

An observational study of synchronous/metachronous tumours in microsatellite unstable/mismatch repair deficient endometrial carcinomas

Suzanne Kotzé^{1,*}, Reubina Wadee^{1,*}¹Department of Anatomical Pathology, University of the Witwatersrand/National Health Laboratory Service (NHLS), 2193 Johannesburg, Gauteng, South Africa*Correspondence: zanao512@gmail.com (Suzanne Kotzé); reubinawadee@gmail.com (Reubina Wadee)DOI: [10.31083/j.ejgo4205131](https://doi.org/10.31083/j.ejgo4205131)This is an open access article under the CC BY 4.0 license (<https://creativecommons.org/licenses/by/4.0/>).

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Objective: Microsatellite instability (MSI) is one of the genetic abnormalities underlying endometrial carcinoma, especially endometrioid endometrial carcinoma (EEC). Microsatellite unstable endometrial carcinomas may be associated with multiple primary malignancies. We aimed to document the incidence and type of associated tumours in MSI/mismatch repair deficient (MMR-d) endometrial carcinomas at a single institution in South Africa. **Methods:** The study assessed EECs for the period 2009–2015 at a Johannesburg hospital and followed on previous research undertaken in our department in which 66/145 MSI/MMR-d EECs were identified using immunohistochemistry and polymerase chain reaction (PCR). After ethical clearance was granted, using the microsatellite unstable/MMR-d endometrial carcinoma case-specific laboratory reference numbers, a retrospective search and cross-reference for associated histologically proven tumours, was performed. **Results:** Three patients (4.5%) out of 66 confirmed MSI/MMR-d endometrial carcinoma cases had associated tumours, including an invasive squamous cell carcinoma of the cervix and mucinous breast carcinoma. One patient had two tumours, namely uterine leiomyosarcoma and rectal adenocarcinoma. The incidence of EEC patients with an associated tumour is significantly lower ($p = 0.0045$) than that documented in the United States of America (USA). **Conclusion:** Our study's incidence of associated tumours in MSI/MMR-d endometrial carcinoma patients was significantly lower than studies from the USA. The associated tumours in our study included traditional Lynch syndrome tumours such as colonic adenocarcinoma and demonstrated less commonly associated tumours, namely cervical carcinoma and uterine leiomyosarcoma. These findings highlight the need for further research in our population to assess risk factors and the true incidence of Lynch syndrome-associated tumours.

Keywords

Lynch syndrome; Microsatellite instability; Endometrial carcinoma; South Africa; Metachronous tumours; Synchronous tumours

1. Introduction

According to the most recent GLOBOCAN 2020 database release, uterine carcinoma was the 16th most common cancer diagnosed worldwide [1]. In the USA, the American Cancer Society estimates a 7% incidence of uterine carcinoma in 2021

[2]. In 2018 the incidence of uterine cancer was 3.2% in sub-Saharan Africa [3]. According to the latest published statistics in South Africa, cancer of the uterine corpus had a similar incidence of 3.77% [4, 5].

Multiple primary malignancies are defined as the development of more than one tumour, synchronous or metachronous, in the same individual fulfilling specific criteria delineated by Warren and Gates [6, 7]. These criteria include: a malignancy must be confirmed histologically; and each tumour should be separate and not a metastasis from the other [7]. The International Association of Cancer Registries (IACR)/International Agency for Research on Cancer (IARC) guidelines recommend that a tumour is synchronous if occurring within six months after the first neoplasm, or is metachronous when occurring after six months [6]. The incidence and risk of patients with endometrial carcinoma developing other tumours is approximately 10% to 23% [8, 9]. With improved diagnostic and treatment modalities, patients may now survive longer, thus providing time for such patients to develop second or multiple tumours [10].

A genomic analysis performed by The Cancer Genome Atlas Research Network (TCGA) classified endometrial cancers into four molecular groups: copy-number low, copy-number high, *POLE* ultramutated and microsatellite instability hypermutated [11]. Microsatellite instability (MSI) is one of the genetic abnormalities underlying endometrial carcinoma, especially endometrioid endometrial carcinoma and is associated with inherited and sporadic endometrial carcinomas [12, 13]. It has been demonstrated that patients with *POLE* mutations have the best prognosis of the four categories, while those with MSI and copy-number low mutations have an intermediate prognosis and patients with *p53* and copy-number high mutations have the poorest outcomes [11, 14]. MSI is present in up to 90% of endometrial carcinomas associated with the heritable Lynch syndrome and in approximately 30% of sporadic endometrial carcinomas [12]. Sporadic endometrial carcinomas are secondary to *MLH1* promoter methylation via epigenetic silencing [13, 15].

