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Efficacy of treatment on antioxidant status in cervical cancer patients: A case control study

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ABSTRACT

Background and aim: To evaluate the effect of treatment on antioxidant status of cervical cancer patients with suspected and healthy.
Method: The study was included 59 cancer patients with cervical cancer were compared to suspected ($n = 25$) and healthy controls ($n = 25$). Of the 59, 30 patients undergoing chemotherapy alone, for 3 months and 29 patients were undergoing radiotherapy along with chemotherapy. Blood samples were collected after the treatment from all the groups and estimated the level of serum malondialdehyde, non-enzymatic antioxidant glutathione and enzymatic antioxidant including super oxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase.
Results: The levels of serum malondialdehyde and glutathione reductase were significantly ($P < 0.05$) increased and all antioxidant enzymes were decreased in cervical cancer patients when compared to normal controls and suspected cases. The estimated antioxidant status was increased significantly after the treatment of radiotherapy along with chemotherapy than the chemotherapy alone.
Conclusion: The results suggest that the elevated lipid peroxidation and impaired antioxidant status was significantly increased after the radiotherapy with chemotherapy than the chemotherapy alone.

1. Introduction

Cancer is the second leading cause of death in worldwide. Eleven million new cases of cancer are diagnosed every year.[1] It is estimated that there are approximately 2–2.5 million cases of cancer in India at any given point of time. Amongst the gynaecological cancers, cervical cancer is very common in women of the developing countries like India. Viral infections, chemical carcinogens and oxidative stress are the main causative agents of cervical cancers.

The treatment of cervical cancer is based on the two cornerstones that is radiotherapy (RT) and chemotherapy (CT) alone, in some conditions radiotherapy followed by chemotherapy was used. Either RT or CT, they prove to be immense benefit in eliminating all malignant cells/tissues, the normal cell/tissues functions are also altered. RT causes many biochemical complications which include free radical mediated damage to normal cellular DNA, membrane structures and alterations in the immune system.[2,3] Most widely used anti-cancer drugs cisplatin and other drugs are known to generate free radicals.[4] On the other hand, the anti-tumour effect of either cytostatic drugs or radiation is thought to be caused by oxidative damage or functional impairment of DNA leading to cancer cell damage or cell death.

In recent years a large body of experimental and clinical data has provided compelling evidences for involvement of oxidative stress in large number of pathological states including carcinogenesis.[5] Reactive oxygen species (ROS) cause extensive tissue damage through reactions with all biological macromolecules includes lipids, proteins and nucleic acids, leading to the formation of oxidized products like malondialdehyde. Oxidative stress has been reported in multiple cancers including lung, breast, cervical, oral and colon cancers.[2] However, the deleterious effects of reactive oxygen species and lipid peroxides are protected by an array of endogenous antioxidant defence systems, by acting as a potent scavenger of free radicals as well as inhibitors of neoplastic process.

Under normal circumstances, mammalian cells possess comprehensive array of antioxidant defences comprising of both

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Table 1
Characteristics of patients, suspected and healthy subjects.

S. No	Character	Patients	Suspected cases	Healthy subjects
1	**Age in (years)**	40–71	42–68	40–65
2	**Cervical cancers (N)**	59 (30 + 29)	25	25
3	**Cancer stage**			
	I/IIA	7		
	IIB	11		
	IIIA	17		
	IIIB	13		
	IV	11		
4	**Histological features**			
	SCC	21		
	DSCC	9		
	M.D/WDSCC	7		
	Du SCC	11		
	In. SCC	5		
	Un-identified	6		
5	**Treatment**			
	Chemotherapy (cisplatin + mitomycin)	30		
	Radiotherapy + CT	29		

Note: SCC: squamous cell carcinoma; **DSCC**: differentiated squamous cell carcinoma; **M.D/WD SCC**: moderately and well differentiated squamous cell carcinoma; **Du SCC**: ductal squamous cell carcinoma; **In. SCC**: intermediate squamous cell carcinoma.

