The Effect of Carrageenan on Lymphoma Patients under Chemotherapy (A case series study)

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Abstract
Background There are three types of carrageenan available (iota, kappa, and lambda) which differ in composition and degree of sulfation in polymeric structure. A malignant lymphocytes that accumulate in lymph nodes causes a group of diseases called Lymphomas causing the characteristics lymphadenopathy. These may pelt over into the blood or infiltrate organs outside the lymphoid tissue. They are Hodgkin›s lymphoma and non–Hodgkin›s lymphoma and this is based on the histologic presence of Reed-Sternberg cells in Hodgkin›s lymphoma. **Objectives** The present study designated to determine the oral manifestations after application of carrageenan in patients with Hodgkin›s& Non-Hodgkin›s lymphoma receiving chemotherapy and to determine the effect of topical carrageenan on the levels of salivary immunoglobulin A, interleuekin-6 and tumor necrosis factor-α in Hodgkin›s & non-Hodgkin›s lymphoma patients. **Results** The oral manifestations was in Non-Hodgkin›s lymphoma patients (64%) and in Hodgkin›s lymphoma patients (36%) taste alteration, followed by burning mouth syndrome, dry mouth and lastly candidiasis with significant differences after application of carrageenan p>0.05.The levels of salivary immunoglobulin A, interleuekin-6 and tumor necrosis factor-α were slightly decreased but not significantly differences after carrageenan application. **Conclusions** The carrageenan effects on oral lesion may allow the use of K –carrageenan as a base or primary compound in dental practice besides the other compounds like chlorhexidine. The salivary immunological markers level slightly decreased in lymphoma patients after receiving carrageenan which indicate that there is no absorption from oral mucosa.

Keywords Carrageenan, Oral manifestations, Immunological Markers

Introduction

The red seaweed extracting (Carrageenan) is a sulfated polyglycan (FAO 1990). It is mainly used as food additive due to its gelling, thickening and emulsifying properties making it a vegeterian alternative to gelatin (Bixler 1996; Shah and Huffman 2003), in addition Carrageenan is widely used as excipient in personal lubricants, cosmetics, toothpaste and pharmaceutical products (Buck et al., 2006). There are three types of carrageenan available (iota, kappa, and lambda which differ in composition and degree of sulfation in polymeric structure.

The use of carrageenan was approved by Food and Drug Administration in the United States (FDA, 2006) and by the European Parliament and Council (Commission Directive, 1995) and by the Joint Food and Agriculture Organization of the United Nations/ World Health Organization Expert Committee, JECFA (Cohen and Ito, 2002).

Carrageenan is extracted from these seaweeds by heating it with water containing a dilute alkali which increases gel strength of the product. It is then recovered either by alcohol precipitation; usually isopropanol or gel pressing (only for kappa-carrageenan) (Imenson 2000; McHugh, 2003). It may act as topical microbicide. Especially as antiviral (Gonzalez et al., 1987). Carrageenan containing toothpastes act as coherence of liquid portion and improve texture with good rinseability. (FAO Training Manual, 1990).