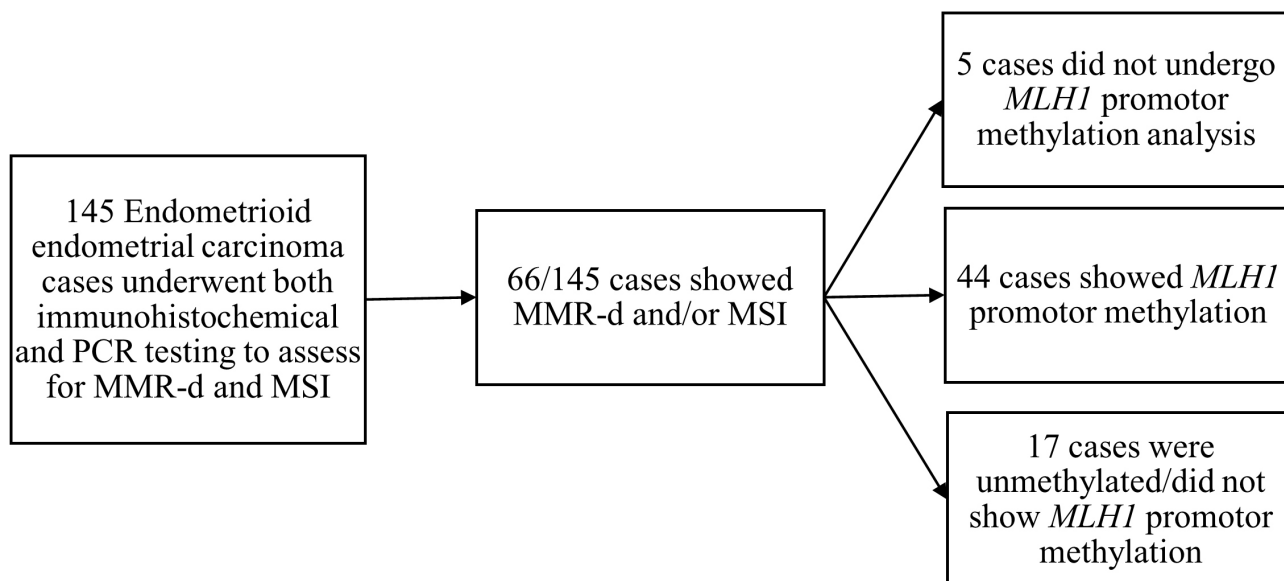


Fig. 1. Flow chart illustrating the number of cases that underwent MSI/MMR-d and *MLH1* promotor methylation analysis [20].

Lynch syndrome is an autosomal dominant syndrome with germline mutation of DNA mismatch repair (MMR) genes resulting in a predisposition to multiple cancers, especially colorectal and endometrial tumours [16–18]. Endometrial carcinoma may be the first tumour diagnosed and may suggest underlying Lynch syndrome [19]. Endometrial carcinoma is associated with multiple primary malignancies such as cancers of the breast, ovary and most commonly, colorectum [8, 9, 13].

To the best of our knowledge, a study documenting tumours associated with microsatellite unstable endometrial carcinoma in South Africa has not been performed. The burden of these tumours on our population is currently unknown. We aimed to assess the incidence of tumours associated with MSI or MMR deficient, endometrial carcinomas at a single institution in South Africa.

2. Materials and methods

This study follows previous research undertaken in our department of Anatomical Pathology, on endometrioid endometrial carcinomas (EECs) [20]. As microsatellite instability is more frequently seen in association with endometrioid endometrial carcinomas than non-endometrioid subtypes, the study was limited to endometrioid endometrial carcinomas [20]. Previous research by Wadee and Grayson [20] identified a total of 66 mismatch repair deficient and/or microsatellite unstable EECs using immunohistochemistry and polymerase chain reaction (PCR) tests. Wadee and Grayson [20] considered a case to be mismatch repair deficient (MMR-d) if the tumour showed loss of nuclear staining of one or more of the MMR antibodies (MLH1, PMS2, MSH2 and MSH6). A case was identified as microsatellite unstable by PCR if one or more markers showed a difference in the size of the alleles [20]. A case was classified as MSI-H (microsatel-

lite instability-high) if two or more markers showed dissimilarity in allele size, MSI-L (microsatellite instability-low) if one marker showed difference in allele size, and microsatellite stable (MSS) if none of the markers showed a difference in allele size [12]. The PCR markers used were NR-21, NR-24, NR-27, BAT-25 and BAT-26 [20].

These 66 EECs also underwent *MLH1* promotor methylation analysis in the study by Wadee and Grayson [20]. Of the 66 endometrial carcinomas, 44 were methylated, 17 were not methylated and one did not have enough DNA to be tested. Four cases were not tested for *MLH1* promotor methylation as they did not show loss of MLH1 immunohistochemical staining [20] (Fig. 1, Ref. [20]).

