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IMMUNOMODULATION AND CLINICAL BIOMARKERS: A REVIEW

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ABSTRACT

Immune modulation is a fast growing area that includes the modulation of host immune response by biological agents, nutritional intervention, and pharmaceutical substances. This exhaustive analysis integrates progress in various associated fields including immunology, microbiology, haematology and clinical biochemistry, based on a body of research undertaken mostly in the Kurdistan region of Iraq and international colleagues. Immunomodulatory effects of mushrooms (*Ganoderma lucidum*) and cultivated edible species on natural killer (NK) cells and broader immune pathways; the role of vitamins B6 and C and certain minerals in boosting immune function and lowering the risk of obesity; antibiotic resistance in Gram-negative pathogens; advancements in viral diagnostics; host immune responses to pathogenic fungi; the impact of lifestyle, nutrition, and chronic disease on inflammatory and biochemical biomarkers; prevalence studies of COVID-19 and anaemia; and molecular-genetic techniques in clinical diagnosis. Collectively, these discoveries provide a multi-dimensional view of immunological health and illness, with implications for pharmaceutical development, public health and precision medicine.

KEYWORDS: Immune modulation, Biological agents, Mushrooms, Antibiotic resistance, ESBL, Renal failure.

1. INTRODUCTION

The first line of defense in multicellular animals, directing the response to a variety of infectious pathogens, neoplastic changes and environmental insults is immune system. The notion of immunomodulation, defined as the deliberate amplification, reduction or

normalisation of immunological function, has attracted a great deal of interest in basic science and clinical medicine during the past few decades.[1,13,14] Immunomodulatory therapies are diverse, including naturally occurring substances such as medicinal mushrooms and plant extracts, manufactured pharmaceuticals, dietary micronutrients, and state-of-the-art nanotechnology-based drug delivery systems.[6,8,10]

Simultaneously with advancements in immunomodulation, clinical microbiology and infectious disease confront challenges posed by antibiotic resistance, the advent of novel pathogens, and diagnostic complexities associated with viral and fungal infections.[3,4,5] The COVID-19 pandemic has underscored the critical necessity for rapid and dependable diagnostic systems and a comprehensive understanding of host-pathogen immunological interactions.[27,28,29]

This study consolidates findings from extensive researches on immunological, microbiological, haema tological, biochemical, and epidemiological dimensions. The objective is to deliver a comprehensive narrative that connects laboratory research with clinical practice, particularly for populations in the Kurdistan Region of Iraq and broader global health contexts.

2. IMMUNOMODULATORY MECHANISMS

A wide array of molecules derived from plants, fungi, animals, and microorganisms, as well as synthetic biologics such monoclonal antibodies and cytokines, can influence immune function as biological agents. These medications exert their effects by several mechanisms, including the stimulation or inhibition of cytokine production, regulation of T-cell and B-cell activity, modification of the phagocytic activity of macrophages and neutrophils, and influence on the cytotoxicity of natural killer (NK) cells.[1,13] A complete list of all immunomodulatory drugs can be broken down into two main groups. Immunostimulants are substances that make the immune system work better. They are most useful in people who don't have strong immune systems. People with autoimmune diseases and people who have had organ transplants use immunosup pressants.[1,14]

2.1 Defense and Detection of Viral Infection

The basic foundation of both innate and adaptive immunity is represented by leukocytes. One key way to prevent and fight viral infections is to boost the formation and activity of white blood cells including lymphocytes, neutrophils, monocytes and NK cells.[16,34] The

implementation of specific immunostimulatory strategies has demonstrated an increase in both the quantity and functional efficacy of leukocytes, hence enhancing the organism's resistance to diverse viral infections.[14,16] The pathway of immunological regulation is particularly sensitive to lifestyle factors. Dysregulation of leukocyte populations and suppression of significant immunological indicators have been linked to smoking, poor diet, chronic stress, and physical inactivit.[32,33,42] However, rigorous dietary interventions and micronutrient supplements can improve or restore immunological function. This is a flexible and economical approach to illness prevention.[13,42] The COVID-19 pandemic of SARS-CoV-2 affected the inhabitants of the Middle East, notably the Kurdistan Region of Iraq, in a severe way. The research in the Raparin district reported on the prevalence, distribution, and determinants of COVID-19 infection among this community. These studies provide crucial baseline epidemiological data and identify high-risk population groups, enabling public health response and resource allocation.[27,28,29]