Malignant lymphocytes that accumulate in lymph nodes causes a group of diseases called Lymphomas causing the characteristics lymphadenopathy. These may pelt over into blood (Leukemic phase) or infiltrate organs outside the lymphoid tissue. They of tow type (Hodgkin›s lymphoma and non–Hodgkin›s lymphoma).depending in the presence of (R.S)cells in Hodgkin›s (Hoffbrand, 2011) Non-Hodgkin's lymphomas (NHL) are a group of lymphocyte proliferative disorders from B, T, or natural killer (NK) lymphocytes origin about 80% to 85% of the cases is of B-cell type and 15% to 20% of T-cell origin; NK type is rare (Jemal et al., 2010). Hodgkin's lymphoma (HL) is type with RS cells histologically. (Hoffbrand, 2011) with two major types of HL: classic HL and nodular lymphocyte predominance (American Cancer Society, 2011) of clonal B-cell origin for both lymphocyte predominant and "classic" HL (Küppers and Clonotypic, 2009).The patient with malignancy suffer from an oral problem from both the disease and the treatment options (Broadfield and Hamilton, 2006). Chemotherapeutic agents have a bad role in damaging not only the malignant cells but also the normal tissue in the patient's body, these agents can cause a lot of oral complications, including taste alteration, burning sensation, infection, hemorrhage, dry mouth, and neurologic and nutritional problems (Toscano et al., 2009). Salivary immunoglobulin A is the major immunoglobulin with specific immune functions in the oral cavity (Thaweboon et al., 2008). Interleukin-6 (IL-6) is a cytokine with a broad biological activity, especially in immune regulation and blood syntheses, inflammation and oncogenesis (Kishimoto, 2010). Tumor necrosis factor-α (TNF-α) is a proinfammatory cytokine. Having the cytostatic and cytotoxic role of certain tumor cells, role in differentiation, growth, and/or has a partial role in inflammatory signal and immunological event (Slattery et al., 2011 and De Paepe et al., 2012).

Materials and methods

The ethics committee of the Ministry of Health, IRAQ had been approved in this study. The study samples consisted of (25) Hodgkin &Non-Hodgkin lymphoma patients (12 male and 13 female). Carrageenan is extracted from these seaweeds by heating it with water containing a dilute alkali which increases the gel strength of the product. It is then recovered either by alcohol precipitation; usually isopropanol or gel pressing (only for kappa-carageenan) (Imenson 200; McHugh, 2003). All of the processes follow the same basic steps (Imeson, 2000; McHugh, 2003; Blakemore and Harpell, 2009).

Preparation of a K-carrageenan gel

The pharmaceutical formula in (percent) was, carrageenan powder (0.9%). sucrose (20%)potassium citrate (0.35%), citric acid (0.45%). By disposable tray filled with carrageenan gel holding it for 5mimute in patient mouth two times a day for one week period and for each time the patient instructed to use carrageenan after finishing breakfast and before bed time.

All the Hodgkin›s lymphoma patients was diagnosed by Hematologists in the oncology unit of (IMAMEIN KADHIMEIN MEDICAL CITY) confirmed as Hodgkin by histological examination of an excised lymphnode, Using Adriamycin, bleomycin, vinblastine and dacarbazine (ABVD Regimen), cyclical chemotherapy.
Non- Hodgkin›s type diagnosed by biopsy or strict biopsy of the lymph node or other involved tissue, supported by immunophenotypic and genetic analysis in order to distinguishes the disease from a reactive node.
Using R-CHOP Regimen {rituximab in combination with cyclophosphomide, hyroxodaunorubicin, vincristine (Oncovin) and prednisolone} chemotherapy combination.
Whole unstimulated saliva was collected to determine the level of salivary immunological markers which measured by Enzyme Link Immunosorbent Assay. Those patients were examined to detect the presence of oral manifestations and to evaluate the level of salivary markers (secretory immunoglobulin A, Interleukin-6 and tumor necrosis factor-α) of age range (20-50) years. Taking saliva at three times interval:
1- At the time of diagnosis
2- After one week (without carrageenan) and instruct to use carrageenan
3- Then after one week (with carrageenan).

Results

The oral manifestations were in non-Hodgkin›s lymphoma patients (64%) and in Hodgkin›s lymphoma patients (36%) taste alteration, followed by burning mouth syndrome, dry mouth and lastly candidiasis with significant differences after application of carrageenan at p> 0.05 that were resulting in tables 1, 2, 3 and 4 and are shown in Figure 1,2 and 3 throughout not present of «T. A.» responses after treatment that mean there are 6 cases without T.A at the time of diagnosis represented by A, which still without T.A represented by B=0 and there were 18 cases with T.A. But only one still have represented by B=1. The levels of salivary immunoglobulin A, interluekin-6 and tumor necrosis factor-α were slightly decreased but not significantly differences after carrageenan application

