

Oral squamous cell carcinomas in patient with graft versus-host disease following allogenic bone marrow transplantation. A case report during mutant Covid-19 pandemic

Khaled S. Ben Salah^{1,2,3}, Abdulfattah A Elturki^{4,5,6}, Yuossef Swaisi^{4,5,6}, Fatma M. Emaetig^{1,7}, Mohamed A. Elfagieh^{8,9}, Firas K. Abdulmalik¹, Yaser O. HOWAYW¹⁰, Mohamed A. Harrama¹⁰, Eltaher Elshagmani^{11,12}, Ebrahim H. ElMahjoubi¹, Matthew E. Agwae¹³, AbdulHakim W. Zaggut²

- 1- Department of Oral pathology and Oral Medicine, Faculty of Dentistry, Misrata University, Misrata, Libya.
- 2- Department of Oral and Maxillofacial Surgery, National cancer institute (NCI), Misrata, Libya.
- 3- Department of Pathology, National cancer institute (NCI), Misrata, Libya.
- 4- Department of Medicine, Faculty of Medicine, Misurata University, Libya.
- 5- Department of Medicine, National cancer institute (NCI), Misurata, Libya.
- 6- Department of Heamatology, National cancer institute (NCI), Misrata, Libya.
- 7- Department of Pathology, Faculty of Medicine, Misrata University, Misrata, Libya.
- 8- Department of Surgical Oncology, National cancer institute (NCI), Misrata, Libya.
- 9- Department of Surgery, Faculty of Medicine, Misrata University, Misrata, Libya.
- 10- Department of Oral and Maxillofacial Surgery, Misurata Medical Centre, Misrata, Libya.
- 11- Department of Microbiology, National Cancer Institute (NCI), Misurata, Libya.
- 12- Department of Microbiology, Faculty of Medical Technology, Misurata University, Libya.
- 13- Cancer Research Centre, Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, 200 London Road, Liverpool L3 9TA, UK

Correspondence to:

Khaled Saleh Ben Salah, BDS, MCs, PhD (UK)

k.bensalah@nci.edu.ly

Abstract

Aplastic anaemia is a severe haematological disorder characterized by an inadequate number of hematopoietic stem cells, resulting in pancytopenia, formed by a hypocellular bone marrow. Disorders of this nature are widely treated with haematopoietic stem cell transplantation (HSCT). A potential chronic complication following (HSCT) is the growth of secondary malignancies. Notably, patients suffering from chronic graft versus host disease (cGvHD) secondary to HSCT have been shown to be more susceptible to oral squamous cell carcinoma (OSCC). Here, we present a rare case of a 30 year old Libyan woman treated with HSCT for aplastic anaemia, with subsequent complications of cGvHD and OSCC after few months of HSCT. These carcinomatous lesions were detected in the buccal gingiva and retromolar pad area at age of 31. The present case report emphasizes on the connection between oral cGvHD and OSCC, and the potential appearance of OSCC after HSCT at any time of patient life. Thus, closer follow-up is mandatory for all patients treated with HSCT who developed cGvHD, and efficient cGvHD prevention and therapeutic approaches are needed.

Keywords: haematopoietic stem cell transplant; graft versus host disease; oral squamous cell carcinoma; OSCC.

Introduction

Aplastic anaemia is a severe haematological illness characterized by hypocellular bone marrow, leading to an inadequate amounts of hematopoietic stem cells, in turn resulting in poor erythrocyte, granulocytes and platelets production (pancytopenia) [1]. In severe cases of this condition, it is typical for neutrophil and platelet counts to fall below 500/ μ L and 20,000/ μ L respectively. Aplastic anaemia appears to be associated with a geographic variation in its prevalence. For example, it is rare in countries of the west, with an average incidence of 2 persons per million, while appearing more prevalent across Asia and countries of the developing world [1]. In Libya, only few patients have been diagnosed and registered either due to a lack of diagnostic tools or wrong diagnosis altogether. Characteristically, the cause of this disease is unknown, but a link with certain drugs, benzene exposure, insecticides, viruses, and hepatitis have been reported [2]. The aetiology however generally remains unknown in half of cases of aplastic anaemia. General weakness and tiredness, contusions, nosebleed, and gingival bleeding are primary symptoms of aplastic

