Research, Society and Development, v. 10, n. 11, e61101118521, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i11.18521

Genetic polymorphism of TNF-α and its implications in previewing the course of pathologies, including oral diseases

Polimorfismo Genético do TNF-α e suas implicações na visualização do curso de patologias, incluindo doenças bucais

Polimorfismo Genético del TNF-α y sus implicaciones en la visión previa del curso de las patologías, incluidas las enfermedades orales

Received: 07/21/2021 | Reviewed: 07/27/2021 | Accept: 08/20/2021 | Published: 08/22/2021

Luciano Barreto Silva
ORCID: https://orcid.org/0000-0002-1508-4812
Faculdade de Odontologia do Recife, Brazil
E-mail: lucianobarreto63@gmail.com
Rodolfo Scavuzzi Carneiro da Cunha
ORCID: https://orcid.org/0000-0001-7110-848X
Faculdade de Odontologia do Recife, Brazil
E-mail: Scavuzzi@gmail.com
Gabriel Henrique De Oliveira Queiroz
ORCID: https://orcid.org/0000-0002-7795-3964
Faculdade de Odontologia do Recife, Brazil
E-mail: qgabriel116@gmail.com
Guilherme Marinho Sampaio
ORCID: https://orcid.org/0000-0003-4441-7601
Faculdade de Odontologia do Recife, Brazil
E-mail: guilhermemarinhosampaio@gmail.com
Hadassa Fonsêca da Silva
ORCID: https://orcid.org/0000-0002-9432-9522
Faculdade de Odontologia do Recife, Brazil
E-mail: hadassafonseca1@gmail.com
Sandra Sayão Maia
ORCID: https://orcid.org/0000-0001-6808-9775
Faculdade de Odontologia do Recife, Brazil
E-mail: sandrinhasayao@hotmail.com
Paulo Melo Júnior
ORCID: https://orcid.org/0000-0001-9926-5348
Faculdade de Odontologia do Recife, Brazil
E-mail: paulo.reis@upe.br
Edleine Dellalibera
ORCID: https://orcid.org/0000-0002-8943-183X
Centro Universitário Maurício de Nassau, Brazil
E-mail: edileine.del@gmail.com
Ana Paula Veras Sobral
ORCID: https://orcid.org/0000-0002-0801-9385
Universidade de Pernambuco, Brazil
E-mail: anapvsobral@yahoo.com.br

Abstract

Objective: the aim of this work was to explain the implications of the genetic profile (TNF-α polymorphisms in the region-308) in the outcome of different pathologies; and explain how this profile is obtained in the laboratory. *Methodology*: The salting out technique, according to Miller et al. (1988) was used to explain the reader how the genetic profile of TNF-α is obtained to foresee the outcome of pathologies that are modulated by this cytokine. *Results*: the product of the amplified digestion of TNF-α genes was obtained, photographed and explained for the reader. *Conclusions*: the genetic profile may be useful as a predictor for the outcome of different pathologies, so that doctors and dentists may choose the best therapy for each patient.

Keywords: Cell-free nucleic acids; Polymorphism, genetic; Polymorphism, single nucleotide; Periodontitis.

Resumo

Objetivo: o objetivo deste trabalho foi explicar as implicações do perfil genético (polimorfismos do TNF-α na região 308) no desfecho de diferentes patologias; e explicar como esse perfil é obtido em laboratório. Metodologia: A técnica de salting out, segundo Miller et al. (1988) foi utilizado para explicar ao leitor como o perfil genético do TNF-α é

obtido para prever o desfecho de patologias que são moduladas por essa citocina. Resultados: o produto da digestão amplificada dos genes do TNF-α foi obtido, fotografado e explicado ao leitor. Conclusões: o perfil genético pode ser útil como preditor de desfecho de diferentes patologias, para que médicos e dentistas possam escolher a melhor terapia para cada paciente.
Palavras-chave: Cell-free nucleic acids; Polimorfismo genético; Polimorfismo de nucleotídeo único; Periodontite.