Table (1): Distribution of the studied Oral Manifestation (T.A.) based on the (Before, and after) carrageenan application with Comparison Significant

T.A.	Resp.	No. & %	After		Total	C.S. (*) P-value
			A	P		
Before	A	No.	6	0	6	P= 0.000 HS
		%	100%	0.0%	100%	
	P	No.	18	1	19	
		%	94.7%	5.3%	100%	
Total		No.	24	1	25	
		%	96%	4%	100%	

(*) HS: Highly Sig. at P<0.01; Testing based on the association table by McNemar test.
Abbreviations of: A=absent; and p=present, T.A=Taste Alteration.

Table (2): Distribution of the studied Oral Manifestation (B.M.S.) based on (Before, and after) with Comparison Significant

B.M.S.	Resp.	No. & %	After		Total	C.S. (*) P-value
			A	P		
Before	A	No.	6	0	6	P= 0.000 HS
		%	100%	0.0%	100%	
	P	No.	18	1	19	
		%	94.7%	5.3%	100%	
Total		No.	24	1	25	
		%	96%	4%	100%	

(*) HS: Highly Sig. at P<0.01; Testing based on the association table by McNemar test.
Abbreviations of: A= absent; and P= present, B.M.S = Burning Mouth Sensation.

Table (3): Distribution of the studied Oral Manifestation (D.M.) based on (Before, and after) with Comparison Significant

D.M.	Response	No. & %	After		Total	C.S. (*) P-value
			A	P		
Before	A	No.	1	0	1	P= 0.000 HS
		%	100%	0.0%	100%	
	P	No.	23	1	24	
		%	95.8%	4.2%	100%	
Total		No.	24	1	25	
		%	96%	4%	100%	

(*) HS: Highly Sig. at P<0.01; Testing based on the association table by McNemar test.
Abbreviations of: A= absent; and P= present, D.M. =Dry Mouth.

Table (4): Distribution of the (Candidiasis) Status based on (Before, and after) with Comparison Significant

Candidiasis	Response	No. & %	After		Total	C.S. (*) P-value
			A	P		
Before	A	No.	1	0	1	P= 0.000 HS
		%	100%	0.0%	100%	
	P	No.	23	1	24	
		%	95.8%	4.2%	100%	
Total		No.	24	1	25	
		%	96%	4%	100%	

(*) HS: Highly Sig. at P<0.01; Testing based on the association table by McNemar test. Abbreviations of: A= absent; and B= present