Patterns of transmission congruent with global observations, including household clustering and age-stratified severity, were demonstrated by survey investigations in the Raparin administrative area. Documentation of COVID-19 factors in this regional scenario adds to the broader epidemiological literature and underscores the difficulty confronted by health services in resource-limited settings under pandemic conditions.[29]

The host immune response is strongly linked to the pathogenesis of COVID-19. Mild disease is associated with a successful clearance of the virus by a well-orchestrated innate and adaptive immune response, while severe disease is associated with dysregulated immunological activation – the so called ‘cytokine storm’ leading to acute respiratory distress syndrome (ARDS) and multi-organ failure.[14]

The immunological foundation of COVID-19 severity is helping to steer therapy development, from corticosteroids for hyperin flammation to monoclonal antibodies that neutralise the spike protein. Ongoing research is important to develop immune modulation techniques that can reduce excessive inflammatory responses while maintaining antiviral immunity.[1,14] The correct and prompt detection of viral infections is key to proper clinical care and infection control. Traditional diagnostic approaches, including virus culture, serological assays and antigen detection, have been increasingly complemented and in many cases replaced by molecular approaches.[4]

Viral diagnostics have seen advancements in methods ranging from traditional PCR to real-time quantitative PCR (RT-qPCR), next-generation sequencing (NGS), and CRISPR-based diagnostic platforms. Genomic technologies in particular have transformed our ability to characterise viral diversity, follow transmission chains and identify emerging variations with pandemic potential. The COVID-19 pandemic highlighted the need for quick diagnostic scale-up, with RT-qPCR becoming as the gold standard for detecting SARS-CoV-2 internationally.[4]

2.2 Importance of Vitamin D

Vitamin D has a crucial role at the intersection of nutrition and immunology. Vitamin D receptors are present on various immune cells, including T lymphocytes, B lymphocytes, and antigen-presenting cells, in addition to their traditional role in regulating calcium and phosphorus. Serum vitamin D levels significantly influence immunological markers in healthy guys, especially the equilibrium between pro-inflammatory and anti-inflammatory cytokines.[36]

Reduced levels of vitamin D are associated with increased vulnerability to respiratory infections, autoimmune illnesses, and chronic inflammatory conditions. Consequently, methods for supplementing to sustain adequate serum 25-hydroxyvitamin D concentrations present a possible adjunct to conventional immunotherapy regimens.[14,36]

3. *GANODERMA LUCIDUM*

One of the most thoroughly investigated medicinal mushrooms *Ganoderma lucidum* has amassed a great evidence base supporting its immunomodulatory activities. Bioactive components of G. *lucidum* such as polysaccharides (especially beta-glucans), triterpenoids, and proteoglycans have been demonstrated to greatly increase NK cell activity, a key component of innate antitumor and antiviral immunity.[12]

NK cells are large granular lymphocytes that can eliminate virally contaminated and malignant cells without prior sensitisation. The immunostimulatory effect of G. *lucidum* on NK cells is facilitated through various pathways. This encompasses the upregulation of activating receptors, enhanced synthesis of perforin and granzymes, and expedited secretion of interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α). These results suggest that G. *lucidum* is a promising option for inclusion in current immunotherapy strategies, especially in the case of cancer and persistent viral infections.[12]

3.1 Immunological Activities

In addition to Ganoderma, numerous other kinds of cultivated edible mushrooms have demonstrated significant medicinal and immunological properties. Immunomodulatory polysaccharides, ergosterol, a precursor to vitamin D, and a variety of antioxidant phenolic compounds are found in species like *Lentinula edodes*, *Pleurotus ostreatus*, and *Agaricus bisporus*.[6]