The EEC cases from 2009–2015 were from the Department of Anatomical Pathology, University of the Witwatersrand/National Health Laboratory Services (NHLS) at Charlotte Maxeke Johannesburg Academic Hospital which provides a pathology service to various hospitals and clinics in the southern Gauteng region.

After ethical clearance (certificate number: M190939) was granted, information was obtained from the LabTrak and DISA database of the NHLS using the microsatellite unstable/mismatch repair deficient proven endometrial carcinoma case-specific laboratory reference numbers. Information obtained from these reports was used to cross-reference other associated histologically proven primary tumours both before and after the endometrial carcinoma diagnosis. The following information was used to perform a retrospective search and cross-reference for associated histologically proven tumours: hospital number, name and surname, date of birth, laboratory case number and episode number.

When an associated tumour was identified, the pathology report was used to ascertain the type of tumour, site of the associated tumour and patient age at diagnosis of the associ-

ated tumour. The date of authorization or sign-out was used as the tumour diagnosis date to establish consistency as the procedure date is often not stated on the clinical request form. No personal identifiers were documented on the data collection sheet, thus ensuring patient anonymity. The slides of the identified associated tumours were retrieved, reviewed, and the diagnoses confirmed. Multicentric and metastatic tumours were excluded as per criteria delineated by Warren and Gates [6, 7].

To assess the difference in means of both the average age of patients with and without associated tumours and the average age of patients with methylated and unmethylated endometrial carcinomas, a two-tailed *t*-test was used. To determine any statistically significant differences between the proportion of associated tumours of our study and a previous study, a two-tailed Z-proportion test was used. A significance level of 5% was used.

In accordance with the journal's guidelines, we will provide our data for the reproducibility of this study in other centers if such is required.

3. Results

Three patients (4.5%) out of 66 confirmed microsatellite unstable/mismatch repair deficient endometrioid endometrial carcinoma cases, had associated tumours. These tumours included an invasive, moderately to poorly differentiated, squamous cell carcinoma of the uterine cervix, uterine leiomyosarcoma, a mucin-producing rectal adenocarcinoma and a mucinous carcinoma of the breast (Fig. 2). One patient had two associated tumours, namely uterine leiomyosarcoma and rectal adenocarcinoma. Table 1 shows that of the three endometrial carcinoma patients who had associated tumours, one patient's tumour showed retention of all MMR proteins but had microsatellite instability in one of 5 PCR markers, while one patient's tumour showed retention of all MMR proteins but had microsatellite instability in two of the 5 PCR markers; whereas the third patient's tumour had loss of both MLH1 and PMS2 immunohistochemical markers and had microsatellite instability in one of the 5 PCR markers used [20]. Thus, only one patient had an MSI-High EEC while 2 patients had MSI-Low tumours, which are considered more similar to the MSS status than to the MSI-high status.

One patient developed a metachronous cervical squamous cell carcinoma, which was diagnosed on a cervical punch biopsy 12 months after the patient underwent a subtotal hysterectomy for endometrial carcinoma. The exact reason for a subtotal hysterectomy having been performed was not stated. The surgery was conducted by doctors who were not gynaecologic oncologists, as this was undertaken at a small district hospital. At the time of diagnosis of another patient's endometrial carcinoma, an associated (synchronous) uterine leiomyosarcoma was simultaneously diagnosed. This patient was also diagnosed with a metachronous rectal adenocarcinoma 9 months after the diagnosis of EEC. The third patient in our cohort who had developed an associated tumour, was

diagnosed with a synchronous mucinous breast carcinoma 1 month after her diagnosis of EEC (Table 1).

Table 2 shows that the average time between the diagnosis of endometrial carcinoma and other tumours was 7.33 months, with a median of 9 months. There was no significant statistical difference between the average age of those with, and those without a second/associated tumour ($p = 0.244$). The average age of patients without an associated tumour, at the time of diagnosis of their endometrial carcinoma was 66 years, while patients with endometrial carcinomas who developed associated tumours had an average age of 59 years at the time of their endometrial carcinoma diagnosis.

Table 3 shows that the average age of patients with methylated endometrial carcinomas, but no associated tumour ($n = 43$) was 65 years, while a single patient who had a methylated EEC and an associated tumour was 55 years of age when the endometrial carcinoma was diagnosed. Patients who had unmethylated endometrial carcinomas and no associated tumours ($n = 16$), had an average age of 67 years, whilst a single patient with an associated tumour and an unmethylated EEC was diagnosed with the EEC at 70 years of age. There was no significant statistical difference between the average age of those with methylated endometrial carcinomas and the average age of those with unmethylated tumours ($p = 0.331$). Of the three endometrial carcinoma patients who had associated primary tumours, one patient had *MLH1* promotor methylation of the endometrial tumour while one patient's EEC was unmethylated. The third patient's tumour, unfortunately, did not have enough DNA available to undergo methylation analysis.