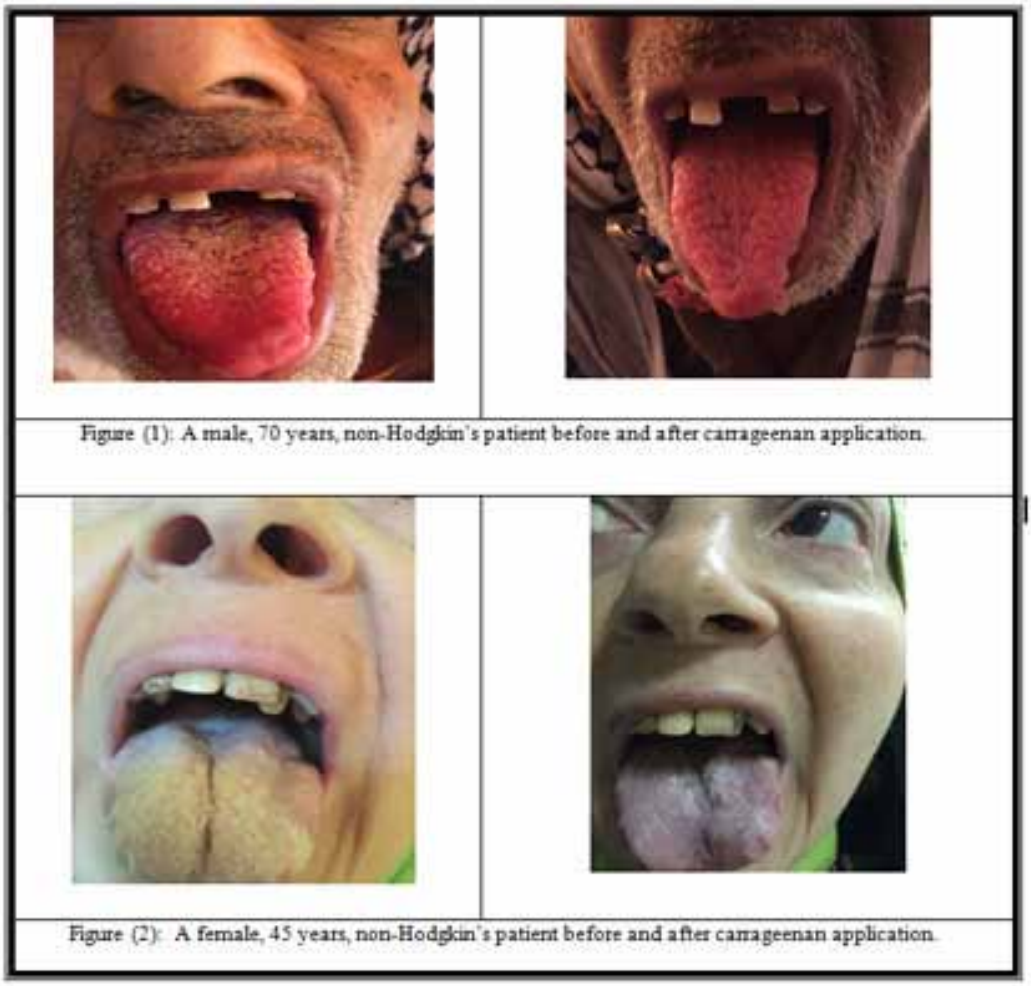

Figure (1): A male, 70 years, non-Hodgkin's patient before and after carrageenan application.

Figure (2): A female, 45 years, non-Hodgkin's patient before and after carrageenan application.

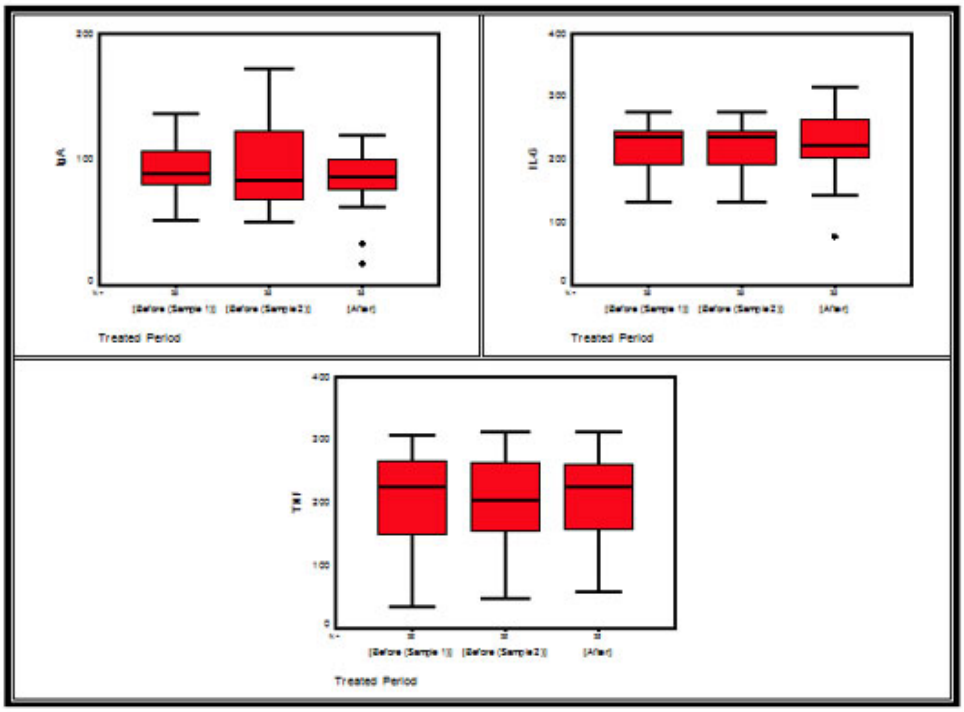

Figure (3): Stem – leaf plots for immunization parameters, (iga, il-6, and tnf) along treated periods (before sample1, before sample 2, and after).

