EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 12
305-309
Year: 2019

EVALUATION OF CIRCULATING INTERFERON- γ IN ORAL LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA

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Article Received on 29/08/2019 **Article Revised on 19/09/2019** **Article Accepted on 09/10/2019**

ABSTRACT

Background: Human immune system plays an important role in host defense against various pathogens. Oral squamous cell carcinoma is a common malignancy of multifactorial etiology which is characterized by an altered host immune mechanism. Interferons are important immune mediators with effective anti-tumor mechanisms. Assessment of the clinical utility of these immune mediators may aid in efficient management and improved prognosis in oral leukoplakia and OSCC. **Objectives:** The objective of the study was to evaluate the clinical significance of interferon-γ by analyzing and comparing their serum levels in oral leukoplakia and oral squamous cell carcinoma. **Materials and Methods:** Serum samples collected from study participants categorized into apparently normal controls (n=10), oral leukoplakia (n=10) and clinically and microscopically diagnosed oral squamous cell carcinoma (n=10) were analyzed using ELISA technique to determine the interferon- γ levels (pg/ml) in the three groups. Statistical analysis using ANOVA and student t-test at $p<0.05$ was performed to evaluate the differences between the groups. **Results and Conclusion:** Comparison of the serum interferon- γ levels between the three groups revealed a statistically significant increase in their levels in oral leukoplakia than in apparently normal controls and the levels were decreased in oral squamous cell carcinoma patients than in oral leukoplakia. Decrease interferon- γ levels suggest a reduced effectiveness of host immune response in oral squamous cell carcinoma. Assessment of interferon- γ in oral leukoplakia and oral squamous cell carcinoma may aid in its clinical utility as an adjuvant therapeutic strategy for better prognosis.

KEYWORDS: Leukoplakia, oral; Mouth neoplasms, Oral squamous cell carcinoma; Immune system; Interferons; Interferon gamma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is an important epithelial malignancy representing 95% of all forms of head and neck cancer, and its incidence has been reported to increase by 50% in the past decade.[1] The development of OSCC could either be de novo or more commonly follows a set pattern of development preceded by the presence of oral precursor lesions. Oral leukoplakia is the most frequently encountered potentially malignant disorder of the oral mucosa with an increased potential for malignant transformation.[2] The process of oral carcinogenesis is a multistep process characterized by the sequential stimulation of additional genetic defects followed by clonal expansion.[3, 4] Despite diagnostic and therapeutic advances over the decades the disease remains a challenge for medical professionals with the five-year survival rate being less than 50%.[5] In addition, a high percentage of patients have a poor response to therapy and high recurrence rates. The process of carcinogenesis involves seven fundamental changes in cell physiology that determines malignant phenotype and one of the important hallmarks of the transformed cell is its ability to evade the host immune response. Immunosurveillance is a body mechanism to identify the tumor cells and their subsequent destruction. The ability of the tumor cells to escape the immune system of the body has been identified as an important hallmark for tumor development.[6]

The immune system represents a complex assortment of interacting cells and proteins which can be broadly divided into innate and adaptive types.[7] The role of immune system in human carcinogenesis is often regarded as a double-edged sword wherein the body's immune system is known to nullify the harmful effects of cancer cell proliferation while it may also promote tumor growth. Data available in the literature provides evidence towards the dual action of the host immune system. This

concept of dual immune response composed of host protection and tumor promotion formed the basis of tumor immunoediting. According to the cancer immunoediting hypothesis, the immune system can either block the tumor growth, development and survival or can facilitate tumor outgrowth by sculpting tumor immunogenicity or by inhibiting host-protective anti-tumor responses.[8]