enzymatic and non-enzymatic forms. The non-enzymatic antioxidant forms include tocopherols, retinols and ascorbate and enzymatic antioxidant includes super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) are directly metabolize ROS.[6] These antioxidants prevent free radical formation or limit their damaging effect and thus offer protection to cellular components. Impaired antioxidant defence system observed in cancer patients at multiple sites reflects the excessive free radical production. This is evidenced with the low antioxidant levels in all cancer cases including cervical cancer. Osmotic fragility, the sensitivity to change in osmotic pressure characteristic of red blood cells, has been found to be altered in various pathological conditions. The integrity of the red blood cells may be determined by measuring the changes in erythrocyte osmotic fragility. Measurement of osmotic fragility of erythrocytes has been applied to the diagnosis of haemolytic diseases, studies of membrane permeability, and alterations leading to destruction of erythrocytes.[7]

These reports provoked interest to test, weather radiotherapy, CT or other combination of treatment shows good results in the form of antioxidant defence mechanism. Hence, the present study evaluates the status of oxidative stress and antioxidant defence mechanism in patients of cervical carcinoma treated with CT and radiotherapy with CT.

2. Materials and methods

2.1. Study population

The present study consists of the following groups, Group A consisted healthy samples ($n = 25$), Group B composed of suspected cases ($n = 25$) with some gynaecological warts and some squamous intra epithelial lesions, Group C ($n = 30$) consisted of cervical cancer patients before chemotherapy (CT) and Group D consisted of cervical cancer patients after chemotherapy, Group E consisted of before radiotherapy plus CT ($n = 29$) and Group F consisted of cervical cancer patients after treatment with radiotherapy with CT.

All the cancer and treated cases were obtained from the Department of Gynecology, S.V. Medical College, Tirupati and Department of Radiation Oncology, Guntur General Hospital, Guntur. The study protocol was approved by the Institutional Ethical Committee (IEC), from S.V. Medical College, Tirupati and all the patients consent form was signed for their approval to this study. Cervical cancer patients of different stages with average age of 51.92 (40–71 years), age matched suspected (42–68 years) and healthy (40–65 years) controls were chosen for the study (Table 1). Cancer patients with other diseases like diabetes, cardiovascular diseases, liver diseases, kidney diseases and other types of tumours etc. were excluded from the study.

2.2. Blood sample collection

The blood samples were collected under aseptic conditions for the analysis of various antioxidant enzymes. Blood was collected without any anticoagulant and allowed to clot for 1 h. Clotted sample was centrifuged at 3500 rpm × 30 min at 4 °C (in cold centrifuge). Serum was separated and stored at −20 °C for further analysis.

2.3. Estimation of lipid peroxidation product and antioxidant enzymes

Plasma circulating lipid peroxides in terms of MDA was estimated by the spectrophotometric procedure as described by Satoh (1978).[8] Standard absorbance of MDA (2.5 nmol) was used to calculate the amount of lipid peroxides in the samples and results were expressed as μl/l. SOD activity was measured by the method of McCord and Fridovin (1969)[9] and the unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560 nm of Nitro Blue Tetrazolium (NBT) reduction by 50% in 1 min under the assay conditions and results were expressed as mg/gm of protein.

Catalase enzyme activity was (CAT) determined by the method of Aebi and Suter (1974)[10] and results were expressed as mg/gm of protein. One unit of CAT decomposes 1.0 mM of hydrogen peroxide per minute under specified conditions.

Glutathione peroxidase activity[11] and glutathione reductase activity was measured in the serum by the method described by Goldberg and Spooner (1983).[12] Glutathione S transferase (GST) was estimated by CDNB method and it was calculated by using the molar extinction coefficient (9.6 mM^{-1} cm^{-1}) of GST.[13] The activity of glutathione was measured by Anderson (1984).[14] Osmotic fragility of the fresh blood taken from each group was determined by the method of Parpart (1994)[15] and mean corpuscular fragility was calculated by recording the saline concentration, which would have resulted in 50% haemolysis.

2.4. Statistical analysis

The results were expressed as mean ± S.D and statistical comparisons were performed by independent samples *t*-test. The results with *p*-value <0.05 are observed to be statistically significant (Table 2).