Discussion

Algas, Algue Rouge Marine, Carrageen, Carrageenan, extracted from red seaweed were explored for evaluation their good influence and effect. Kappa/iota and xi/theta carrageenan stimulate the formation of swollen hyphal segments in the species of Alternaria infectoria, when exposed to carrageenan, (Merlos, M., et al., 1996). The observed phenotype was the same which occur in antifungals toward the wall of fungal cell. The effect of the extracts obtained from red seaweed has been investigated in this study for the first time while other studies are essential to evaluate the mechanism of action and to characterize the bioactive compounds of this material. The results of this study showed that the κ-carrageneenan that used in this study, have the same properties of the standard κ-carrageneenan and it can be prepared as a gel or solution. Both pharmaceutical preparations exert biological activities in vivo studies including humans. Therefore, the results are discussed in term of the new findings that disclosed by this study. This is the first type of study using carrageenan on lymphoma patients.

Carrageenans are consists chiefly of potassium, sodium, calcium, magnesium and ammonium sulfate esters of galactose and 3, 6-anhydrogalactose copolymers. These hexoses are alternately linked α-1,3and β-1,4in the polymer (USP-NF25; training manual 6, 1990). Carrageenan is extracted from red seaweed of the genera Chondrus, Eucheuma, Gigartina and Iridaea (Manjamalai, A., et al., 2011). Its form gives it a gelling, thickening and emulsifying properties, enabling it to be widely used in the food industry In addition to that; its biologically active compounds have made it more appealing to be used in the industries above.

There is no doubt that κ-carrageneenan is prepared in term of gel and in such pharmaceutical preparation is commonly applied in pharmaceutical and food industries. In this study an attempt is tried to prepare a gel, rather than a solution. So the differences of this study from others study in the

•Absorbance wavelength is related to the method of preparation.
•The experimental tools that used for identification of κ-carrageenan.
•The high value of viscosity of κ-carrageenan that reported in this study.
•The high concentration that used for preparation of gel rather than solution (Relleve et al., 2005).
•On the other hand, the conductivity of the κ-carrageenan gel, which is equal to 2430 mS/cm, indicated the presence of impurities or salts and this explained the appearance of precipitate on the long-standing of the gel.

1. Oral manifestations

In April 2014 Natal showed that the Anticancer chemotherapy actually involves the use of drugs that avert proliferation of the tumor cells and/or cause their destruction, the problem in this type of therapy is acting blindly of all anticancer agents, unfortunately they act on normal cells which is on proliferation cell cycle, such as bone marrow cells, hair follicle cells and the epithelial cells of the gastrointestinal tract. side effects of these therapy begin with damage to the primary oral tissue due to its indiscriminate effect on the cycle of cell replication in the basal cells layer proliferation, performed intraoral examinations to diagnose oral lesions so, the most frequent lesions were mucositis, candidiasis, periodontitis and gingivitis, saliva and changes in sense, taste and functional changes, infection of oral and dental and risk of dental disease and necrosis of the jaw (Joel et al., 2012). Infection varied according to oral hygiene of the patient (Andre M Kallab., 2013). while the mediate effects in turn are

due to function of non-oral part that have an accompanying effect on the oral cavity, like the suppression of bone marrow, the immune cells loss, and the salivary protective elements loss (Begonya et al., 2011) Severe oral complication can adjust given the patient of exact therapy protocols, like reduction of the dose or modifications of treatment schedule to permit for oral lesions resolution. But in cases of severe oral life threatening, the patient couldn't be able to have cancer therapy anymore; so it is usually stopped. These rupture in therapy due to oral complications can affect patient life by threating it (National Cancer Institute, 2014).
In the present study, all of the oral manifestations below had been mentioned in cases after taking treatment that's mean those manifestations may be secondary to the chemotherapy and not to the pathology of the disease itself.