Edible mushrooms have been shown to possess therapeutic activities such as immunosti mulation, anti-inflammatory effects, antibacterial properties, hepatoprotection, and potential antidiabetic benefits. The multifunctional attributes of edible mushrooms render them significantly valuable in both traditional medicine and the development of functional foods, nutraceuticals, and pharmaceutical formulations. Their extensive cultivation renders them accessible and sustainable sources of bioactive immunomodulatory compounds.[6,7]

3.2 Truffles

Tubers and analogous species, such as truffles, have historically been valued for their gastronomic applications; however, recent studies suggest they may also possess health benefits. Truffles are nutritionally abundant in proteins, unsaturated fatty acids, polysaccharides, and many bioactive compounds, including phenolics and sterols. These compounds exhibit antioxidant, anti-inflammatory and potentially immunomodulatory properties.[15]

The importance of truffles for human health also includes their antibacterial qualities, with extracts showing effectiveness against harmful bacteria and fungus. Truffles are an emerging source of bioactive compounds with potential use in integrative medicine and health promotion with an increasing attention on the functional food concept.[15]

4. VITAMINS AND MINERALS

Micronutrient status has a major effect on immunological competence. Vitamin B6 (pyridoxine) is vital for lymphocyte proliferation, antibody production and cytokine generation. Deficiency results in reduced T-cell function and antibody responses. Ascorbic acid (vitamin C) stimulates the phagocytic activity of neutrophils and macrophages, boosts B and T lymphocyte proliferation and differentiation, and acts as a potent antioxidant to protect immune cells from oxidative damage.[2]

In animal models, integrated supplementation of vitamins B6 and C along with specific minerals including iron, zinc, and selenium has demonstrated synergistic immunomodulatory effects that outperform therapy with individual vitamins. This combination is significantly associated with a reduction in obesity-related metabolic risk factors, underscoring the strong, reciprocal relationship between immunological function and metabolic health.[2]

5. MICROBIAL ENZYMES AND PROPERTIES OF SOME PLANTS

5.1 Beta-Lactamase

Antimicrobial resistance is a worldwide concern and one of the greatest threats to public health in the 21st century. A wide range of Gram-negative bacteria develop beta-lactamase enzymes that give resistance to beta-lactam antibiotics by hydrolysing the beta-lactam ring, so making the drugs ineffective.[3,21]

The presence of resistance determinants in ambient water sources was discovered through the molecular characterisation of beta-lactamase genes in *Klebsiella* spp. isolated from the Euphrates River in Iraq, underscoring the significance of aquatic ecosystems as distributors and reservoirs of antibiotic resistance genes. The discovery of blaTEM and blaSHV mutations in environmental Klebsiella highlights the need for One Health surveillance methods that incorporate environmental, animal, and human monitoring.[3]

5.2 Extended-Spectrum Beta-Lactamases (ESBLs)

Therapy options are severely limited by extended-spectrum beta-lactamases (ESBLs), a more sophisticated form of beta-lactamase activity that confers resistance to monobactams and third-generation cephalosporins. The therapeutic importance of ESBL producing organisms is increased by the synergistic role of ESBL synthesis with bacterial structural characteristics such outer membrane porins, efflux pumps and biofilm development.[21]

To maintain the efficacy of carbapenems and other antibiotics of last resort, the emergence of ESBL-producing enterobacteriaceae in the clinical setting necessitates high detection diagnostic methods and careful antimicrobial stewardship. The spread of ESBL-encoding plasmids in clinical isolates has been characterised using PCR-based molecular epidemiology.[21]