The cellular mediators of immunity and immunosurveillance includes tumor necrosis factor related apoptosis inducing ligand (TRAIL), interferons, macrophages and dendritic cells, natural killer cells, B-lymphocytes and T-lymphocytes.[9] Literature studies have identified potential role of the various cellular mediators of immunity in oral leukoplakia and oral squamous cell carcinoma. TRAIL is a type II transmembrane protein which plays an important role in immunosurveillance against tumor progression by selectively inducing apoptosis in tumor cells.[10] Macrophages are efficient phagocytic cells which have immunosurveillance ability as well as promote tumor growth. Macrophages operate to both directly destroy tumors and augment the functions of natural killer cells and T-cells.[11] Natural killer cells represent cellular populations of the innate immune compartment that protects the host from tumor formation. Robust infiltration of NK cells is nearly always associated with a favorable prognosis.[12]

One of the important cellular mediators which also possess therapeutic implications is the family of interferons. A significant finding with this aspect is that endogenously produced interferon γ (IFN- γ) was shown to protect the host against the growth of transplanted tumors and the formation of primary chemically induced and spontaneous tumors. [13] Interferons are a multigene family of inducible cytokines that was first discovered as an anti-viral agent.[14] Subsequently it was found that these cytokines have an important role in cell growth inhibition, modulation of cell differentiation and in the elimination phase of cancer immunoediting.[15,16] Interferons are commonly grouped into two types. Type I IFNs are also known as viral IFNs and include IFN-α (leukocyte), IFN- β (fibroblast) and IFN-ω. Type II IFN is also known as immune IFN and includes IFN-γ [14]. IFN-γ is a 17kDa peptide synthesized by natural killer cells, CD4Th1 cells and CD8 cytotoxic suppressor cells that is mainly induced by mitogenic or antigenic stimulus.[15]

Extensive research over the past several years has demonstrated that IFN-γ is vital to the promotion of tumor surveillance in immunocompetent hosts. The role of IFN-γ in tumor immunosurveillance has been documented in breast cancer,[15] laryngeal cancer,[17] malignant melanoma,[18] and to some extent in oral cancer. [19] In addition to its role in tumor immunoediting, IFN-γ has also been evaluated as an important therapeutic agent against tumor cells.

One of the drawbacks in the treatment of OSCC and oral leukoplakia is the delay in the diagnosis and management compounded by the failure of the treatment in many instances. The need of the hour is to identify potential therapeutic targets which can complement the existing treatment strategies. With this background the aim of this study was to evaluate the clinical significance of serum IFN-γ in oral leukoplakia and oral squamous cell carcinoma.

MATERIALS AND METHOD

The study involved analysis of serum interferon-gamma levels of patients clinically diagnosed with oral leukoplakia and histopathologically confirmed OSCC. A total of 30 study participants were included in the study and were divided into three groups. Group I (n = 10) was apparently normal individuals without any oral lesions, tobacco habits, and systemic illnesses; Group II (n = 10) included clinically diagnosed cases of oral leukoplakia and group III (n=10) included participants that were clinically and histologically diagnosed cases of oral squamous cell carcinoma. The patients with known history of systemic illnesses and medications; patients with a history of therapy for oral leukoplakia and OSCC (surgery, chemotherapy, and radiotherapy); and patients with recurrent oral lesions were excluded from the study. Institutional ethical committee clearance was obtained before the commencement of the study (YMT/DE/EM/140/17). The study details were explained to the patient and written informed consent was obtained.

Five milliliters of blood were collected by venipuncture from forearm region under aseptic precautions and transferred into plain vials. The obtained samples were centrifuged at 1000 rpm for 15 min. The supernatant (serum) was then separated and analyzed immediately. In situation where the analysis could not be performed immediately, the serum was stored at -20^{0}C before analysis.

Sample analysis was done using Diaclone interferon gamma ELISA kit (Diaclone SAS F-25020 Besancon Cedex, France). The prepared samples using standard technique were added to the pre-coated wells of IFN-γ, incubated at room temperature for 2 hours and then analyzed as per manufacturers' instructions. Analysis was performed on a spectrophotometer using 450nm as the primary wavelength and 620nm as the reference wavelength in relation to the normal curve and the levels were obtained in graphical form and numerical values for each sample. The levels of interferon gamma were expressed as pg/ml (picogram/milliliter) for all analyzed samples. Statistical analysis to determine the difference between the three groups was done using one-way analysis of variance (ANOVA) and intergroup comparison was done using students t-test (unpaired) at a 'p' value of <0.05 ('MedCalc Statistical Software' Version 17.8.2).