Based on earlier research performed in our department by Wadee and Grayson [20], 41 out of 145 cases had mismatch repair protein deficiency and 46 out of 145, had microsatellite instability detected by PCR. There was MMR-d/MSI discordance identified in 25 cases [20]. Of the 41 cases shown to have MMR protein deficiency, 20 cases showed both MLH1 and PMS2 loss, 2 cases had both MSH2 and MSH6 deficiency, 16 cases demonstrated isolated MLH1 loss, 2 had isolated MSH2 deficiency and a single case showed loss of 3 markers (MLH1, MSH6 and PMS2) [20].

We compared our current results in addition to results from the previous study in our department by Wadee and Grayson, to a study by Buttin *et al.* [20, 21]. We found a statistically significant ($p = 0.0045$) difference in the incidence of associated tumours between the present study and that of Buttin *et al.* [21] (Table 4, Ref. [20, 21]). Furthermore, Table 4 shows a statistically significant difference ($p = 0.0000$) in our patient cohort when comparing the proportion of *MLH1* methylated endometrial carcinoma cases to *MLH1* unmethylated cases. There was no significant statistical difference between the proportion of methylated and unmethylated cases in our patient cohort compared to Buttin *et al.* [21].

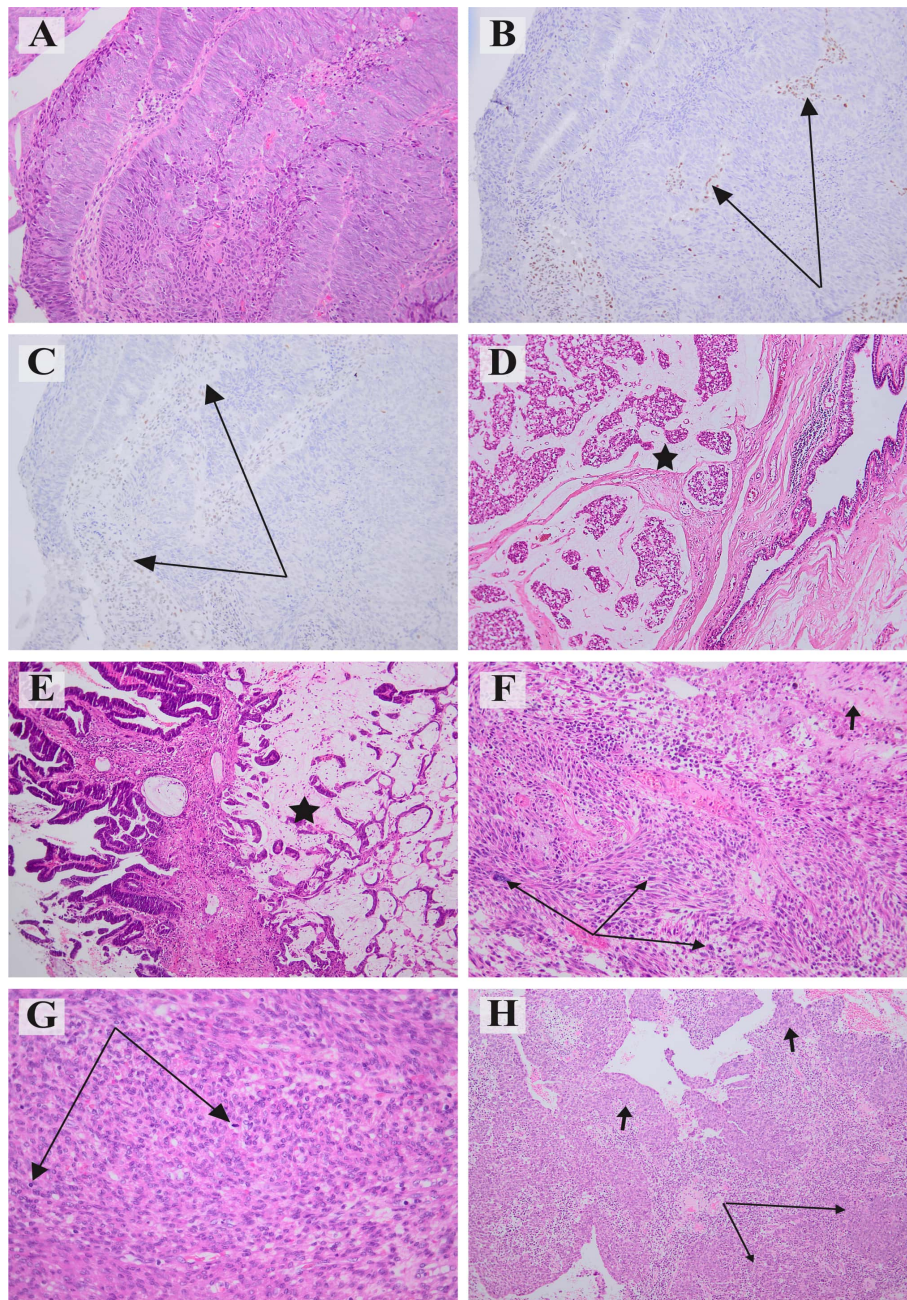


Fig. 2. Composite photomicrograph of various tumours. (A) Demonstrates a Haematoxylin and Eosin stained endometrioid endometrial carcinoma. (B) Shows an MLH1 immunohistochemical stain on the endometrioid endometrial carcinoma, in which lymphocytes and stromal cells have retained staining (two long thin arrows) while the tumour cells show loss of staining. (C) Demonstrates loss of PMS2 staining in tumour cells from the endometrial carcinoma with retention of staining of stromal cells and lymphocytes (two long thin arrows). (D) Demonstrates a mucinous breast carcinoma with tumour cells in pools of mucin (star). (E) Shows the rectal adenocarcinoma with mucin seen at the star. (F–G) Show the uterine leiomyosarcoma. An area of necrosis is identified (block arrow) and marked cellular atypia is shown (three long thin arrows) in (F). Two mitotic figures are seen (two long thin arrows) in (G). (H) Demonstrates an invasive moderately to poorly differentiated squamous cell carcinoma of the cervix. Surface dysplasia is noted (block arrows) and islands of tumour cells within the stroma are seen (two long, thin arrows). Original magnification: (A–C,F) $\times 200$, (D–E,H) $\times 100$ and (G) $\times 400$.