In addition to investigations on antibiotic resistance, research on plant-derived antimicrobial chemicals provides interesting possibilities for new medicinal treatments. Extracts of oak and

oak gall are known to have a strong antibacterial activity against certain pathogenic bacteria including Gram-positive and Gram-negative bacteria. The primary phytochemicals responsible for the bactericidal actions include tannins, gallic acid and ellagic acid and they exert their effects by affecting cell membrane integrity and inhibiting critical enzyme activities.[18]

Likewise, preparations of mint (Mentha spp.) have inhibitory action against various pathogenic microorganisms [19], and pomegranate (*Punica granatum*) extracts have been demonstrated to inhibit bacterial viability due to their high amount of punicalagins, ellagic acid and flavonoids. These results are promising for future studies on plant extracts as adjuncts or alternatives to existing antibiotics, especially in view of increasing antimicrobial resistance.[20]

6. FUNGAL INFECTIONS AND THERAPEUTIC METHODS

Pathogenic fungi are an increasing clinical problem, particularly in immunocompromised populations such as HIV/AIDS, transplant recipients and chemotherapy patients. Responses to fungus include innate responses such as pattern recognition involving Toll-like receptors and dectin-1 and adaptive responses such as Th1 and Th17 cells.[5]

Modern antifungal agents for invasive fungal infections include azoles, echinocandins, and polyenes, which act on different aspects of fungal cell biology. However, the appearance of resistant strains, especially in Candida and Aspergillus spp., demands the development of new antifungal medicines. Promising areas for future research include immunotherapeutic strategies that increase host antifungal defences, such as granulocyte transfusions and antifungal vaccinations.[5]

7. RENAL FAILURE AND MEDICAL PARAMETERS

End-stage renal disease (ESRD) and chronic kidney disease (CKD) are linked to immunological dysfunction, which is marked by immunosuppression, heightened vulnerability to infections, and persistent inflammation. Tumor necrosis factor-alpha, interleukin-6 (IL-6), C-reactive protein (CRP), and immunoglobulin levels are important immunological biomarkers that are changed in renal failure.[11]

Monocyte activation, neutrophil and NK cell activity, and lymphocyte proliferation are all significantly impacted by the uremic environment. Among the significantly elevated

cardiovascular and infectious morbidity in dialysis users are such immunological disorders.[11,25,26]

Despite being life-sustaining, haemodialysis causes a unique collection of haematological and biochemical abnormalities. Dialysis has a clinically significant impact on haematological parameters, such as haemoglobin, haematocrit, red cell indices, white cell counts, and electrolyte balance, which calls for close observation.[25] Studies comparing low-flux and high-flux dialysis membranes show that they have differing effects on the occurrence of dialysis-related problems and dialysis adequacy (Kt/V). The rate of blood flow during dialysis is directly correlated with solute clearance efficacy and patient prognosis. Optimizing dialysis parameters based on individual patient physiology is a crucial method to enhance the quality of care for patients with end-stage renal disease.[26]

A prevalent haematological disorder with a multifaceted etiology is anaemia. Research in the Kurdistan Region indicates that certain subpopulations, including youth in the Ranya area and female athletes in Sulaymaniyah, exhibit a significant prevalence of anaemia, particularly iron deficiency anaemia.[23,24,39]

The concurrent demands of monthly iron depletion and heightened iron utilisation associated with endurance training render iron deficiency particularly clinically relevant in female athletes.[23,24]

In adults, chemotherapy-induced anaemia has extra pathophysiologic complexity, including reduction of erythropoiesis, chronic illness anaemia physiology, and dietary shortages. Management options include erythropoiesis-stimulating agents (ESAs), iron supplements, and in selected situations, red cell transfusion.[22]