anaemia. There is a greater risk of infections associated with complications related to persistent pancytopenia. In patients with aplastic anaemia, sepsis caused by both bacterial and fungal infections are the major reasons for expiration. Clinically, involvement of the mouth is commonly reported, but without sufficient details about frequency, risk factors and significance of these oral conditions [1]. Haematopoietic stem cell transplantation (HSCT) is the treatment of choice for patients with aplastic anaemia associated with significant success rate [3, 4]. A frequent complication from allogeneic transplantation is Graft versus host disease (GvHD) due to an immunologic reaction from grafting immunocompetent cells to an immunodeficient, with multiple organ involvement [5]. It has been reported that about 25–40% of survivors who lived for long period of time after HSCT showed GvHD [6]. Skin, liver, gastrointestinal tract, lungs and eyes are the most affected body organs in systemic GvHD. However, approximately 80% of cases show oral manifestations including atrophy, erythema, lichenoid lesions and dryness and pain in the oral cavity [5]. GvHD causes a significant deterioration in the quality of life of survivors and it is likely to happen when the recipient (host) obtains a graft from an unrelated donor, or when the host or donor is older (can be paraphrased by: when there is considerable difference in the age between the donor and recipient) [7].

The survival of patients cured of their original malignancy is influenced by emergence of secondary cancers, described as a potentially significant long-term complication following HSCT. Secondary malignancies such as leukaemia in donor-derived cells, Hodgkin disease, non-Hodgkin's lymphoma and granulocytic sarcoma are frequently reported [8]. Significant danger of developing oral squamous cell carcinoma (OSCC) and squamous cell carcinoma of the skin as secondary solid tumours has been described in many recent studies although they remain less frequent [8]. We report a rare case of a Libyan female patient who was diagnosed by her haematologist in 2017 as a case of aplastic anaemia disease at the age of 28 years, complicated by GvHD after HSCT performed 28th August 2019. The importance of this case report lies on several points: firstly, the occurrence of this carcinoma as a late complication of bone marrow transplantation on a background of GvHD after a short period of time estimated totally by about one year and few months. Secondly, there has been the challenge associated with management of such a case at the time of a worldwide pandemic due to Coronavirus (Covid-19). Thirdly, it is a challenging case of a medically compromised patient with multiple systemic diseases.

CASE REPORT

A 31-year-old Libyan woman; mother of two children aged 7 year and 5 year. She presented with a history of aplastic anaemia (AA), diagnosed in 2017. She was treated with several courses of antibiotics to control infections and received blood transfusion every 3 months to correct haemoglobin level which had dropped to 5-6 g/dl with low platelet and low white blood cells counts. In July 2017, her bone marrow aspirate and biopsy showed hypoplastic bone marrow, hyperplasia of thrombocytopoiesis cells, and 20% cellularity. She was treated with steroids, erythropoietin, folic acid and danazol for 5 months. In December 18, 2017; bone marrow biopsy was performed and revealed severely hypoplastic bone marrow with 10% cellularity. Cyclosporine was therefore added to her medication for 3 months, and she had her last dose May 5, 2018. May 7, 2018, her haemoglobin level dropped to 6 g/dl, she received 1 unit Packed red blood cells (PRBC). May 21, 2018, her bone marrow, biopsy and aspirate showed unilateral hypocellular bone marrow with features compatible with Myelodysplastic syndrome (MDS) with multilineage and hypoplastic. Results of her blood investigations are shown in table 1. Ultrasound Scan for her abdomen, Echocardiogram and Doppler did not reveal any significant findings. A diagnosis of hypoplastic MDS (Fanconi anaemia) was arrived at and she was enrolled for a reduced intensity conditioning (RIC) allogeneic bone marrow transplantation (ALLO-BMT).

Test	Result
Flow cytometry	No Paroxysmal nocturnal hemoglobinuria (PNH) clone
Fluorescence in situ hybridization (FISH) for MDS	Negative
Cytogenetic	Pseudodiploid clone with 1q triplication
NGS:BCOR. Chromosomal breakage test:	Positive

Table 1 showing results of blood investigation performed prior to HSCT.