Resumen
Objetivo: el objetivo de este trabajo fue explicar las implicaciones del perfil genético (polimorfismos en la región-308 del TNF-α) en la evolución de diferentes patologías; y explicar cómo se obtiene este perfil en el laboratorio. Metodología: La técnica de salting out, según Miller et al. (1988) se utilizó para explicar al lector cómo se obtiene el perfil genético de TNF-α para prever el resultado de patologías que son moduladas por esta citoquina. Resultados: Se obtuvo, fotografió y explicó al lector el producto de la digestión amplificada de los genes TNF-α. Conclusiones: el perfil genético puede ser útil como predictor del desenlace de diferentes patologías, de modo que médicos y odontólogos puedan elegir la mejor terapia para cada paciente.
Palabras clave: Ácidos nucleicos libres de células; Polymorphisme génétique; Polimorfismo de nucleótido simple; Periodontitis.

1. Introduction

With the discovery of deoxyribonucleic acid, the genetic constitution of the majority living organism, whether microscopic or complex, has been thoroughly scrutinized. For the last ten years, geneticists have been associating the genetic polymorphisms of the vast number of genes that constitute the human GENOME, and have concluded that alterations in the alleles of the nucleotides Adenine, Thymine, Cytosine and Guanine may exacerbate or diminish the intensity of the inflammatory response to certain diseases.

TNF-α is a potent pro-inflammatory cytokine, with relevant modulation capacity in several inflammatory processes, including arboviruses, cancer, rheumatoid arthritis, heart attacks, psoriasis, Alzheimer's disease, among others. Thus, researchers converged their attention to the region-308, a genetic site of TNF-α where the substitution of certain alleles seems to alter the individual response to some diseases, altering their course. The aim of this article was to explain and describe how the genetic profile is obtained for the detection of TNF-α polymorphisms in the region-308 G/A, in order to preview the implications in the inflammatory response, so that it can be used for dental and medical research prevision of the pathological outcome.

2. Literature Review

In the last three decades there has been a growing recognition concerning the importance of the role of genetics in the various branches of the health area, through changes in the protocols, influenced by recent discoveries and clarifications of the genetic features of certain pathologies; modifying their diagnosis and risk prediction, regardless of their being rare and not (Varmus, 2002). Within this cutting-edge new perspective, many studies have increasingly reported different host reactions, according to the genetic profile, to a number of pathologies including arboviruses, cancer, rheumatoid arthritis, heart attacks, psoriasis, Alzheimer's disease and periodontitis. From this point of view, a greater understanding of the genetic fundamentals involved in in the course of the diseases could actually contribute to an increase in the understanding of their etiological aspects, as well as their development and evolution, including their effective response when facing an aggression (Kinane, 2003; Price, 2015).

Among the clinical manifestations that affect the oral health, periodontal disease has been referred to as the oral pathology that most affects Brazilians. Studies dating back to 1965, such as Löe et al. reiterated the importance of the essential role of plaque for the stebelness and progression of periodontal disease, which achieves a respectable percentage of individuals distributed in many populations, its chronic form being the most prevalent (Laine, 2000).

The genetic differences based on polymorphisms, with potential to affect the pulp tissues, began to be investigated in the nineties (Rankinen et al.,2000). The rapid advancement of molecular biology techniques made it possible to identify variant DNA sequences in specific genes, relating gene heterogeneity to different levels of proteins associated with pulp diseases. A change in the sequence of DNA bases of a gene that encodes a protein can influence both its expression and its activity.

In the sequence, periapical inflammatory lesions are one of the most commonly found in routine dental examinations in most of the dental offices. With regard to its pathological course, it is observed, as well as in other diseases such as diabetes, and some arboviruses (dengue, Zika and Chikungunya), in which the effective clinical response to treatment differs among the patients of the same group, that there is an individual variability in the effective inflammatory response, which is genetically determined or by interactions with environmental factors. There are also specific variables related to the individual's habits, such as oral hygiene and deleterious addictions, such as smoking and Alcoholism (Offenbacher, 2008).