Inflammatory indicators and biochemical parameters are significantly impacted by modifiable lifestyle variables such as smoking, physical inactivity, and food preferences. Research on male smokers shows markedly different levels of oxidative stress markers, lipid profiles, and inflammatory biomarkers like CRP, fibrinogen, and interleukin-1 beta when compared to non-smokers.[32] In some areas of Iraq, hookah smoking is prevalent among students and is associated with similar or higher levels of tobacco-related toxicants as cigarette smoking, with similarly observed effects on inflammatory and oxidative biomarkers. Therefore, public health measures targeting tobacco use in young people are critically

important to reduce the long-term burden of chronic inflammatory illness.[33] There is a complex, multidirectional relationship between immune health and dietary practices. Immunological dysregulation is associated with both undernutrition and overnutrition. Undernutrition reduces secretory immunoglobulin and lymphocyte function. A levels, whereas elevated levels of circulating interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-alpha show that obesity is linked to the promotion of chronic low-grade inflammation.[42]

8. SOME MOLECULAR GENETIC TOOLS

Molecular genetic approaches revolutionized clinical diagnostics by providing exact identification of genetic variations, chromosomal aberrations and genotyping of pathogens. Genetic techniques to isolate and analyse chromosomes including karyotyping, FISH and chromosomal microarray analysis form the basis of diagnosis of a broad spectrum of congenital and acquired genetic disorders.[30]

Certain variants linked to bleeding diseases have been genotyped using PCR-based techniques, such as inverse-shifting PCR (IS-PCR). The feasibility of modern molecular diagnostics in regional healthcare settings has been demonstrated by the successful genotyping of intron 1 and intron 22 inversions in the factor VIII gene, the most common mutations causing severe haemophilia A, in Kurdish patients in Iraq.[38] Epigenetic alterations including DNA methylation, histone modification and non-coding RNA expression are essential regulators of immune gene expression and have been progressively implicated in the etiology of autoimmune illnesses. Hashimoto's thyroiditis (HT) is the most prevalent autoimmune disease of the thyroid. HT is caused by loss of immunological tolerance to thyroid antigens, which results from a combination of hereditary predisposition and epigenetic dysregulation.[31]

9. SOME APPLICATIONS OF NANOPARTICLES

9.1 Bone Structure

Regenerative medicine has benefited greatly from nanotechnology, especially in the area of bone tissue engineering. Osteoinductive growth factors, antibiotics, and anti-inflammatory drugs can be delivered to the site of bone repair or defect in a targeted and sustained manner using nanoparticle-based pharmaceutical delivery techniques.[8]

Hydroxyapatite nanoparticles, which imitate the mineral phase of bone, polymeric nanoparticles, carbon nanotubes, and nanostructured scaffolds, which offer both structural support and bioactive cues for osteogenic differentiation, are significant nanotechnology platforms utilised in bone tissue engineering. In terms of biocompatibility, biodegradability, and controlled drug release, these techniques provide significant advantages over traditional bone grafting techniques.[8]

9.2 Agriculture

Nanotechnology is very promising, but its use in agriculture causes valid worries about human health and safety of environment. Nanomaterials employed in agrochemical formulations including nano-herbicides, nano-pesticides and nano-fertilizers may survive in soil and water settings with potential for bioaccumulation in the food chain. Nanotoxicological research demonstrates that specific nanoparticles, influenced by their composition, size, shape, and surface chemistry, can provoke oxidative stress, genotoxicity, and the disturbance of soil microbial communities.[10]

10. REPRODUCTIVE DISORDERS

The immune system must tolerate the semi-allogeneic fetus during pregnancy in order to sustain defense against infection, which is a remarkable feat. Dynamic changes in cytokine profiles, with a predominance of regulatory and Th2-type responses, especially around mid-gestation, and a shift toward a more inflammatory phenotype at term to aid parturition, characterize the gestational immune milieu.[35]

Progesterone-induced blocking factor (PIBF) and human chorionic gonadotropin (hCG) are two examples of immunosuppressive molecules that are produced during pregnancy. Other important immunomodulatory mechanisms include the growth of regulatory T cells (Tregs), changes in NK cell populations in the decidua, and modulation of complement activity. Pregnancy problems like repeated miscarriages, hypertension, and premature birth are linked to dysregulation of these gestational immunological adaptations.[35]

