SHORT COMMUNICATION

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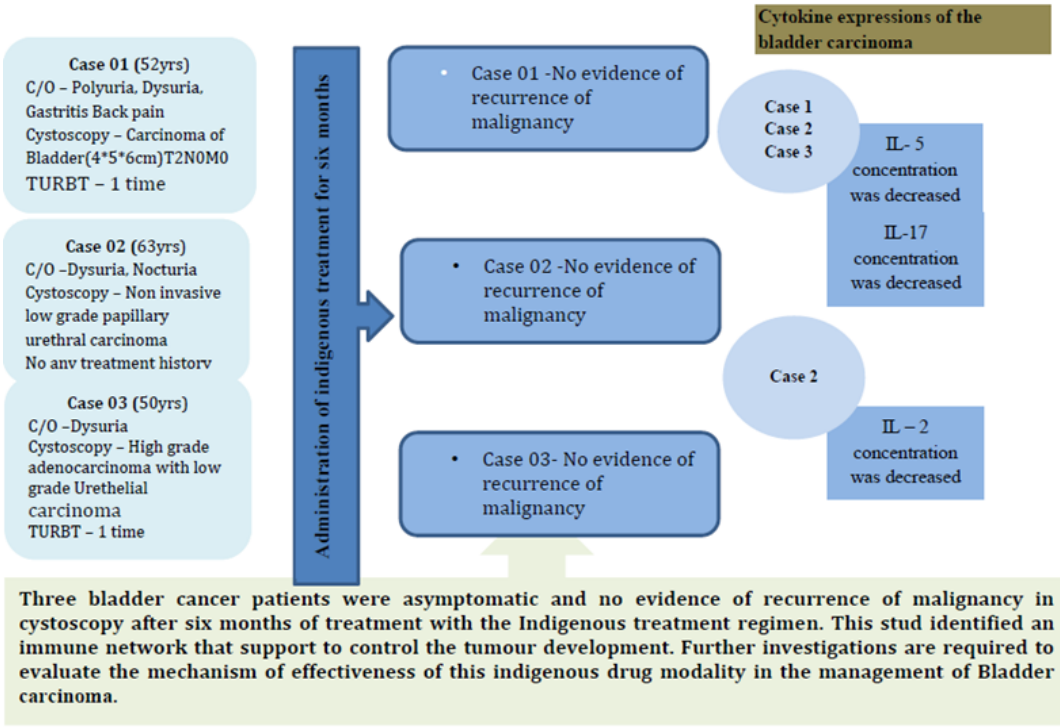

Three bladder cancer patients were asymptomatic and no evidence of recurrence of malignancy in cystoscopy after six months of treatment with the Indigenous treatment regimen. This stud identified an immune network that support to control the tumour development. Further investigations are required to evaluate the mechanism of effectiveness of this indigenous drug modality in the management of Bladder carcinoma.

Highlights

- This study identifies an immune network that supports to control the tumor development.
- Experimental evidence in their respective roles of the treatment regimen in antitumor activity and to bladder carcinoma.
- Three bladder cancer patients were asymptomatic and no evidence of recurrence of malignancy in cystoscopy after six months of treatment with the Indigenous treatment regimen.

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Indigenous treatment regimen in the management of bladder carcinoma: A preliminary assessment on cytokine expression in three male patients

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Abstract: Bladder carcinoma is the nineth most common cancer and 13th most common cause of death from cancer with around 430,000 new cases recorded worldwide in 2012. This study evaluated the efficacy of an indigenous treatment regimen in managing bladder carcinoma in three bladder cancer patients.. Three patients were treated with two indigenous herbal drug compounds: as follows: C1 powder – 10 g/day and IM powder -10 g/day for six months. Cystoscopy was carried out before and after treatment. Serum IL-2, IL-5, IL-17, Interferon-ɣ, and PUMA were quantified using ELISA before and after 45 d and 90 d of the treatments. The IL-2 concentration increased, and IL-5 and IL-17 expressions decreased after the treatment regimen. After six months of treatment, all three patients presented with cystoscopy reports which showed no evidence of recurrence of malignancy and. the bladder cancer patients were asymptomatic. Further investigations are required to evaluate the mechanism of effectiveness of this indigenous treatment regimen in controlling bladder carcinoma.