3. Results

Serum membranes are more liable to lipid peroxidation because of their high polyunsaturated fatty acid (PUFA) content and their direct exposure to molecular oxygen. The compensatory mechanism to counter the ROS results in decreased levels or activities of enzymatic and non-enzymatic antioxidants in serum. In the present study, the *in vitro* lipid peroxidation in the serum of normal subjects, suspected cases and cervical cancer patients were shown in Table 2. The release of lipid peroxide product MDA was

significantly higher in cervical cancer patients and then suspected cases as compared with normal subjects.

It was observed that there is a high significance ($p < 0.05$) between the two groups i.e., healthy and suspected individuals with respect to the each enzyme respectively. Table 2 shows the levels of serum glutathione (GSH), glutathione peroxidase (GPx), catalase activity (CAT), super oxide dismutase (SOD) and glutathione S transferase (GST) were significantly ($p < 0.01$) lower in suspected cases than the normal healthy subjects. The glutathione reductase (GR) enzyme was increased significantly ($p < 0.01$) in suspected cases than the normal healthy subjects. The same parameters were also decreased in much more levels in cervical cancer patients than the suspected and healthy subjects.

The paired sample *t*-test was noticed that there is a statistical association ($p < 0.05$) between pre and post treatment in each group with respect to CT (Group D) as well as radiotherapy along with CT (Group E). Table 2 also indicated that the antioxidant enzymes GSH, GPx, G6PD, CAT, SOD and GST were increased significantly in radiotherapy along with chemotherapy (Group E) than the chemotherapy alone (Group D). The GR enzyme was decreased significantly ($p < 0.01$) in radiotherapy along with chemotherapy (Group E) than the chemotherapy alone (Group D).

Difference between the values of pre and post treatments were designated as individual scores which are significantly associated ($p < 0.05$) between CT (Group D) and radiotherapy along with CT (Group F) with respect to GR, GPx, G6PD, SOD and GST. But there is no significant association between the CT and radiotherapy plus CT with respect to GSH and CAT (Fig. 1).

Fig. 2 shows that the osmotic fragility curves of normal subjects, suspected and cervical cancer patients. The mean corpuscular fragility of blood was significantly higher in cervical cancer patients and then suspected cases when compared to healthy subjects.

4. Discussion

The enzymes GSH, GPx, CAT and SOD, GST catalyze cell defence reactions against the potentially harmful effects of super oxide anion generated by a wide variety of biological processes. We have found that these antioxidant enzymes were significantly ($p < 0.01$) lowered in cancer patients than suspected cases and healthy controls. A well-established work has been carried out on the association between free radical activities, antioxidants scavenging of free radicals and their relation with chemotherapy in patients of the cervical cancer. This increased oxidative stress was because of raised free radical injury. We observed a significant relationship between treatment (RT followed by CT and CT alone) and changes in the status of antioxidant enzymes and lipid peroxides in patients with cervical carcinoma.

Lipid peroxidation was a one of the most frequently used parameters for assessing the involvement of free radicals in cell damage. Lipid peroxidation products diffuse from the inflammatory site and can be measured in the blood.[16] Serum of cervical cancer patients showed a higher release of lipid peroxides (MDA) *in vitro* as compared to suspected cases and then normal subjects. The increased lipid peroxides reflects insufficient antioxidant potential in the serum of cervical cancer patients and also in some of the suspected cases. Improved lipid peroxidation observed in the serum of cervical cancer patients can be correlated with the decrease in catalase activity. High activity of catalase in red blood cell has been reported to play a crucial role in protecting red blood cells against oxidative damage. Addition of catalase has been shown to have a significant role in fortification against H_2O_2-mediated lipid peroxidation.[17] In the present study, serum incubated with sodium azide, an inhibitor of catalase resulted in higher release of MDA *in vitro*, reflecting impaired catalase activity in serum.

Kolanjiappan (2002)[18] found an increased serum lipid peroxidation in cervical cancer and concluded that an increase in lipid peroxidation is one of the risk factor in the pathogenesis of cervical cancer in addition to other causes. Increase in lipid peroxidation products has been reported in patients with laryngeal and oral cancer.[19,20] Elevated serum lipid peroxidation and disturbed antioxidant activities have been reported in patients with malignant lymphoma.[21]

At more levels GST may rapidly detoxify anti-cancer agents, thereby preventing their cytotoxic action. In the previous study, it was reported that glutathione-S-transferase activity in malignant tumours of uterus, breast and ovaries were higher than in normal.[22] Our results lend credence to these reports, that the levels of GST were found to be increased significantly in cervical cancer patients after radiotherapy followed by CT than the CT alone.

