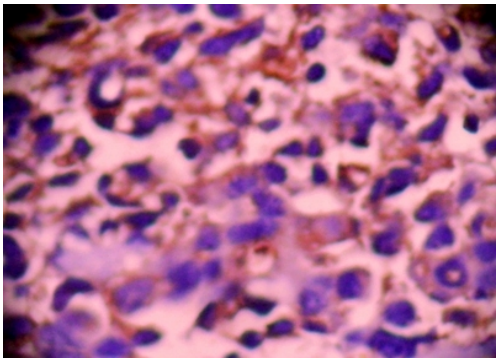


Figure 4. Microphotograph showing diffuse positivity (brown) for synaptophysin in cytoplasm of tumor cells (Immunohistochemistry, 40×).

3. Discussion

Spinal metastases occur 20 times more commonly than primary tumors of spine. Five to ten percent of cancer patients develop spinal metastasis [3]. Spinal metastasis are often multiple and are frequently seen late in the course of disease, in which brain or visceral metastasis are also evident. Intramedullary spinal cord metastases (ISCMs) represent only 0.9% to 2.1% of autopsy cases in patients with cancer [4]. One third of adult spinal cord tumors are intramedullary and rest are extramedullary. Of the tumors metastasizing to the spinal cord lung carcinoma is the most common accounting for 50% followed by breast carcinoma, Lymphoma, melanoma, colorectal carcinoma, Hodgkin disease, head and neck carcinoma and leukemia [5] [6]. Primary tumors less commonly reported to metastasize to spine include schwannoma, mesothelioma, merkel tumor, plasmacytoma, teratoma, as well as basal cell, parotid, nasopharyngeal, laryngeal, esophageal, gall bladder, pancreas, ovarian, endometrial, and urinary bladder tumors. Only about 2% of spinal metastasis is of unknown origin [7].

There is a slight preponderance of metastatic spinal tumors in males (60%) compared to females. In our case, the patient was a male of 38 years of age.

Grossly, the spinal cord appears firm and swollen, frequently in a fusiform manner [7]. Multiple segments are often affected. Metastatic lesions occur at multiple noncontiguous levels in 10% to 38% of cases [8]. Leptomeningeal and intradural involvement can be found in conjunction with the intramedullary tumor [9] [10]. In our case, there was intradural and intramedullary involvement of entire spinal cord at multiple levels causing nodular enlargement with no evidence of any lesion in brain or primary in lung, thyroid, pancreas, stomach, colon, prostate etc.

Extradural metastases account for approximately 95% of secondary spinal tumors. Intradural extramedullary metastases are uncommon. Intramedullary tumors are rare, comprising 3.5% of spinal metastasis. Intramedullary metastasis arises hematogenously from tumor emboli followed by para-vertebral venous plexus, which is characterized by absence of valves or from direct extension from the nerve roots or CSF [11].

Patients with spinal cord metastases arising from primary tumors outside the CNS show rapid onset of symptoms. The clinical features of the metastatic spinal disease are predominantly pain, neurological deficit, progressive deformity, general weakness and bladder and bowel dysfunction as in our case. It is axiomatic that new onset back or neck pain in a cancer patient means spinal metastasis until proved otherwise [12].

In the diagnostic work up, plain radiograph can show spinal alignment, the presence of fracture, and gross involvment by tumor. MRI is now the method of choice to detect the presence and extent of spinal metastasis. MRI imaging proves helpful because multiple lesions that are not suspected clinically are found in 30% of cases [13] [14]. In our case, MRI findings revealed intramedullary T2 hyperintense signal intensity from C7 to T2 and D9 to conus.

When primary cancer is not yet identified, metastatic extension can be difficult to detect [12]. In our case, primary site could not be ascertained despite all investigations and detailed examination of the patient, which remained the main limitation of our study.

Biopsy from the affected site proves to be useful in the confirmative diagnosis, treatment and prognosis of metastatic lesion. A direct biopsy during surgery prior to definitive surgery is advantageous for the patients [15]. In our case biopsy from the nodular lesion was reported as metastatic carcinoma with neuroendocrine differentiation based on routine H & E staining and further confirmed by immunohistochemistry which showed positivity for synaptophysin, chromogranin and cytokeratin. Small cell carcinoma lung was considered in differential diagnosis but could not get supportive clinical and radiological findings to make a definitive diagnosis.

Management of metastatic spinal tumor should be focused on pain relief, preservation of neurologic function, prevention of pathologic fracture and correction of spinal instability for improving quality of life. Treatment includes conservative and operative treatment. Conservative treatment methods are analgesics and braces, chemotherapy, radiotherapy and steroid therapy. Rapidly progressive neurological deterioration can not be recovered by radiotherapy because immediate spinal decompression is not possible.

Prognosis is uniformly poor with life expectancy of months as this represents an advanced stage of disease [6]. Prognosis is based on degree of deficit, duration of symptoms, type and location of tumor and degree of advancement of disease. Our patient had poor prognosis and died within few months due to rapid disease progression [15].

4. Conclusion

This report pertains to a rare case of intramedullary spinal metastatic carcinoma at multiple sites with unknown

primary having poor prognosis. Therefore, in patients with spinal metastasis, a thorough work up is advised to evaluate primary site. This would help to delineate the nature and the extent of the systemic disease. Treatment and prognosis depends upon age of the patient, site and extent of involvement in spinal cord, alignment of spine and histological typing of the tumor. Depending on this, various modalities such as surgery, radiotherapy and chemotherapy are provided to improve quality of life and prognosis. Loss of sphincter control is a poor prognostic feature and is mostly irreversible. This report is of interest to multiple specialities. No treatment has been proven to increase the life expectancy of patients with lung cancer and spinal metastasis. Pain relief and maintenance of quality of life must be balanced against the patient life expectancy, presence of co-morbidities, immunological, nutritional and functional status.

Consent

A written informed consent was obtained from the patient's next kin for the publication of this case report and the accompanying images.

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