The patient was made to undergo ALLO-BMT with CY/FLU/ATC/TBI conditioning. 28 August 2019, an allogeneic bone marrow transplant was performed, the matched donor being her younger sister aged 16 year at operation time with age difference of 14 years between the two sisters. She shared similar blood group with the donor, different RH (patient B positive,

donor B negative) and same Cytomegalovirus (CMV) status, DEP test negative for the donor.

A few months post-allogenic bone marrow transplantation, the therapeutic procedure was complicated by the onset of severe cGVHD with clinical manifestations including chest infection, acute kidney injury, mucositis, epistaxis and gum bleeding, BK cystitis, mouth GVHD and skin GVHD. Post-transplant evaluation using bone marrow, biopsy and aspirate revealed unilateral normocellular bone marrow with trilineage differentiation and maturation. No morphologic evidence of significant dysplasia. Chimerism: 98% >>>97%. Bone marrow cytogenetic: normal. Repeated bone marrow; final diagnosis: bone marrow, aspirate and biopsy: slightly hypocellular bone marrow with trilineage hematopoiesis. Last chimerism: 96%. Her skin GVHD and mouth GVHD improving on tapering steroid and Cellcept 1000mg BID.

January 05. 2021, the patient was referred to us at the National Cancer Institute of Misrata where new blood investigations have been requested for her and results shown in table 2.

Test	Result	Test	Result
HbA1C	4.14	WBC	9.2 x 10 ³ /μl
CA19.9	35.1 U/ml	RBC	4.10 x 10 ⁶ /μl
CEA	3.60 ng/ml	HGB	14.3 g/dL
Blood urea	74 mg/dl	HCT	42.3%
Blood Creatinine	0.46 mg/dl	MCV	103.2 fL
Serum potassium	3.28 mmol/L	MCH	34.9 pg
Blood Sodium	138 mmol/L	MCHC	33.8 g/dL
ESR	7 mm/2hrs	PLT	139 x 10 ³ /μl
CRP Titer	14.0 mg/l	LYM%	19.1%
Serum Creatinine	0.7 mg/dl	MXD%	9.3%
Total bilirubin	0.6 mg/dl	NEUT%	71.6%
S.G.O.T	25 U/I	LYM#	1.8 x 10 ³ /μl
S.G.P.T	30 U/I	MXD#	0.9 x 10 ³ /μl
ALK.Phosphat	261 U/I	NEUT#	6.5 x 10 ³ /μl
Mg	2.1 mg/dl	RDW-SD	56.5 fL
Vitamin B12	427 bg/ml	RDW-CV	14.7%
Folic Acid	8.9	RDW	14.5 fL
HBsAg by Elisa	Negative	MPV	11.3 fL
HCV Ab by Elisa	Negative	P-LCR	35.5%
HIV by Elisa	Negative	PCT	0.16

Table 2. Results of blood investigations before taking the oral incisional biopsy.

She was first seen at the Maxillofacial Unit at Misrata medical centre in January 12. 2021 for oral evaluation. The patient complained of mouth soreness, unpleasant discharge, discomfort and pain in the posterior part of the lower right jaw. Clinical examination of the oral cavity revealed severely inflamed, erythematous and ulcerated gingiva. The buccal mucosa, bilaterally, showed red and white lichenoid-type lesions (Fig 1), and a plaque-like lesion on the right lateral side of the tongue. Examination of the teeth revealed presence of grade 3 mobility of lower molars on the right side. The possibility of epithelial dysplasia was considered. Two incisional biopsies, on the right retro-molar area and on the right buccal gingiva, were performed. Apart from the chlorhexidine mouthwash prescribed, no extra treatment was provided.





(Fig 1). Intra oral photographs for the lesions at time of presentation.