RESULTS

The age and gender distribution of the study participants were as given in table 1. Mean serum IFN-γ levels in the three groups was 1.94±0.42 in apparently normal individuals, 2.58±0.93 in oral leukoplakia patients and 2.05±0.60 in patients diagnosed with oral squamous cell carcinoma (Fig 1). From the data, it was observed that the serum IFN-γ levels were increased in oral leukoplakia and OSCC group than in normal controls. However, ANOVA analysis between the three groups did not reveal any statistical significance (Table 2). On inter-group comparison using student t-test, the levels were significantly high in oral leukoplakia than in apparently normal individuals and no statistically significant difference was obtained between OSCC patients with oral leukoplakia and apparently normal group (Table 3).

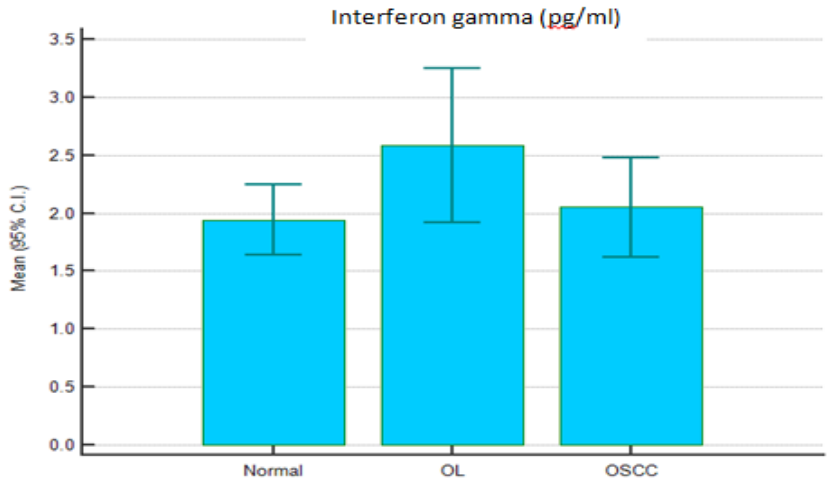

Figure 1: Box plot showing serum IFN-γ levels in the three groups as determined by ELISA.

Table 1: Age and gender distribution among the study participants of the three groups.

Group	Age		Sex	
	Median	Range	Male	Female
Group I (n=10)	33	29-37	6	4
Group II (n=10)	39.5	32-45	7	3
Group III (n=10)	47	40-52	6	4

Table 2: ANOVA analysis of serum IFN-γ levels between the three groups.

Group	Mean ± SD	ANOVA
Group I (n=10)	1.9 ±0.42	p=0.100*
Group II (n=10)	2.5±0.93	
Group III (n=10)	2.0±0.60	

Table 3: Student t-test (unpaired) for inter-group comparison of serum IFN-γ levels

Group	I	II	III	Significance
Group I	-	**P=0.05***	**P=0.6**	(2)
Group II	**P=0.05***	-	**P=0.1**	(1)
Group III	**P=0.6**	**P=0.1**	-	-

DISCUSSION

An important hallmark of tumor development and its subsequent progression is related to the effect of host immune system in combating the aggressive behavior of tumor cells. Experimental and epidemiological studies have indicated that a deficient immune system is associated with poor prognosis of various human tumors. [11] Amongst the various cellular immune mediators studied, the role of interferons has garnered attention owing to its ability to protect the host against the tumor growth as well as the possibility of employing these compounds in targeted oncotherapy.[13] This study was thus designed to evaluate the serum interferon- γ levels in oral leukoplakia and OSCC with an aim to understand its clinical significance.