The immunological system and the role of cytokines in inflammation

The immune system is a sophisticated and interconnected organ that makes use of cellular elements, protein substances and vascular alterations pertinent and necessary for homeostasis. This dynamic is able to establish the physiological process of tolerance and effective responsiveness of the host and its environment, and any deregulation of this equilibrium is capable of producing a potential disease. The innate immune system, which basically uses first-line cells for the nonspecific defense, plays a fundamental role in the early stages of organic injury, adequately responding to indications of cell death, the presence of antigens as Viral DNA and bacteria, as well as cytokines released by nearby or distant cells (Kannarkat et al., 2013). Its main representatives are monocytes, neutrophils and dendritic cells, which are cells specialized in presenting antigens (APCs) on the surface of their membranes to T lymphocytes. The activation of the innate immune system leads to the immediate production of soluble mediators for the recruitment of specific cell types. These mediators are chemokines and cytokines, and constitute the effective stimulus for an adaptive immune response, or simply humoral immunity, represented by cells of prolixous and sophisticated mechanisms, lymphocytes, which orchestrate inflammatory or pro-inflammatory reactions to modulate and control the organic microenvironment (Allen Reish and Standaert, 2015; Shlomchik and Weisel, 2012; Salmond & Zamoyska, 2011; Chaplin, 2003).

Thereon, cytokines appear with a special emphasis on its important role in the mentioned immunologic intermediations. They are signaling molecules that, similarly to hormones, act on specific receptors on the surfaces of cell membranes, stimulating or inhibiting cell division, the secretion of their own molecules, and even inducing apoptosis. Cytokines can be produced by several different cell types, and because their being cellular signaling proteins, they are quickly produced and released into the bloodstream to be coupled to their respective receptors, located in membrane of corresponding cell types. Their family is vast in components, being their main representatives the interleukin (ILs), Interferon (IFN), tumor necrosis factor (TNF), colony stimulating factor (CSF), and Growth Factor (GF) (Alam, 2003). Thus, the establishment of an inflammatory periapical lesion means the existence of cells responsible for chronic inflammation, traditionally the plasmocytes, which have previously been activated in producti on and recruited to the site of injury. Therefore, the establishment of periapical lesions is directly related to the release of intercellular mediators, antibodies and effector molecules towards the periapical tissues (Deo, 2010), exerting a crucial role in the pathogenesis of inflammatory lesions around the teeth apexes (Abbas, 2010; Moran, 2009).

TNF alpha

TNF-α is a proinflammatory cytokine able to activate chemotaxis in immunological cell types, modulating inflammation. It is produced as a prohormone substance constituted of 233 amino acids with rapid action when unleashed into

blood stream, and closely linked in the cell membranes and then processed into a 157 residue mature protein by cleavage of a 76 residue signal peptide. It is involved in many diseases with contradictory effects.

Not until 1975 was TNF-α discovered and researched. It was firstly described as a soluble factor able to cause the necrosis of tumors, and thereafter came its designation (Carswell, 1975). In spite of this fact, which instantly called the medical attention, it was initially referred to as Cachectin or Differentiation Inducing Factor (DIF). This recently new substance had two bioactive forms: transmembrane TNF-α (tmTNF-α) and soluble TNF-α (sTNF-α) (Agarwal, 2000; Chen, 2011; Li, 2006; Hu, 2014). The latter seem to be able to bind the ligand and inhibit the cytotoxic activities of TNF alpha (Seckinger, 1989).

TNF-α and inflammation

Inflammatory signs have been clinical and didactically characterized by the presence of heat, flushing, tumor and pain, and dysfunction of the organs involved. It can be classified as acute generally, when it lasts for at least two days to two weeks, and as chronic when prolonged. Both types of inflammation have well-defined histopathologic features with the predominance of different cell types in each of them. The typical case of acute inflammation is characterized by initial vasodilation, followed by plasma exudation and concomitant emigration of neutrophils in the affected areas. However, not all examples of acute inflammation exhibit neutrophil infiltration and may be associated with chronic inflammation, and the transition between acute and chronic encompasses prolixous processes through chemotactic interactions and recruitment of specific cell types, depending on the aggressiveness of the agent and the responsiveness of the host (Silva, 2015). This process is modulated by the action of several cytokines, especially TNF-α, with lots of studies describing its role in inflammatory diseases, chronic or acute ones, including pulpitis and periapical lesions. Its liberation in the blood stream resulted in cytotoxic or cytostatic effects to human cells, leading to hemorrhagic necrosis, and performing, in experiments with mice, carcinogenic cell regression (Pennica et al., 1984).