The microscopic examination of the specimens showed overlying squamous epithelium with ulceration, and invasive malignant tumour composed of nests of malignant squamous epithelium. The tumour cells exhibit moderate nuclear pleomorphism with frequent mitosis, and the presence of keratin pearls. The histopathologic examination also showed presence of fibrous desmoplasia. No fungal infection was reported. The final diagnosis was moderately differentiated squamous cell carcinoma (G2). The patient presented 2 weeks later with continuing discomfort in her mouth. On examination, the oral lesions showed essentially identical features as the first examination with good healing of surgical biopsy site. Clinical examination of head and neck lymph nodes revealed no indication of their involvement. Orthopantomography (OPG) examination showed right sided invasive bone destructive lesion with osteolytic nature (Fig 2). Computerized tomography scan revealed presence of an ill-defined osteolytic bone destructing lesion (Fig 3), with soft tissues enhancing components on the medial and lateral mandibular aspects, measuring 5.8 x 2.7 x 1.8 cm, mainly seen at the right mandibular body, extending beyond the symphysis menti of the left side. Multiple bilateral enlarged cervical lymph

nodes at levels II and III, the largest one seen at right submandibular region measuring 10 x 5 mm. The nasopharynx, oropharyngeal and laryngeal air spaces appeared normal. No glottic, supra or infra glottis mass lesions were observed. Intact cartilaginous skeleton of the larynx. Normal appearance of thyroid glands, submandibular, parotid salivary glands. Abdomen and pelvis CT scan revealed presence of right labial soft tissue mass lesion measuring 3.5 x 2.5 cm.

The patient was referred to medical oncology department to provide the necessary management. Regarding medical management of this patient, immunosuppressive agents including steroid discontinued and then referred to gynaecologist to take biopsy from genital growth to exclude the presence of another potential tumour. Moreover, due to lack of oncological guidelines in the management of such a patient, and the peculiarities of the oral lesion, the patient was referred to radiotherapy department. Additionally, there was also the concern and challenge that; stoppage of immunosuppressive therapy may result in worsening of GVHD with multisystem involvement.



Figure 2. Orthopantomograph showing presence of ill-defined osteolytic destructive lesion related to lower teeth on the right side.

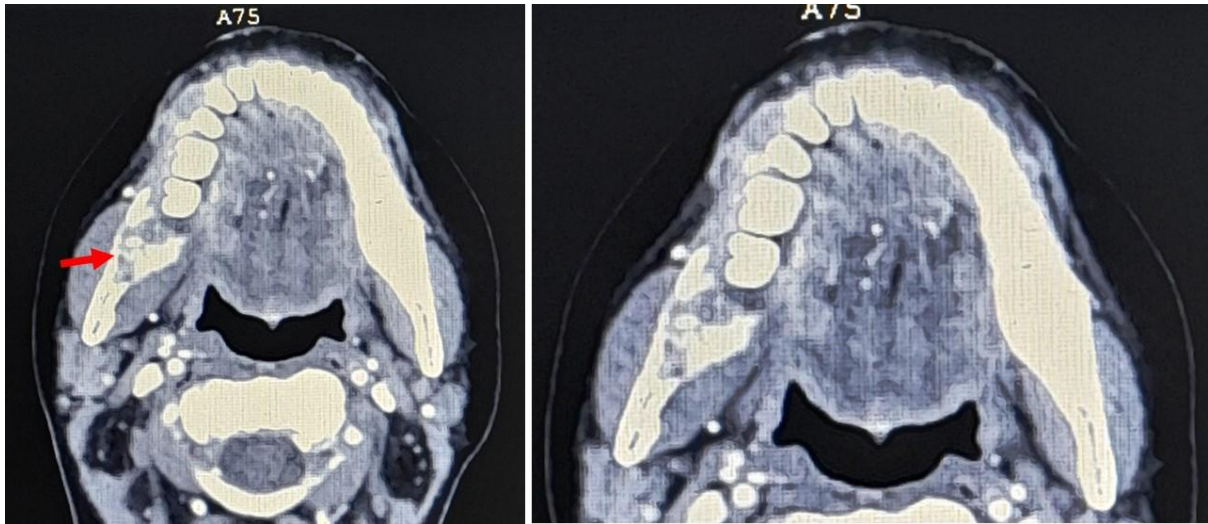


Figure 3. Computerized tomography scan revealed presence of an ill-defined osteolytic bone destructive lesion with soft tissues enhancing components on the medial and lateral mandibular aspects, mainly seen at the right mandibular body.