In the present study, GSH an antioxidant was significantly decreased in patients with cervical cancer when compared to suspected cases and healthy controls. The decrease in the GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in these patients. Similar reports of decreased GSH levels in cancers have been reported earlier by Ahmed (1999)[23] in patients with cervical cancer. They have observed that GSH levels were mainly reduced in poorly differentiated tumours than in well and moderately differentiated tumours.

Table 2
Antioxidant status in normal, suspected subjects and cervical cancer patients pre and post treatments with various comparisons (mean ± S.D.).

Parameter	Group A	Group B	Chemotherapy		Radiotherapy with CT	
			Group C	Group D	Group E	Group F
GSH	51.34 ± 5.39	35.25 ± 10.11[a]	15.32 ± 3.41[b]	28.36 ± 9.34[d]	33.54 ± 13.12[c]	33.54 ± 13.12[e]
GR	22.91 ± 7.30	45.51 ± 7.30[a]	57.13 ± 3.71[b]	52.00 ± 4.11[d]	48.01 ± 6.01[c]	48.01 ± 6.01[e]
GPx	61.09 ± 5.80	54.7 ± 4.28[a]	38.28 ± 6.44[b]	46.75 ± 7.42[d]	49.68 ± 10.30[c]	49.68 ± 10.30[e]
G6PD	5.98 ± 0.76	3.83 ± 1.08[a]	2.52 ± 0.72[b]	3.34 ± 0.93[d]	3.76 ± 0.89[c]	3.76 ± 0.89[e]
CAT	4.17 ± 0.69	3.44 ± 0.62[a]	2.08 ± 0.50[b]	3.05 ± 0.79[d]	3.38 ± 0.67[c]	3.38 ± 0.67[e]
SOD	5.20 ± 0.66	4.32 ± 0.94[a]	2.66 ± 0.74[b]	3.33 ± 0.79[d]	3.79 ± 0.69[c]	3.79 ± 0.69[e]
GST	4.84 ± 0.69	3.85 ± 0.77[a]	2.87 ± 0.74[b]	3.38 ± 0.64[d]	3.72 ± 0.98[c]	3.72 ± 0.98[e]
MDA	6.86 ± 0.94	9.97 ± 1.68[a]	15.15 ± 1.75[b]	11.89 ± 1.71[d]	15.43 ± 1.63[c]	10.61 ± 2.32[e]

Note: Groups: **Group A**: healthy subjects; **Group B**: suspected cases: **Group C** & **D**: pre and post treated chemotherapy; **Group E** & **F**: pre and post treated radiotherapy with chemotherapy. **GSH, CAT** & **SOD**: mg/gm of protein; **GR**: U/l; **GPx**: nmol/NADPH/min/mg protein; **GST**: μ mol/min; **MDA**: μl/l.

[a] Significance between Group B and Group A ($p < 0.01$*).
[b] Significance between Group C and Group A ($p < 0.01$*).
[c] Significance between Group E and Group A ($p < 0.01$*).
[d] Significance between Group C and Group D ($p < 0.01$*).
[e] Significance between Group E and Group F ($p < 0.01$*).