Biological aggression to the host organism, represented by microbial infection, is the most important in terms of induction and perpetuation of the pulp pathology. Although bacteria can reach the living pulsing root canal by different ways, dental caries is undoubtedly the most commonly observed pathway in the clinical routine. Thus, the approximation of the microbial activity towards the dental pulp causes a reversible pulpitis initially, with pain provoked and of short duration.

TNF-α is produced by cells with genetic programming for its production and release in cases of injuries. Eventual alterations in the constituent bases of the DNA in the chromosomes may alter the production in quantity and quality. Thus, the effective immune response capacity of any mammal organism is directly related to its genetic programming, which, being known in advance, may work as an immune response predictor, determining the best clinical approach when facing it.

The Genetic Polymorphism of TNF Alpha

From the genetic point of view, the discovery of deoxyribonucleic acid, carried out by the German biochemist Johann Miescher in 1869, and represented in acronym as its correspondent in English DNA (Desoxy-acid), or simply Deoxyribonucleic acid, offered the world the real possibility of accurately determining millionth the identity of a certain living being, through the analysis of its genetic code, glimpsing the possibility of anticipating the onset of diseases and making predictions of its intensity and effects on an organism, simply by scrutinizing the genetic programming. Thereafter, the body´s response in each specific case may be expected and adaptations in the clinic protocols may be accomplished. DNA is known in the scientific world as the "molecule of life", which exists in most living beings, with the exception of some types of viruses.

The genetic polymorphisms existing within the DNA can be conceptually defined as variations in the DNA chain itself, which can create or dismiss sites of recognition of certain restriction enzymes, creating variations that seem to be related

only to a certain constituent base. If such variation is detected in frequency higher than 1% of the population, then there is a polymorphism (Balasubramanian, 2004), which acts as genetic markers, since its transmission is associated with other genes, located in the chromosomal region near them, a fact named linkage, whose importance relates to the fact that if a gene close to a marker is able to unleash a certain disease, all affected individuals in the family will receive both the marker, as well as the causing gene of that same disease. Therefore, polymorphisms may cause different responses to certain pathologies, exerting a predisposition, and modifying in the carriers their immunological responses of varying intensity when affected.

Many genetic tests developed in studies conducted in humans use genomic DNA, extracted from blood samples collected from patients affected by different pathologies, where it aims to separate the white cell layer (Buffy-coat), or total blood. To act so, it is necessary that the patients involved are precisely diagnosed, then grouped in certain specific studies, and that a blood sample of each of them is collected with anticoagulants, so that they remain fluid, to become possible to aspiration by pipettes. Following the procedure, subsequent separation of cells and plasma from non-coagulated blood through centrifugation takes place and, therefore, the removal of the nucleated cell layer (Garc, 1996).

Some genetic polymorphisms have been identified as being related to the intensity of the host response to certain endogenous and exogenous substances, modifying the spectrum of normality when compared with the majority of the population, this being a modifying factor that should be studied to better understand the process of organic responses in the patients, favoring the prospective planning of the behavior of certain population samples in the face of different pathologies. In dentistry, studies involving genetic polymorphisms have approached the relationship of the host response with periodontal diseases, but few relate them to periapical lesions.