Discussion

The risk of developing secondary cancers in HSCT patients is considerably high [6], with a greater than ten times increased frequency of oral, oesophageal and thyroid cancers [8]. It has been reported that in people who are 10 years old at the time of HSCT, the risk reaches its maximum figure and continues to be high in people of age group between 10-29 years old, while it diminishes in people who are above 30 years [3]. In patients with a primary diagnosis of aplastic anaemia and Fanconi anaemia [9], the risk of solid cancer was stated to be higher, whereas other reports have mentioned presence of higher risks for patients with a primary diagnosis of acute and chronic leukaemia [8]. The development of secondary solid tumours peaks between 8 and 9 years after HSCT [6]. In Libya, few aplastic anaemia cases have been registered and this may be due to lack of facilities for proper diagnosis. Also, stem cell transplant is not readily available locally and patients are not usually able to travel overseas for such procedures, therefore, SCC secondary to GvHD is extremely rare. In our case, the patient was aged 28 year at the time she was diagnosed with aplastic anaemia and did undergo bone marrow transplantation when she was 30 years and 4 months. Few months later she had the manifestation of GvHD and in the space of one year, starting with the appearance of the GvHD manifestation, she developed at least two OSCCs. We were not able to confirm histopathologically the clinical changes in the buccal mucosa on both sides because of her general condition at time of biopsy. The risk factors involved in the development of secondary tumours in patients treated with HSCT have been investigated by many researchers. Occurrence of new malignant lesions are broadly considered to be associated with chemotherapy agents or irradiation for pre-transplant cytotoxic therapy [6]. No relationship has been established between cytotoxic therapy and the risk of solid cancer in this case [3]. Risk factors including smoking, alcohol consumption and older age are found unrelated in patients treated with HSCT. In parallel with these data, our patient had never smoked and did not consume alcohol or other hazardous habits considered to be known risk factors. Blood investigation results of the three viral screening performed by Elisa tests were negative. Viral screening routinely done before any stem cell transplant to avoid flare up of HCV or HBV.

Chronic GvHD (cGvHD) is thought to be a possible risk factor for the development of secondary cancers after HSCT. OSCCs have been identified by many researchers in cases with widespread cGvHD with its chronic inflammation affecting the skin and mucosa [10]. Some authors [10, 11] have reported two cases that developed OSCCs, one of them, having previously had cGvHD in the buccal mucosa [11]. The other OSCC appeared in the gingiva at a

site of previous lichenoid mucositis. Moreover, an association between cGvHD and cancer has been demonstrated in different anatomical locations. For instance, cutaneous SCC and melanoma have developed on skin previously affected by GvHD [12]. Also, reports described pulmonary tissue previously diagnosed with GvHD-related lung disease have been affected by pulmonary mucoepidermoid carcinoma [13]. In accordance with the literature, our patient has been affected by the GvHD after receiving the HSCT therapy and developed OSCCs in at least two oral sites in addition to a labial growth as the gynaecological examination and CT scan studies revealed. It is very important to exclude SCC in genitalia because of the multifocality nature of the disease.

cGvHD of the oral cavity manifests as lesions that are clinically and histologically similar to oral lichen planus, a moderately common oral cavity condition with a limited but substantial risk for malignancy. Both conditions are of similar pathogenesis, where T-lymphocytes chronically target the epithelium of the oral cavity. Such chronic immunologic damage to the oral mucosa by T cells is thought to lead to some form of malignant transformation. For the management of cGvHD therefore, HSCT recipients must use immunosuppressive medications for prolonged periods of time, and several reports have stressed the association between the development of solid cancers and long-term use of immunosuppressive drugs [3, 6]. The relationships between chronic inflammation and immunosuppression state that results from therapy is not totally clear, however, immunosuppression can hinder tissue repair in the field of chronic inflammation, such as cGvHD, thus raising the risk of malignant tumour development. In the current case, the patient has used immunosuppressive drugs and this is in agreement with the above mentioned data but the period elapsing between HSCT and the development of OSCCs as secondary solid tumours was shorter than that stated in the literature which is a relatively short period of time compared with reported peaks between 8 and 9 years after HSCT [6]. The difference in age between the donor and recipient is an important factor and as described in the literature can result in HSCT therapy failure; even if both donor and recipients are very close relatives [7]. In the present case, the patient has received the bone marrow from her younger sister because she was the only family member who was compatible. However, the fourteen years difference in age between the two sisters could be one of the reasons behind the HSCT failure and development of GvHD in our patient. Regarding medical management of this patient, immunosuppressive agents including steroid discontinued and then referred to gynaecologist to take biopsy from genital growth to exclude another potential tumour. Moreover, due to lack of oncological guidelines in the management of such patient, and the