Table 1. Clinicopathological characteristics of patients with associated synchronous or metachronous tumours.

Patient's study assigned case number	Age of patient at diagnosis of endometrial carcinoma (years)	Age of patient at diagnosis of associated tumour (years)	Time between diagnosis of endometrial carcinoma and associated tumour (months)	Histopathological type of synchronous or metachronous tumour	MLH1 methylation status of endometrioid endometrial carcinoma	MMR protein expression and MSI status of endometrioid endometrial carcinoma
11	70	71	12	Invasive, moderately to poorly differentiated squamous cell carcinoma of the cervix (metachronous tumour)	Unmethylated	All 4 MMR markers retained MSI-Low for 1/5 PCR markers
22	53	54 53	9 0	Rectal adenocarcinoma (metachronous tumour) Uterine leiomyosarcoma (synchronous tumour)	Not enough DNA to be tested	All 4 MMR markers retained MSI-High for 2/5 PCR markers
50	55	55	1	Mucinous breast carcinoma (synchronous tumour)	Hypermethylated	Mismatch repair deficient for both MLH1 and PMS2 MSI-Low for 1/5 PCR markers

Table 2. Age of patients with endometrial carcinoma and metachronous or synchronous tumours.

Factor	Metachronous or synchronous tumours		p-value
	Identified (n = 3/66)	Not identified (n = 63/66)	
Age at diagnosis of endometrial cancer (years)			
Mean ± SD	59.33 ± 9.292	65.75 ± 9.225	0.244
Time to development of metachronous or synchronous tumour (months)			
Mean ± SD	7.33 ± 5.686		
Median (IQR)	9 (1–12)		

Table 3. Age and methylation status of endometrial carcinomas in our study.

Factor	Associated tumour		No associated tumour		Total	%	p-value
	n	%	n	%	n	%	
	n = 3	4.55%	n = 63	95.45%	n = 66	100%	
Methylated endometrial carcinomas	1	33.33%	43	68.25%	44	66.67%	0.331
Age at diagnosis of endometrial carcinoma (years) Mean ± SD	55		65.23 ± 9.486				
Median			63				
Unmethylated endometrial carcinomas	1	33.33%	16	25.40%	17	25.76%	
Age at diagnosis of endometrial carcinoma (years) Mean ± SD	70		67.44 ± 8.794				
Median			67				
Cases which did not undergo methylation assessment	1	33.33%	4	6.35%	5	7.58%	
Reason	Insufficient DNA		Not tested due to intact MLH1 immunohistochemistry				

Table 4. Comparison between studies undertaken at our institution and those of Buttin *et al.* [20, 21].