The genetic polymorphisms called single nucleotide polymorphisms (SNPs) constitute 90% of the variations found in the human genome, which may be present in the coding region, or in the gene regulatory region, a fact that could generate changes in amino acid sequence of the coded protein, or at its production rate, with special interest for the aim of this work. According to Petersdorf and Malkki (2013), because they are biallelic, SNPs can be detected by using techniques that discriminate against any different combinations in the nucleotides adenine (A), thyme (T), cytokine (C) and guanine (G). According to the studies of these authors, real-time quantitative assays are sensitive and specific for DNA quantification, such as polymerase chain reaction (PCR), which offers a variety of markers and probes for the detection of genetic alterations. Among the SNPs, the -308 G/A (rs1800629), in the promoter region, is described by increasing the expression of the gene and consequently the level of production of TNF-α, due to the transition of the wild allele G to the variant allele A (Qidwai, Khan, 2011).

By the facts exposed above, it is feasible to speculate that the presence of polymorphisms may indeed exacerbate the inflammatory responses of periapical tissues, modifying the course of the disease and the response to treatment in the presence of pulp necrosis (Morsani, 2011). The evolution of untreated pulp diseases, then, will lead to the onset of periapical lesions, even if not demonstrated radiographically in some situations; The course of these pathologies can be drastically intensified by the existence of genetic polymorphisms in the genomics production sites of the cytokines TNF-α.

Obtention of the genetic profile: biological material collection

Obtaining the biological material usually occurs by accomplishing peripheral blood collection through vacuum venous puncture, by a qualified and trained professional following all the biosafety up-to-date norms. It is worthy mentioning that there are several techniques available, depending on the location from which to extract human DNA. The aim was to explain the reader how DNA is extracted, quantified and digested for the determination of the genetic profile.

In our routine procedures, for each patient, two blood tubes of 5 mL each are collected, one with EDTA K3 anticoagulant (purple lid) in order to extract human genomic DNA. The collection follows the recommendations of the NCCLS

(National Committee for Clinical Laboratory Standard). The samples were packed and transported in thermal bags, kept between 2 º and 8 ºC.

Genomic DNA Extraction

For didact matters, the DNA in this paper was extracted by the salting out technique, according to Miller et al. (1988) (Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *NucleicAcidsResearch.* 1988; 16 (3): 1215.), and quantified by optical density reading.

Determination of Polymorphism – 308 G/A of the TNF-*α* gene

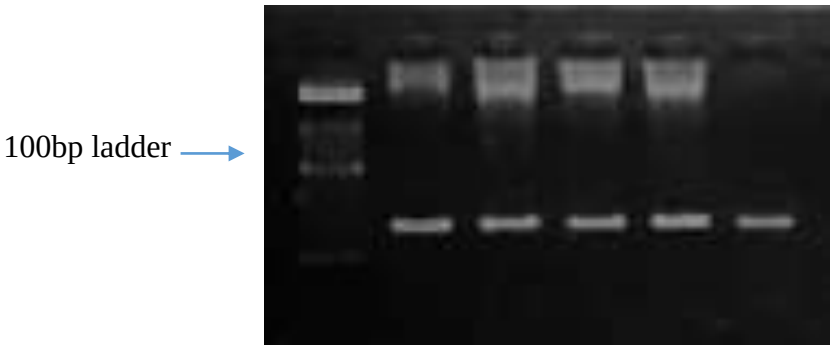
Genotyping was performed using the PCR-RFLP method. The sequences of the initiators used were 5 '-AGGCAATAGGTTTGAGGGCCAT-3 ' (Forward) and 5 '-TCCTCCCTGCTCCGATTCCG-3 ' (reverse), according to Cabrera et al, (1995). Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J, Blackwell JM. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J Exp Med. 1995 Nov 1; 182 (5): 1259-64. PubMed PMID: 7595196; PubMed Central PMCID: PMC2192198. The amplification reactions had a final volume of 10µL, containing: 4µL of Master Mix® 2X-Promises®, 0.5 µL of each initiator (10pmol/µL), 4µL of nuclease free Water (Promega®) and 2µL of DNA (50 to 100ng/µL). The cycling conditions were: initial denaturation for 3 minutes at 94ºC, followed by 30 cycles composed of denaturation at 93º C for 1 minute, pairing at 55 º C for 1 minute and elongation phase for 1 minute at 72ºC. The final extension was held at 72 º C for 5 minutes (STANULLA et al., 2001). PCR products were visualized by electrophoresis in 2% agarose gel and 1X TBE buffer, stained with ethidium bromide (5 M g/mL). Figure 1.