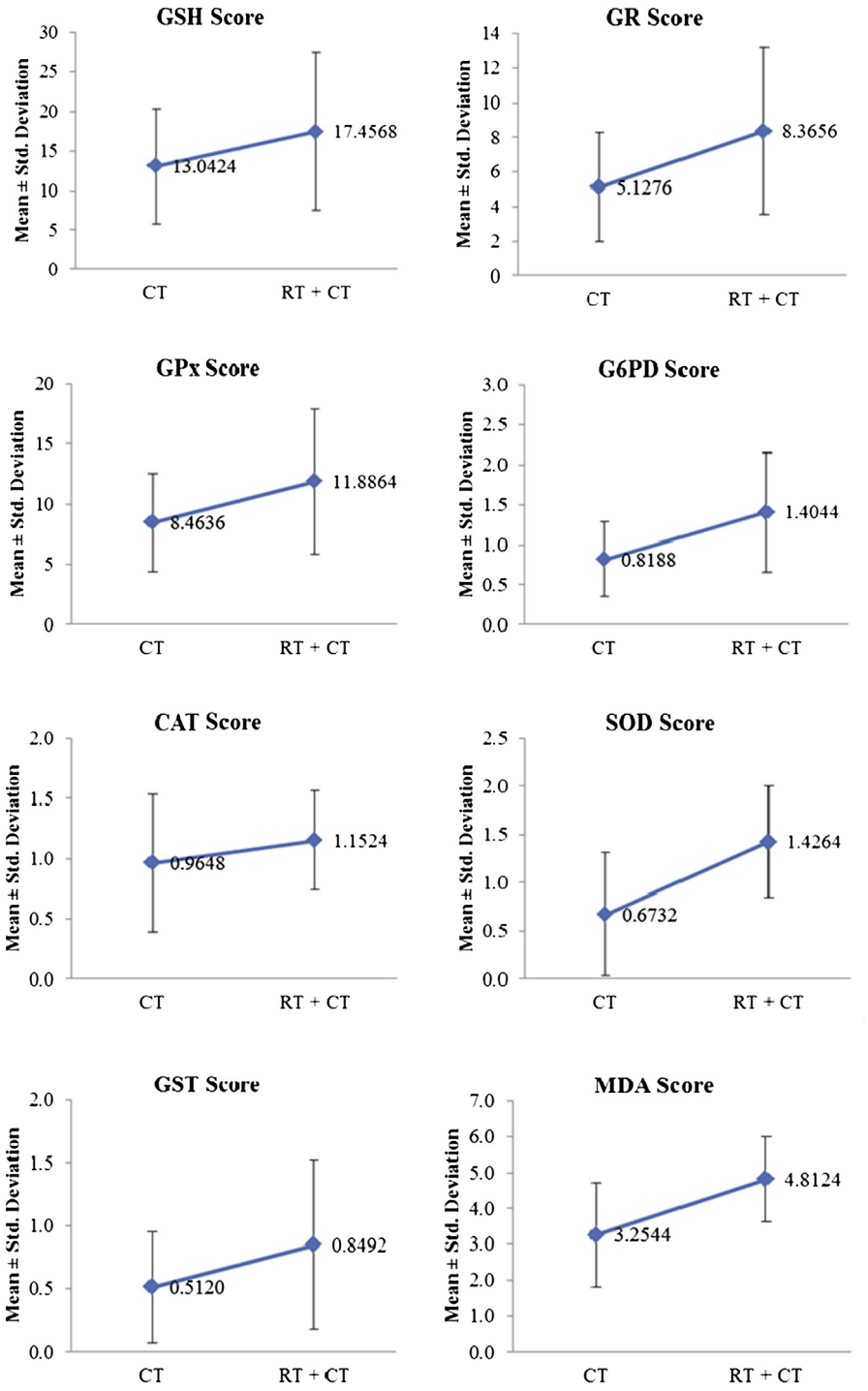

Fig. 1. Effect of chemo and radiotherapy + CT on Serum GSH, GR, GPx, G6PD, CAT, SOD, GST and MDA respectively in cancer represented by Whisker line graphs.

The level of glutathione reductase was significantly high in cervical cancer patients, than the normal and suspected cases. The increased level was significantly lowered after radiotherapy with CT than the CT alone treatment. After radiotherapy with CT, the lowered enzyme activity was representing the improvement in antioxidant defence mechanism.

GPx – an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and also in maintaining functional integrity of the cell membrane.[24] The decrease in the activity of GPx could be due to its induction to counter the effect of increased oxidative stress. The significant increased level of GPx activity was observed in patients treated with radiotherapy with CT than the CT alone treatment.