Approximately 50% of infertility cases in couples are attributable to male causes, with sperm DNA fragmentation identified as a crucial etiological factor inadequately assessed by conventional semen analysis.[40] Assessment of sperm DNA integrity by methods such as the sperm chromatin structure assay (SCSA), TUNEL test and Comet assay gives clinically useful information on the potential for fertilization and pregnancy outcomes.[40]

Two immunological components of male infertility are dysregulation of local testicular immune privilege and anti-sperm antibodies (ASA), which can impede sperm motility and cervical mucus penetration. The examination of both immunological markers and DNA integrity provides a comprehensive evaluation framework for the infertile male.[40]

Reproductive conditions such endometriosis, polycystic ovarian syndrome (PCOS), and repeated implantation failure have important immunological components in addition to infertility. The evaluation of immunological and physiological biomarkers in patients with reproductive disorders, encompassing natural killer cell activity, cytokine profiles, and hormonal assays, establishes a foundation for comprehending the immune contributions to these conditions and pinpointing potential therapeutic targets.[41]

11. DISCUSSION

The corpus of research reviewed here constitutes a unified and growing programme of scientific investigation, extending from the basic biology of immune control to the clinical therapy of complicated disease states. From this synthesis many themes arise that are overarching.

First, there is proof that immunological competency is significantly influenced by nutritional and lifestyle factors. Immune function is significantly impacted throughout the life course by modifiable factors, such as micronutrient supplementation of vitamins B6, C, and D, dietary adjustment of inflammatory markers, or avoidance of smoking and sedentary behavior. These results directly affect public health initiatives and preventative medicine.[2,13,32,36,42]

Second, a class of naturally occurring immunomodulatory drugs with significant therapeutic promise is represented by medicinal mushrooms, including *Ganoderma lucidum* and developed edible species.[6,12] They are appealing candidates for incorporation into evidence-based integrative medicine frameworks due to their diverse biological activities, which include NK cell activation, anti-inflammatory action, antioxidant qualities, and possible anticancer effects. This mycological therapeutic landscape is further expanded by the recognized importance of truffles for human health.[15]

Third, antimicrobial resistance necessitates a coordinated One Health response that includes increased surveillance, prudent antibiotic use, and quick development of alternative

antimicrobial strategies, including plant-derived compounds, whether it is caused by beta-lactamases in environmental and clinical isolates or amplified by ESBL production.[3,18,19,20,21]

Fourth, the COVID-19 pandemic has offered an unmatched lens through which to study the epidemiology of infectious diseases in local contexts, host-pathogen immunological dynamics, and diagnostic innovation. Both pandemic preparedness and the more general treatment of respiratory virus infections are informed by the lessons learnt.[27,28,29]

Fifth, nanotechnology-based platforms have the potential for transformative impact in medication delivery and tissue engineering, but also present serious environmental safety concerns that require careful management through thorough risk assessment and regulatory oversight.[8,10]

Lastly, the shift toward precision medicine approaches that take into account individual genetic variation and the epigenetic landscape in disease diagnosis and management is demonstrated by genetic and epigenetic insights from molecular diagnosis of haemophilia A in Kurdish patients to epigenetic profiling in Hashimoto's thyroiditis.[31,38]

CONCLUSION

Characterization of natural immunomodulatory agents derived from medicinal mushrooms and plant extracts, documentation of antimicrobial resistance in environmental and clinical contexts, advancements in viral diagnostics and epidemiological surveillance, especially in the context of Covid-19, haematological insights in chronic kidney disease and anaemia, and the use of molecular-genetic tools for clinical diagnosis in underserved populations are some of the field's major contributions. Future studies should therefore focus on understanding the mechanistic basis of natural product immunomodulatory pathways, developing safe nanotechnology-based therapeutic platforms, and applying precision medicine approaches by integrating genomic, epigenomic and immunological profiling in clinical practice. Strengthening diagnostic and research facilities in the Kurdistan Region of Iraq and other similar contexts will be key to realizing these aims and converting scientific findings into tangible health gains in the public.

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