Figure 1. Electrophoresis in 2% agarose gel stained with ethidium bromide.

Source: Authors.

After application in the gel, it was subjected to an electric current of 200 Volts, for 20 minutes, visualized in Transilluminator-UV and photographed with digital camera. Figure 2.

Figure 2. DNA amplification. The image confirms DNA presence. The ladder used was 100bp.

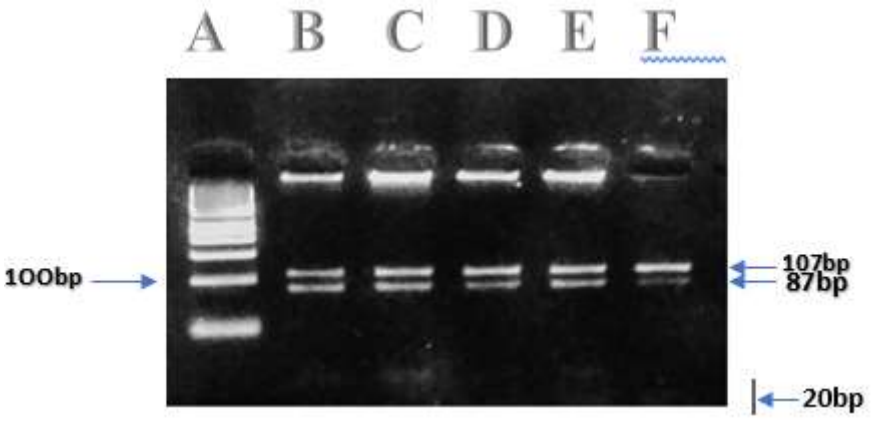


Source: Authors.

Following visualization, the PCR product was submitted to enzymatic digestion for the identification of polymorphism. 6µl of amplified, 1.5 µl of 10X buffered Buffer (NEBuffer™ 3.1), 0.1 µl of Ncol enzyme (NcoI-HF®, 20,000 U/mL), 0.2 of BSA (10mg/mL, Invitrogen®), 2.2 µl of ultrapure water were used. The reaction was incubated at 37°C for 16 hours. Digestion products were analyzed using 3% agarose gel electrophoresis and 1X TBE buffer stained with ethidium bromide (5 M g/mL). After application in the gel, it was subjected to an electric current of 200 Volts, for 30 minutes, visualized in Transilluminator-UV and photographed with digital camera.

The interpretation of the polymorphism took place as follows: The PCR product generated a 107bp fragment, which was subjected to enzymatic digestion. This, in turn, could generate: two fragments, one of 87bp and another of 20bp when the restriction occurred, which were identified as the wild allele TNFA-1 (-308G); Or only a fragment of 107bp when there was no restriction, which was identified as the mutant allele TNFA-2 (-308A). To identify the size of the digested fragments, a molecular weight marker of 100bp (250 µg, Invitrogen®) was used in the same electrophoretic run. The possible genotypes were: GG (low producer of TNF-α, wild genotype), GA (intermediate producer of TNF-α, heterozygous genotype) and AA (high producer of TNF-α, mutant genotype). Figure 3.

Figure 3. Product of the amplified digestion for the TNF-α gene. TNF-α gene alleles are marked between 87bp and 20bp (wild genotype/GG) and 107bp, 87bp and 20bp (heterozygous genotype G/A).



Source: Authors.

The wild genotype G_{-308}/G_{-308} is usually the most common genetic profile of TNF-α. The individuals belonging to this profile are low producers, and therefore are expected to express a less intense inflammatory reaction when compared to the

intermediate profile, G_{-308}/G_{-308}. The high producer profile is the rare mutant A_{-308}/A_{-308}, which produces higher amounts of TNF-α, and therefore the individuals with this genotype are expected to react intensely to inflammation.