peculiarities of the oral lesion making her a poor candidate for surgery, she was referred to radiotherapy department. Additionally, the challenge is that; stoppage of immunosuppressive therapy may result in worsening of GVHD with multisystem involvement. Furthermore, another factor that had a strong influence on our case management, beside the above mentioned points, is the pandemic of Corona virus (Covid-19). As many surgical procedures in public and private practice has been limited to very restricted emergency cases only. Also, treatment of such case is not readily available locally and patient is not able to travel overseas because of the tough restrictions applied by the health authorities during the pandemic of Covid-19 and presence of different mutations of corona virus.

In summary, a potential risk to have oral squamous cell carcinoma is linked to HSCT patients with cGvHD at high risk at any time of their age after HSCT. The known link between GvHD of oral cavity mucosa and OSCC should be a warning to doctors to pay attention to closer follow-up and the biopsy of suspected lesions for these patients. Policies for avoiding cGvHD in patients with symptomatic GvHD should be related to the production of more successful and less carcinogenic treatments. Increased indications and patients undergoing allogenic stem cell transplant may predict an increase in the incidence of secondary cancers and needs further studies and clinical trials and development of special guidelines of management.

References

1. Young, N.S., *Acquired aplastic anemia*. *Jama*, 1999. **282**(3): p. 271-8.
2. Brown, K.E., et al., *Hepatitis-associated aplastic anemia*. *N Engl J Med*, 1997. **336**(15): p. 1059-64.
3. Curtis, R.E., et al., *Impact of chronic GVHD therapy on the development of squamous-cell cancers after hematopoietic stem-cell transplantation: an international case-control study*. *Blood*, 2005. **105**(10): p. 3802-11.
4. Georges, G.E. and R. Storb, *Hematopoietic stem cell transplantation for acquired aplastic anemia*. *Curr Opin Hematol*, 2016. **23**(6): p. 495-500.
5. Eggleston, T.I., V.B. Ziccardi, and H. Lumerman, *Graft-versus-host disease. Case report and discussion*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 1998. **86**(6): p. 692-6.
6. Demarosi, F., et al., *Squamous cell carcinoma of the oral cavity associated with graft versus host disease: report of a case and review of the literature*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2005. **100**(1): p. 63-9.
7. Bauters, T., et al., *Highly effective treatment with tacrolimus ointment in an adolescent with oral graft-versus-host disease*. *Pharm World Sci*, 2010. **32**(3): p. 350-2.
8. Kolb, H.J., et al., *Malignant neoplasms in long-term survivors of bone marrow transplantation. Late Effects Working Party of the European Cooperative Group for Blood and Marrow Transplantation and the European Late Effect Project Group*. *Ann Intern Med*, 1999. **131**(10): p. 738-44.
9. Deeg, H.J., et al., *Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients*. *Blood*, 1996. **87**(1): p. 386-92.
10. Otsubo, H., et al., *Gingival squamous cell carcinoma in a patient with chronic graft-versus-host disease*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 1997. **84**(2): p. 171-4.
11. Abdelsayed, R.A., et al., *Oral precancerous and malignant lesions associated with graft-versus-host disease: report of 2 cases*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2002. **93**(1): p. 75-80.
12. Lishner, M., et al., *Cutaneous and mucosal neoplasms in bone marrow transplant recipients*. *Cancer*, 1990. **65**(3): p. 473-6.
13. Sánchez, J., et al., *Bronchial mucoepidermoid carcinoma after allogeneic bone marrow transplantation*. *J Clin Pathol*, 1997. **50**(11): p. 969-70.