Keywords: Bladder carcinoma; indigenous treatment; ELISA

INTRODUCTION

Bladder carcinoma is one of the most common cancers and a common cause of death worldwide. Around In 2012, 430,000 new bladder cancer cases were recorded (Vieira, *et al.,* 2015). Males are affected at a rate of two to three times more than females. The vast majority (90 – 95%) of primary bladder carcinoma is transitional cell origin. The main signs and symptoms of bladder carcinoma are blood in the urine, painful urination, frequent urination, urgent urination, urinary incontinence, and pain in the lower back (Margaret, 2000).

Since cancers are rising as critical life-threatening risk in the modern world, it has become crucial to develop effective therapies against cancers. Although there are several therapies against cancers, the threat is still rising. Traditional and ancient health care systems such as Ayurveda in Asia are capable of boosting the immune system and reputed as reliable therapeutic approaches for years. On the other hand, modern cancer treatments such as radiotherapy can harm healthy cells together with cancer cells causing subsequent health risks. Thus, the development of natural therapies can be benefited most of the world to combat cancers.

Authentic Ayurveda texts described Cancer as *Arbuda*, or *Granthi* (Murthy, 2001; Martha, *et al*., 2015). According to the Ayurveda system of medicine, one of the leading causes for *Arbuda* or *Granthi* is the deficiency of immunity (*Vyadhi Kshamathva*). Therefore, the major line of Ayurveda treatment for cancer patients is the enhancement of immunity. Immunomodulators are considered one of the most potent tools in the management of health and disease by modern medicine. Studies have shown that herbal medicine plays as immunomodulators in the body to control the tumour (Tiwari, *et al*., 2015). The chemotherapy and radiotherapy target rapidly multiplying cells to stop the growth and division of cancer cells as well as normal cells. It has shown that the complementary and alternative medicines used by the global population varied from 9% to 65% for treating cancer (Ernst, 2000). In this context, complementary and alternative medicines play an important role in reducing tumor growth, minimizing disabilities, reducing complications, minimizing side-effects of chemotherapy and radiotherapy, enhancing immunity and improving quality of life (Vineeta. *et al*.,2014).

Stimulation of immune effect or cells and stromal cells at the tumour site and enhancement of tumour cell recognition by cytotoxic effector cells occur under the influence of several cytokines. Studies on animal tumour models have revealed that cytokines have broad antitumour activity, which has been translated to a number of cytokine- based approaches for cancer therapy (Sylvia and Kim, 2011). In addition, p53 Upregulated Modulator Apoptosis (PUMA) is a key transcriptional target of the tumour suppressor p53 and it carries out a cell death cascade in response to p53 activation (Ariele *et al*., 2013).

Many cancer patients sought the traditional system of medicine in Sri Lanka. Derivatives of medicinal phytochemicals act as anti-tumor agents, chemo-preventive agents of individual medicinal plants and multi-herbal formulae. The objective of this study was to investigate the efficacy of the Indigenous treatment regimen in managing bladder carcinoma.

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MATERIALS AND METHODS

The present study was carried out at the cancer clinic of the Bandaranaike Memorial Ayurvedic Research Institute (BMARI) Navinna, Maharagama Sri Lanka. Ethical approval has been obtained from the Ethic Review Committee, Institute of Indigenous Medicine, University of Colombo, Sri Lanka (ERC No: 15/46)

Two bladder cancer patients who had undergone Trans Urethral Resection of Bladder Tumour (TURBT) several times and one bladder cancer patient without previous treatment volunteered for this study. Detailed history and clinical examinations were conducted at the clinic.

Case 1:

A 52 years old male patient visited the cancer clinic at the BMARI in 2018 with chief complaints of polyuria (12-13/ day), dysuria, gastritis, and back pain. The patient was diagnosed as having carcinoma of the bladder arising from the left lateral wall (4 × 5 × 6 cm) T2N0M0 with CT Urogram and done TURBT in April 2017. He had a recurrence and had done two more TURBTs within eight months.

Case 2:

A 63 years old male who was a retired technical officer visited the cancer clinic in January 2019 with chief complaints of dysuria and nocturia. He has been diagnosed with a non-invasive low-grade papillary urethral carcinoma in his histopathology report in July 2017. He had not received any treatment history for this condition.