Nevertheless, the fact that an individual is classified in any of the three possible genotypes mentioned previously does not necessarily mean that they will correspond strictly to inflammation as expected by the expression of their genetic profile, simply because the profile itself is not able to control all the immunological mechanisms in the system. It also depends on the genetic profile of the other cytokines involved in the modulation of inflammation, such as IL-1β, responsible for amplifying the inflammatory signs, and IL-10, with anti-inflammatory effects.

3. Discussion

Understanding the genetic polymorphisms allowed science to better understand the course of the disease, its tendencies; in order to help find the best therapeutic approach according to the patient´s needs. Predicting the course of diseases had long been a wish of the medical community; with the advent of scrutinizing DNA this could finally come true. In this sense, only by analyzing specific proinflammatory and anti-inflammatory cytokines of a determined organism can geneticists foresee the evolution of the pathology in focus. Virtually all the inflammatory processes are modulated likewise.

As TNF-α is able to amplify the immune response, individuals belonging to the high producing mutant genetic profile (A/A) would, at least theoretically, respond more intensively to inflammation than would low-producing ones. The wild type (G/G) is the most commonly found in the Brazilian population, are low producing individuals whose inflammatory effects tend to be lowered. The mutant intermediary producer (G/A), usually shows amplified inflammatory response; although the real effective immune response capacity can only be determined by the summatory of the main cytokines involved in the process, such as IL-6, which is proinflammatory, contrasting to IL-10, whose anti-inflammatory effects may counterbalance the modulation of the immunological response as a whole. Last by not least, individuals with high producing genetic profile (A/A) tend to respond much more drastically to inflammation and therefore, depending on the pathology that affects different patients, as well as their genetic programming to produce more or less specific cytokines, the differences in the quality of the immune response may be so dramatic that some of them may show very little symptoms of a determined pathology, while others may even die; especially if the agent is a new virus or resistant bacteria.

In this sense, only by analyzing the main cytokines involved in the process can researchers have a better prospect of how inflammation will behave in a determined organism.

4. Conclusions

The genetic polymorphisms of TNF-α gives an overall idea of the intensity of the immune response, when compared exclusively to its lone effect on the immune system of the groups analyzed and categorized, as belonging to each determined polymorphism in each pathology studied. However, knowing the importance of this cytokine, one can expect a matching correspondence and use the genetic profiles to research their effects in diverse diseases, since the patients are properly diagnosed and grouped correspondingly to the pathology in question. This professional cutting-edge therapy may save lives and promote a better life quality for bearers of many different pathologies.

References

Abbas, A. K., Lichtman, A. H., Pillai, S. Cytokines. In: Abbas, A. K., Lichtman, A. H., Pillai, S. *Cellular and molecular immunology*. Saunders/Elsevier, 2010. 267-302.

Aggarwal, B. B. (2000) Tumour necrosis factors receptor associated signalling molecules and their role in activation of apoptosis, JNK and NF-kappaB. Ann Rheum Dis 59: i6-i16.

ALAM (2003). Lymphocytes. *Allergy and Clinic. Immunol.* 2003 February, 111(2).

Allen Reish, H. E., & Standaert, D. G. Role of α-synuclein in inducing innate and adaptive immunity in Parkinson disease. *Journal of Parkinson's disease*, 5, 1-19.

Balasubramanian, S. P. *et al.*Candidate gene polymorphisms in solid cancers. *Eur J SurgOncol*, 30, 593-601.

Cabrera, M. *et al.* Polymorphism in tumor necrosis factor genes associated with mucocutaneousleishmaniasis. *J Exp Med*., 182, 1259-64.

Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., et al. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 72: 3666-3670.

Chaplin, D. D. Overview of the immune response. *J Allergy ClinImmunol*, 111, 5430-5.

Chen, H., Xiao, L., Zhang, H., Liu, N., Liu, T., et al. (2011) The involvement of beta-actin in the signaling of transmembrane TNF-alpha-mediated cytotoxicity. *J Leukoc Biol* 89: 917-926.

Deo, V., & Bhongade, M. L. Pathogenesis of periodontitis:role of cytokines in host response. *DentToday*, 29(9), 60-2, 64-6

Garg, U. C. *et al.* Simple and Rapid Method for Extraction of DNA from fresh and Cryopreserved Clotted Human Blood. *Clinical Chemistry* 42:647-648.