Parameter	Data sources	Number of cases n (%)	p-value
Incidence of associated tumours	Present study	3 (4.5)	0.0045
	Buttin <i>et al.</i> [21]	19 (20.2)	
Number of <i>MLH1</i> methylated cases	Previous study from our department (Wadee and Grayson [20])	44 (72.1)	0.7991
	Buttin <i>et al.</i> [21]	66 (70.2)	
Number of <i>MLH1</i> unmethylated cases	Previous study from our department (Wadee and Grayson [20])	17 (27.9)	0.7991
	Buttin <i>et al.</i> [21]	28 (29.8)	
Present study's cases	<i>MLH1</i> methylated cases (Present study)	44 (72.1)	0.0000
	<i>MLH1</i> unmethylated cases (Present study)	17 (27.9)	

Table 5. Clinical and pathological details of the associated tumour resection specimen.

Parameter	Leiomyosarcoma/Endometrial carcinoma resection specimen
Age (years)	53
Tumour size (mm)	
Leiomyosarcoma	25 × 22 × 19
Endometrial carcinoma	55 × 24 × 10
Lymph node involvement	No lymph nodes submitted for pathological examination
Pathological TNM stage	pT1a Nx Mx, Stage IA

Table 5 shows additional clinical and pathological details of one of the patients who had undergone an excision of an associated tumour. Unfortunately, the resection specimens for the other two patients could not be traced despite an extensive search on our laboratory computer database. This may be as a result of the patients not having undergone surgical excisions at our hospital complex or could be due to patients seeking medical attention at other hospitals which we do not provide a pathology service to, or having been lost to follow-up, or the patients may have died of their disease.

4. Discussion

Our present study showed a statistically lower incidence of associated tumours when compared to a study from a western country. We also demonstrated associated tumours that are different from those traditionally associated with Lynch syndrome.

Of the confirmed microsatellite unstable or mismatch repair deficient endometrial carcinoma patients in our study, 3 cases (4.5%) had associated tumours. These associated tumours included the well-documented colorectal carcinoma and tumours less commonly associated with Lynch syndrome, including mucinous breast carcinoma, uterine leiomyosarcoma and cervical squamous cell carcinoma [19]. The rectal adenocarcinoma and cervical squamous cell carcinoma both occurred metachronously. Synchronous tumours included mucinous breast carcinoma and uterine leiomyosarcoma, the latter having been diagnosed at the same time as the endometrial carcinoma on the excision hysterectomy specimen.

The incidence of associated primary tumours in patients with endometrial carcinoma differs in the literature. In the USA, early research from the 1960's and 1980's showed an incidence of approximately 10%, whilst more recent studies,

including that of Uccella *et al.*, and Buttin *et al.* [8, 21] show an incidence of 20–22%. The initial studies were smaller in number and hospital-based compared to recent studies that included a larger number of patients, regions and better follow-up of patients [8]. Uccella *et al.* [8] assessed the clinicopathological features of patients (n = 1028) with endometrial carcinoma, which included endometrioid and non-endometrioid subtypes, in addition to the associated tumours they developed; and evaluated the risk of patients developing an associated tumour. The study by Buttin *et al.* [21], assessed the incidence and the different types of synchronous and metachronous tumours in MSI unstable endometrial carcinoma patients (n = 94) which also included endometrioid and non-endometrioid subtypes.

Our study showed an incidence of 4.5%, which is far lower than that of the American studies (20–22%) [8, 21]. Our study and the study by Buttin *et al.* [21] assessed the incidence and types of associated tumours of microsatellite unstable endometrial carcinomas. A comparison of the incidence of associated tumours between our study (4.5%) and that of Buttin *et al.* (20.2%) showed a statistically significant difference in proportions (Table 4).

Whilst our sample size was small, both polymerase chain reaction (PCR) and immunohistochemistry were conducted during the previous study in our department by Wadee and Grayson [20], to detect microsatellite unstable/MMR deficient endometrial carcinoma cases, thus ensuring a more accurate identification of cases. A study by McConechy *et al.* [22] showed a high level of concordance (93.3%) between MMR protein immunohistochemistry and MSI PCR analysis, and as such, IHC may be used instead of PCR. However, a recent publication by Wadee and Grayson [20], showed that 5% of microsatellite unstable endometrial carcinoma cases would not have been identified if PCR was not used, as these

cases did not show loss of immunohistochemical staining. Similarly, Buttin *et al.* [21] also used both PCR and immunohistochemistry.

The low incidence in our study (4.5%) compared with American studies may be explained by the smaller study number (66 patients) and that our cohort was limited to microsatellite unstable/MMR-d, *endometrioid* endometrial carcinomas, which contrasts to both that of Uccella *et al.* and Buttin *et al.* [8, 21].