Case 3:

A 50 years old male visited the cancer clinic with the chief complaint of dysuria at the end of January 2020. He was diagnosed as high-grade adenocarcinoma with low-grade urothelial carcinoma T2N2M0. He had done TURBT in mid-January 2020.

Trial drugs preparation

C1 Powder- Fine powder of roots of *Smilax glabra* Roxb

IM Powder – Fine powder of *Tinospora cordifolia* (Willd.) Miers, the fruit of *Embelica officinales Gaertn*., root of *Withania somniferra (L) Dunal*, and *Curculigo orchioides*.

Two Indigenous herbal formulations C1 powder and IM powder, were given to the patients, as shown in Table 1.

Table 1: Drug regimen.

Oral drug	Dosage form	Dose	Drug vehicle	Duration
C1 powder	Powder	5.0 g - morning 5.0 g - evening	Bee honey	six months
IM powder	Powder	5.0 g - morning 5.0 g -evening	Warm water	six months

A dose of 5.0 g of C1 powder was given two times per day with bee honey and 5.0 g of IM powder was given two times per day with warm water. All treatment regimens were continued for six months for the patients. During the whole course of medication the patient was instructed to avoid fried food, oily food, pork, beef, tuna fish, prawns, cuttlefish, prickle, coffee, chocolate, and alcohol.

The level of Interleukin-2 (IL-2), Interleukin-5 (IL-5), Interleukin 17 (IL-17), (Interferon)-ɣ, and PUMA was evaluated by *Enzyme Linked Immunosorbent Assay* (ELISA) before and after 45 d and 90 d of the treatment.

Blood sample collection and storage

Whole blood was collected into EDTA and centrifuged for 10 min at 2,000 rpm and was aliquot into small tubes and stored at -80 ℃ until use.

Enzyme – Linked Immuno Sorbant Assay (ELISA)

IL-2, IL-5, IL-17, (Interferon)-ɣ, PUMA quantitative ELISA kits were bought from Elab-science Biotechnology Co. Ltd (Elab-science) and a sandwich ELISAs were performed according to product manuals. on a microplate pre-coated with the antibodies specific to IL-2, IL-5, IL-17, (Interferon)-ɣ, PUMA standards or samples were added to the appropriate micro ELISA plate wells and combines specific antibodies. Then a biotinylated detection antibody specific for IL-2, IL-5, IL-17, (Interferon)-ɣ, PUMA and Avidin- Horseradish Peroxdase peroxidase (HRP) conjugate added to each micro plate well successively and incubated. Free components washed away. 100 µL of substrate solution was added to each well and incubated for 10 min at 37 ℃ in the dark. Absorbance was read at 450 nm filter using a plate reader.

RESULTS

The cytokine expressions of the bladder carcinoma patients were recorded in Table 2.

IL-2 concentration increased after the treatment in Case 1. IL-5 and IL-17 expressions decreased after the treatment modality in all three Cases. Response of the treatment was recorded by Cystoscopy reports of the patient as indicated in Table 3.

Follow-up Cystoscopy after six months showed no recurrence of malignancy. In the first two months of follow up, the symptoms were reduced and this reduction improved further at the next four months. At the end of six months, patients were asymptomatic and general condition was also improved.

DISCUSSION

In this treatment regimen, different forms of recipes were included. High concentration of IL-2 led to suppress bladder carcinoma (Edith and Hartwig,1989). IL-5 is detected by RT- PCR in the bladder cancer cells, and it promotes the growth of bladder cancer. In addition, Interferon-gamma inhibits the incidence and growth of bladder tumor (Se-Jung *et al*.,2012). IL -17 expression level is increased in tumor tissue (Jian*et al*., 2018). Bladder cancer cells reduce

Table 2: Cytokine expression of bladder cancer patients.