The study results showed a significantly increased levels of serum IFN- γ in oral leukoplakia than in normal controls. The levels were decreased in OSCC than in oral leukoplakia although the difference was not statistically significant. Since the present study is the one of the initial studies performed to evaluate the serum IFN- γ levels in oral leukoplakia, no literature data exist to support such a finding. One of the possible causes for an increase levels in circulating fluid could be due to an enhanced defense mechanism of the host immune system. The subsequent decrease in OSCC group than in oral leukoplakia could be attributed to the defective immune response associated with tumor progression and possibly the failure of the immune system to control tumor cell proliferation. The inter-group comparison between apparently normal controls and OSCC group did not reveal any statistical significance. While IFN- γ levels were not significantly decreased in OSCC, it is possible that the immune response was ineffective towards the uncontrolled cell proliferation owing to the growth promoting factors of the tumor cell and its surroundings. Literature findings on comparison of IFN-γ levels between control and OSCC group have

suggested a decrease levels in OSCC thereby pointing towards a poor host immune response. A study by Wang et al (2014) has shown that decreased levels of IFN- γ along with increased IL-10 is associated with poor prognosis of patients with oral squamous cell carcinoma and may serve as better prognostic indicator.[20] A study by Naganawa et al (2015) showed that IFN- γ levels were progressively increased in advanced stages, in metastatic cancers and with increased severity of lymph node metastasis. The finding was attributed in part to natural killer cell-mediated activity.[21]

Literature data were reported on the role of IFN- γ in breast cancer, laryngeal cancer, prostate cancer and melanoma with an intention to evaluate their mechanism of action as well as its interaction with other pro- and anti-tumoral factors. A study by Garcia-Tunon et al (2007) has found a decreased expression of interferon gamma and its receptors in breast cancer than in normal controls and benign tumors of the breast. It was concluded that the decreased expression could be a tumor cell response which subsequently could not inhibit the uncontrolled cell proliferation.[15] In another study by Lee et al (2013), the levels of IFN- γ were significantly reduced in hepatocellular carcinoma and was associated with higher tumor stage, size and increased tumor recurrence.[22]

The action of IFN- γ in tumor rejection has been explained by different pathways. Experimental findings have concluded that tumor cell is a physiologically relevant target of IFN- γ in the tumor rejection process. [23] IFN- γ is also known to enhance tumor cell immunogenicity by upregulating components of the MHC class I antigen processing and presentation pathway which can cause tumor rejection. [24] IFN- γ is known to have profound anti-proliferative and/or pro-apoptotic effect on certain tumor cells. [9] Other work suggests that γδ T-cells are an important source of IFN-γ during the development of protective anti-tumor response. [25] It was also found that host cells are important targets of IFN- γ during the development of protective anti-tumor immune response. Studies have shown that IFN- γ in combination with granulocyte monocyte-colony stimulating factor (GM-CSF) controls chronic infection thus reducing tumor development. [26] Another possible mechanism is that IFN- γ can induce angiostatic effects in tumors by targeting non-transformed host cells that are near the tumor site.[27]

CONCLUSION

To conclude, a preliminary study was performed to determine the serum IFN- γ levels owing to the scarcity of literature data available to determine its clinical utility. The serum levels of IFN- γ were significantly elevated in oral leukoplakia than in normal controls while the levels were decreased in OSCC group than in oral leukoplakia. Alteration of host immune system could be considered as an important parameter in the progression of OSCC as well as to determine the malignant potential of oral leukoplakia. The need of the hour is to identify the therapeutic potential of immune regulators such as IFN-γ to enable the host to effectively combat the tumor cell and its associated morbidity. Targeted therapy aimed at action of IFN- γ against tumor cells should be clinically evaluated as a treatment regimen to support the standard treatment protocols thereby preventing morbidity and mortality. Furthermore, detailed analysis of a larger sample; comparison between various grades of oral leukoplakia and OSCC and their clinical correlation should be carried out to identify the precise role of IFN-γ in a clinical set-up.

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