Other aspects that may have limited our patient sample size include the possibility of patients having been lost to follow up or having been registered on the laboratory system with different names and surnames which would not have been traceable on our laboratory computer database. In our setting of a developing country, loss to follow up of patients is a common occurrence as we provide a service to state/public sector patients. The patients may not have easy access to transportation to get to hospitals, or the financial means to use transport or may not be able to take time off from work to come to the hospital. In addition, patients may decide to access health care facilities closer to their homes which may be in areas that we do not provide a pathology service to. Furthermore, the possibility of patients having died exists, and such knowledge may not be conveyed to the patients' treating physicians and this may then not be communicated to pathologists.

Buttin *et al.* [21] further subdivided the MSI positive group into *MLH1* methylated and *MLH1* unmethylated, categorizing tumours into sporadic and possible germline tumours respectively. Comparisons between *MLH1* methylated and unmethylated cases in the studies by Wade and Grayson and Buttin *et al.* [20, 21] showed no statistically significant differences ($p = 0.7991$) (Table 4).

The statistically significant difference in proportions when comparing the *MLH1* methylated cases with *MLH1* unmethylated cases in our present study suggests that the proportion of possible germline tumours is significantly lower than the proportion of sporadic tumours in our cohort. Germline mutations in MMR genes have been documented in 2–6% of endometrial carcinomas [19]. Currently, the incidence of Lynch syndrome differs between populations, ranging from 0–10% [15]. In South Africa, Wade and Grayson's study showed that 6.9% of patients with EEC may have underlying Lynch syndrome, which is slightly higher than the expected 2–6% [15]. Unfortunately, confirmatory germline testing is currently not available in the public sector of Gauteng, South Africa [15]. Another South African study by Vergouwe *et al.* [23] showed that in contrast to Western studies, there is a higher rate of MMR deficiency in colorectal carcinoma suggesting an increased proportion of inherited disease in regions of South Africa where the incidence of colorectal carcinoma is relatively low. As such, the incidence of possible Lynch syndrome associated tumours in South Africa was expected to be higher than the 4.5% identified in the present study.

The development of multiple tumours occurring in a patient with endometrial carcinoma, especially in the setting of Lynch syndrome, is well-established [8, 24]. These include tumours from the colorectum, ovary, small bowel, stomach, pancreaticobiliary tract, skin, brain and the urinary tract [17, 24, 25]. Endometrial and colorectal carcinomas are the most commonly described Lynch syndrome-associated tumours and the association between the two carcinomas is well documented [17, 21]. Only one patient in our study had an associated colorectal carcinoma. Although gastrointestinal tumours were the first neoplasms described in Lynch syndrome, endometrial carcinoma is the sentinel tumour in up to 50% of patients [19, 24].

One patient with endometrial carcinoma in our study had an associated mucinous breast carcinoma. The incidence of Lynch syndrome-associated breast carcinomas is marginally increasing, but the association between breast carcinoma and Lynch syndrome varies in the literature and is controversial [17, 26]. Stoll *et al.* [26] showed that women with Lynch syndrome do not have an increased risk of breast cancer compared to the general population, while Win *et al.* [27] showed that patients with MMR mutations were at a risk of developing breast carcinoma that was at least four times greater than that of the general population.

Furthermore, we identified one patient who had endometrial carcinoma and an associated uterine leiomyosarcoma. Generally, sarcomas are not commonly associated with Lynch syndrome. Sporadic sarcomas are rarely associated with microsatellite instability. However, a Brazilian study recently indicated that sarcomas, although rare, are linked to the Lynch syndrome spectrum of tumours [28, 29]. An increased lifetime incidence is reported; but the exact risk for sarcoma development has not been fully elucidated [28].

We identified one patient with an endometrial carcinoma who had an associated cervical squamous cell carcinoma, which is not considered a traditional Lynch syndrome-associated tumour [30]. However, several case studies have documented cervical carcinoma in association with germline mutations of the MMR genes [30]. Antill *et al.* [30] indicated that cervical carcinoma, including squamous cell carcinoma, could be considered part of the extracolonic manifestations of Lynch syndrome. Antill *et al.* [30] state that it is plausible that the tumour pathway of cervical carcinoma in MMR gene mutation patients may be secondary to the known carcinogenic effect of human papillomavirus (HPV) exposure, but microsatellite instability should be investigated as a possible underlying molecular pathway. In our study, microsatellite instability as an underlying molecular pathway has been confirmed for the endometrial carcinoma. While it is possible that this is the underlying pathway of the associated cervical carcinoma, such testing was not performed on any of the associated tumours, including the cervical tumour, as this was a retrospective, observational study and we did not have ethical clearance to perform any new additional studies/investigations.