Hu, X., Li, B., Li, X., Zhao, X., Wan, L., et al. (2014) Transmembrane TNF-alpha promotes suppressive activities of myeloid-derived suppressor cells via TNFR2. *J Immunol* 192: 1320-1331.

Kannarkat, G., Boss, J. M., & Tansey, M. G. The role of innate and adaptive immunity in Parkinson´s disease. *Journal of Parkinson´s Disease*, 3(4), 493-514.

KINANE, D. F., SHIBA, H., & HART, T. C. The genetic basis of periodontitis. *Periodontol 2000*, 39, 91-117.

Laine, M. L, Farré, M. A, Crucius, B. A, Winkelhoff, A. V, & Peña, S. *J. Periodontol.* 2000. https://doi.org/10.1902/jop.2000.71.8.1315.

Li, Q., Li, L., Shi, W., Jiang, X., Xu, Y., et al. (2006) Mechanism of action differences in the antitumor effects of transmembrane and secretory tumor necrosis factor-alpha in vitro and in vivo. *Cancer Immunol Immunother* 55: 1470-1479.

Löe, H, Theilade E, & Jansen B. Experimental gingivitis in man. *Lic. Odont.* May 1965. https://doi.org/10.1902/jop.1965.36.3.177.

Miller, S. A., Dykes, D. D., & Polesky, H. F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*., 16(3), 1215.

Moran, Y. *et al*..Distribución de polimorfismos genéticos de interlequina-1 enindividuos de laregióncentrooccidental de Venezuela. *ActaBiolColomb*., 14(1), 185-94.

Morsani J. M. *et al*. Genetic predisposition to persistent apical periodontitis. *J Endod*., 37(4), 455-9.

Offenbacher, S., Barros, S. P., & Beck, J. D. Rethinking periodontal inflammation. *J Periodontol*., 79(8), 1577-84.

Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature. 1984,312(5996):724-9.

Petersdorf, E. W., Malkki, M., Hsu, K., et al, International Histocompatibility Working Group in Hematopoietic Cell Transplantation. 16th IHIW: international histocompatibility working group in hematopoietic cell transplantation. *Int J Immunogenet* 2013,**40**(1):2-10. **PubMedGoogle Scholar** Petersdorf EW,

Price, A. L., Spencer, C. C. A., & Donnelly, P. Progress and promise in understanding the genetic basis of common diseases. *Proc R Soc B.*, 282(20151684), 1-10, 2015.

Qidwai, K. (2011). Tumour Necrosis Factor Gene Polymorphism and Disease Prevalence. *Scandinavian Journal of Immunology*, 74(6), 522–547.

Rankinen, T., Perusse, L., Gagnon, J., Chagnon, Y. C., Leon, A. C., Skinner, J. S., et al. Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. *J Appl Physiol*. 2000,88:1029-35.

Salmond, R. J., & Zamoyska, R. The influence of mTOR on T helper cell differentiation and dendritic cell function. *Eur J Immunol*., 41, 2137-41.

Seckinger, P, Isaaz, S, & Day, J. M. Purification and Biologic Characterization of a Specific Tumor Necrosis Factor a Inhibitor. *The Journal of Biological Chemistry*. 264(20), 11966-11973.

Shlomchik, M. J., & Weisel, F. Germinal center selection and the development of memory B and plasma cells. *Immunol Rev*., 247(1), 52-63.

Silva, L. Stem Cells in the Oral Cavity. *Glob J Stem Cell Biol Transplant*., 1(1), 12-6, 2015.

Stanulla, M., Chhalliyil, P., Jani-Sait, S. N., & Aplan, P. D. Mechanisms of MLL gene rearrangement: site-specific DNA cleavage within the breakpoint cluster region is independent of chromosomal context.*Hum Mol Genet*. 10(22), 2481-91.

Varmus, H. Getting ready for gene-based medicine. *N Engl J Med*., 347(19), 1526-7.