The decreased level of SOD, CAT activity may be associated with free radical generation which causes damage by cross linking or damaging the nuclear DNA leading to mutations. It may also be due to scarcity of trace elements like zinc, manganese etc. which acts as a cofactor for this enzyme.[25] The present investigations were evaluated the status of SOD in cervical cancer patients treated with CT alone and radiotherapy followed by CT and they found that the

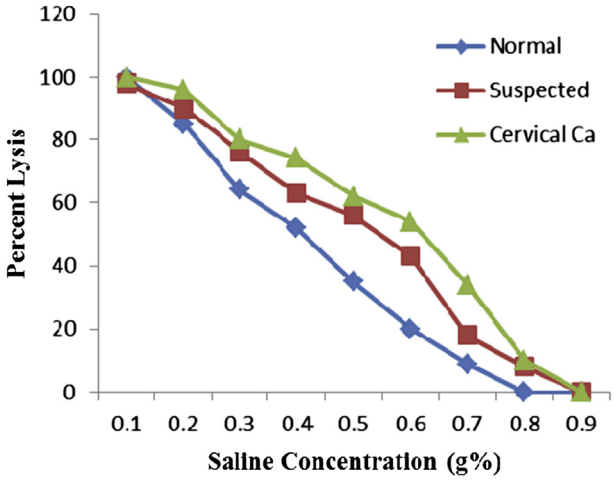

Fig. 2. Osmotic fragility curves of normal, suspected and cervical cancer patients. The degree of haemolysis was calculated by comparing with 0.1% NaCl solution which represented 100% lysis.

SOD, CAT activities of the cancer patients were lower than those of the suspected cases and healthy subjects.[26]

In cancer, extensive free radical production leads to shortage of NADPH, which results in accumulation of oxidized glutathione (GSSG) and free radicals. The low availability of the NADPH substrate may be responsible for the decrease in the activity of GRx. The glucose-6-phosphate dehydrogenase (G6PD) has responsible for the catalytic reduction of GRx to NADPH. The observed decline in the activity of G6PD in cervical cancer may be due to an increase in the production of oxidized GSH (GSSG). Significant increase in the activity of the G6PD was observed after the treatment of cancer patients with radiotherapy followed by CT than the CT alone treatment. Radiation therapy uses high energy X-rays to kill cancer cells. These X-rays may be given externally which is called external beam radiation therapy. For cervical cancer, this type of radiation therapy is often given along with low doses of chemotherapy with a drug called cisplatin. The majority of epithelial cancers are only moderately radiosensitive, and require a significantly higher dose of radiation (60–70 Gy) to achieve a radical cure. In the present study dosage of 50–60 Gy radiation was given to the patients and along with the chemotherapy (cisplatin). During the treatment of radiation the cancer infected epithelial cell were shrinkage and reduce the size of the tumour along with pain. Hence, the RT along with CT kills and decreases the size of cancer cells which facilitate the significant alterations (increased) in the development of antioxidant system, which is not possible in case of CT alone. According to 'spatial cooperation' theory[27] (Steel, 1979) the action of radiation and chemotherapeutic drugs is directed towards target site in the body and work independently of each other. Radiation tends to target localized tumours and provide a systemic effect in addition to the local effect of the radiation. The chemotherapy drugs are likely to be more effective in eliminating micrometastases and help sensitize radiation. Hence, the combinational treatment of radiation with CT in cervical cancer causes sensitization to antioxidants.

Osmotic fragility has been found to be increased in various pathological conditions including cancer.[28] In the present study, the erythrocytes of cancer patients were more fragile than those from suspected and normal subjects. The increased osmotic fragility may be due to the increased lipid peroxidation,[29] which has been implicated in the alterations of membrane structure and functions that leads to the increased osmotic fragility.

5. Conclusion

In conclusion, enhanced lipid peroxidation and impaired antioxidant status was observed in patients with cervical cancer compared to suspected and healthy controls. Significant change was observed in antioxidant levels between the patients treated with radiotherapy and CT than the patients treated with CT alone. Which indicated that radiotherapy with CT, have an impact on the antioxidant system and sensitization to antioxidant defence mechanism. But the dosage and time intervals of radiotherapy were depending on the case study of the individual patients.

Contribution details

The first author contributes the sample collection, clinical and experimental studies, the second author contributes the manuscript preparation, editing, the third author contributes the concept, design, definition of intellectual content, data analysis and review the manuscript.

Conflicts of interest

All authors have none to declare.

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