The average time to development of an associated tumour was 7.33 months. A previous case series from China reporting the clinicopathological features of multiple primary malignant tumours with endometrial carcinoma, found an interval of 51.27 months between the development of the first and second tumour [9]. Another study by Amer reported an average time to develop a second tumour after the first, was 117 months; but this study was not limited to patients with only endometrial carcinoma and associated tumours as it included a wide range of tumour types [31]. Only one month passed between the development of the breast carcinoma and the endometrial carcinoma in our study, compared to Uccella *et al.*'s [8] median of 6 years. In addition, the diagnosis of the rectal adenocarcinoma in our study was made 9 months after that of the endometrial carcinoma, compared to Uccella *et al.*'s [8] median time interval of 4 years between diagnosis of the two tumours. It is clear that the time interval between the diagnosis of an associated tumour and the primary tumour in our study is far less than those of other studies. The reason for this is unknown and although our cohort is too small to draw substantial conclusions, further studies investigating possible risk factors contributing to the reduction of the time interval between multiple tumour diagnoses are needed. Furthermore, this finding may suggest that closer follow up of patients with MSI unstable tumours is indicated.

Only 1 patient had an MSI-High EEC while 2 patients had MSI-Low tumours, which have been considered more akin to the MSS status than to the MSI-high status [22]. Methylated tumours point toward a sporadic occurrence whereas unmethylated tumours that have MMR-d may indicate an underlying germline mutation [20]. Hereditary tumours tend to present at a younger age [32]. Our study, however, illustrated that the patient with a *methylated* EEC and synchronous breast carcinoma was 55 years of age at the time of her EEC diagnosis, whereas the patient with an *unmethylated* EEC and metachronous cervical carcinoma was 70 years of age at the time of EEC diagnosis. As such, despite the patient's age (70 years) the occurrence of an *unmethylated* tumour and a metachronous tumour may still suggest an underlying germline mutation and would warrant further investigation. The third patient in our cohort who had EEC and associated neoplasms had a total of 3 malignancies (metachronous and synchronous). Despite there not being sufficient DNA to perform methylation assessment, the occurrence of 3 neoplasms in this patient, of which one neoplasm was an MSI-high endometrial tumour, suggests that further investigations for possible underlying Lynch syndrome are also warranted.

The incidence of Lynch syndrome in the rest of Africa is not well recorded [33]. A 2019 population-based study from Zimbabwe was the first to study hereditary colorectal carcinomas extensively and found a 3.3% prevalence of Lynch syndrome [34]. A study in Libya assessing the incidence of synchronous and metachronous tumours, reported an incidence of 1.14%, with breast carcinoma being both the most common primary, as well as the second identifiable tumour

[35]. To the best of our knowledge no other study from the African continent has investigated the presence or absence of tumours associated with microsatellite unstable/MMR-d endometrial carcinomas.

A Zambian case series reported HIV as a risk factor for the development of multiple primary malignancies [36]. South Africa currently has the highest number of people living with HIV in the world [37]. The influence that HIV has on Lynch syndrome-associated tumours is not well known. Microsatellite alteration has been described in AIDS-associated and non-AIDS associated tumours, for example, lung carcinoma, as microsatellite alterations are present at a higher frequency in HIV associated lung carcinomas compared to sporadic lung carcinomas [38]. A South African study assessing cervical biopsies showed that HPV alone may induce MSI; however, co-infection with HIV may exacerbate disease progression [39]. Our study did not assess microsatellite instability/MMR-d in the associated tumours and did not document the HIV status (as this is not always provided with the patient's clinical information), which is a limitation of our study as the role of HIV in the development of multiple tumours is currently unknown.

5. Conclusions

To the best of our knowledge, our study is the first to document the incidence and type of associated tumours in microsatellite unstable/mismatch repair deficient (MMR-d) endometrial carcinomas in Africa. The results from our study showed a statistically lower incidence of associated tumours when compared to a western country. We have demonstrated associated tumours that are different from those traditionally associated with Lynch syndrome. The precise reasons for this are currently unknown. Further studies, including assessing the role of HIV, the incidence of multiple primary tumours and identifying possible risk factors in our population, are needed. Such information may also evaluate the feasibility and cost-effectiveness of the possible implementation of screening programs and germline testing in a developing country. In addition, this study will contribute to global data and provide a greater knowledge base from which gynaecologic oncologists, pathologists, medical oncologists, and radiation oncologists may draw information, with the ultimate aim of optimal patient care.

Author contributions

SK contributed to the design and drafting of the manuscript and critically reviewed the manuscript for relevant intellectual content. RW contributed to the conception and supervision of the study; and contributed to the drafting of the manuscript and critically reviewed the intellectual content therein. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical clearance (certificate number M190939) was obtained through the Human Research Ethics Committee (Medical) of the University of Witwatersrand. Dr. Wadee had obtained ethical clearance for use of patient tissue samples (M151051) for research towards a PhD.

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Conflict of interest

The authors declare no conflict of interest.

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