Case No.	Pre Rx (Pg/mL)					After 45 d (Pg/mL)					After 90 d (Pg/mL)				
	IL-2	IL-5	IL-17	Gamma IFN	PUMA	IL-2	IL-5	IL-17	Gamma IFN	PUMA	IL-2	IL-5	IL-17	Gamma IFN	PUMA
Case 1	11.8	28.5	331.8	196.1	8.7	15.1	22.2	103.8	103.2	3.7	17.6	20.5	88.7	85.7	3.4
Case 2	27.5	61.6	284.7	108.7	2.42	14.6	15.9	105.5	83.2	1.6	17.9	30.8	201.2	95.9	0.9
Case 3	24.7	49.0	354.3	123.2	2.4	30.2	32.4	203.0	103.2	2.4	22.0	26.1	296.9	101.4	2.0

Table 3: Response of the treatment.

Case No.	Pre-treatment		Post treatment	
	Cystoscopy report	Symptoms	Cystoscopy report	Symptoms
1	There was a papillary growth on left side of bladder measuring 6.2 x 4.9 x 6.7 cm. T2N0M0 Vascularize has seen	Polyuria (12-13/ day), Back pain, Gastritis, Dysphonea and Burning urination	No evidence of recurrence of malignancy	Symptoms disappeared
2	Bladder growth over the right urethral orifice	Dysuria and Nocturia (3 - 4/Night)	Definite residual mass lesion not seen in the bladder	Symptoms disappeared
3	High grade adenocarcinoma with low grade urothelial carcinoma T2N2M0	Dysuria	No evidence recurrence of malignancy	Symptoms disappeared

PUMA expression (Bin *et al*., 2015; Christina *et al*., 2013). According to the results of the ELISA, IL-5 and IL-17 expression were decreased after administration of the treatment modality, which is possible to control bladder carcinoma by this treatment modality.

The main ingredient of C1 powder of this drug modality is *Smilax glabra.* Anti-oxidant, immunomodulatory and anti-cancer effects of S*milax glabra* have been reported (Shiyao *et al*., 2018). Ingredients of IM i.e. *Tinospora cordifolia*, *Phyllanthus emblica*, *Withania somnifera***,** and *Curculigo orchioides* have been found to possess related pharmacological activities such as anti-oxidant, anti-cancer and immunomodulatory properties (Qamar*et al*.,2012; Rubaiyat *et al*., 2016; Kumari and Singh, 2017; Priyanka *et al*., 2019). A recent study has shown that the extract of *Tinospora cordifolia* reduced tumour growth and increased the life span of tumour bearing, which help to the progress of tumour necrosis (Mahiuddin and Shaikh, 2010). *In-vivo* and *in-vitro* studies of *Withania somnifera* have shown a significant increase in the production of T- Lymphocyte that occur reduction of tumor growth (Mahiuddin and Shaikh, 2010). Therefore, *Withania somnifera* helps to suppress tumour growth and symptoms in cancer patients.

Modulation of the immune system denotes any change in the immune response that can involve induction, expression, amplification, or inhibition of any part or phase of the immune response. Immunomodulator is defined as a substance used for its effect on the immune system. According to their effects, there are two types of immunomodulators: immunosuppressants and immune stimulators. They can mount an immune response or defend against pathogens or tumours (Madhuri, and Pandey, 2009). It was revealed that the extract of *Tinospora cordifolia* had the ability and secretion of IL-1 and tumour necrosis factor in Dalton's lymphoma-bearing mice. Also they have shown that the extract of *Tinospora cordifolia* reduced the tumour growth and increased the life span of tumour bearing host (Mahiuddin and Shaikh, 2010). According to above findings *Tinospora cordifolia* can be helped to the progress of tumour necrosis. Another research study has shown that *Curculigo orchioides* extract drastically elevated the gamma interferon and Interleukin 2 and it induced phase II enzymes involved in detoxification pathway (Vishnuand Girija, 2015). Considering the above facts, the trial formulae might have enhanced the immunity (*Vyadhi Kshamatva*) of the bladder cancer patients.

CONCLUSION

Three bladder cancer patients were asymptomatic and no evidence of recurrence of malignancy in cystoscopy after six months of treatment with the Indigenous treatment regimen. This study identified a possible immune network that supports to control the tumor development. Evaluation of the treatment by histopathological findings in order to confirm the prognosis has to be done in future work. Further investigations are required to evaluate the mechanism of effectiveness of this indigenous drug modality in the management of bladder carcinoma.

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DECLARATION OF CONFLICT OF INTEREST

All the individuals participating as investigators in this stud do not have any conflicts of interest to declare relevant to this study.

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