1.1 Taste alteration
The most prevalent oral manifestation in lymphoma patients and was significantly higher in both NHL patients and HL patients with chemotherapy.

2.1 Burning mouth syndrome
Second most prevalent oral manifestation with significant increase ($P<0.05$) in both NHL group and HL group.

3.1 Dry mouth
The this study also there is high significant rate statistically, in both NHL & HL patients

4.1 Oral Thrush during Chemotherapy (candidiasis)
Oral thrush can occur in people undergoing chemotherapy which reported by Alkula, 2011, Arendrup, et al., 2014. Particularly in patient who take steroids. Candida albicans is the causal fungus of Thrush
Result shows that equal distribution between male and female also on H&NH Lymphoma with different stages of the disease, with increase severity of thrush with age. A highly significant different at $P<0.01$ are accounted after application of carrageenan, and that were resulted throughout not present of «Candidiasis» responses after treatment.

2. Salivary immunological markers
2.1 Salivary IgA
Salivary IgA is the eminent immunoglobulin and is considered to be the essential defense mechanism in oral cavity (Sookto, et al. 2013). By preventing adherence of any microbs, on enzymes neutralizion, toxins and viruses; or synergistically action with other factors like lysozyme and lactoferrin (Jafarzadeh et al., 2010). Dimeric IgA in salivary IgA is produced by local plasma cells in the stroma of salivary glands (Per Brandtzaeg, 2013).
Timucin et al., 2009 reported that total serum IgA concentration was found to be within normal ranges in all NHL & HL patients while this study found slight increase in IgA during treatment and decrease slightly after carrageenan application.
This difference may be explained by first: change in oral microflora and this may lead to local increase of salivary IgA secretion, this was documented by Ye et al., 2013 as they said the patients with cancer had a less diverse and different community of bacteria in their oral cavity when compared to control group, second: there is no rela-

tion to be noticed between serum and Salivary immunoglobulins. This may reported that extravascular transfer of immunoglobulin A depends on the mucosal status of the subject and not depending on the serum level Also carrageenan never absorbed by oral mucosa to act systemically as Borthakur, A., in 2007 found that carrageenan induce interlukin-8 only if injected intrapluerally. Golds by et al., 2000 stated the lower level of salivary IgA may be a result of impairing the normal function of the human immune system by chemotherapy which can cause major alterations in the oral defense mechanisms that are likely to play a role in the decrease of salivary contents of immunoglobulins.

2.2 Salivary IL-6
It is a pleiotropic cytokine with a lot of biological activities in inflammation, immune regulation, hematopoiesis, and oncogenesis. (Kishimoto, 2010). A cytokine produced by a variety of cells, including tumor cells, macrophages, and lymphocytes (Adriana et al., 2014). From this study which show a slight decrease in IL-6 after carrageenan application.

2.3 Salivary TNF-α
Tumor necrosis factor-α is cytokine with pleiotropic biological capacities including toxic effects on certain tumor cells, growth influences and regulate inflammatory and immunological events (Slattery et al., 2011 and De Paepe et al., 2012). The present study showed a slight elevation of salivary TNF-α level in patients receiving carrageenan.

Conclusion
1. The carrageenan effects on oral lesion may allow the use of κ –carrageenan as a base or primary compound in dental practice.
2.The most frequent oral manifestations in Lymphoma patients were taste alteration followed by burning mouth syndrome and dry mouth and increase during treatment with chemotherapy
3. The salivary immunological markers level slightly decreased in lymphoma patients after receiving carrageenan.

Conflict of interest: The material used in this research may be advisable to use for other patients on chemotherapy

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