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Review Article

Recent Advances on Nucleolar Functions in Health and Disease

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Abstract

The nucleolus is an internuclear organelle without a visible membrane via the light microscope inside the cell nucleus. It is the main site for synthesis of ribosome as a complex machine for coordinating protein production. It forms around a specific chromosomal feature called the nucleolar organizing region (NOR) which possesses numerous ribosomal DNA (rDNA). Although the nucleolus is best known as coordinator of ribosomal biogenesis and protein synthesis, recently, there is exciting awareness both on better understanding of ribosome biogenesis and non-ribosomal nucleolar functions. A great amount of research has clearly indicated that the nucleolus has functional activities in both ribosomal and non-ribosomal conditions such as development, aging, cell cycle, gene stability, lifespan regulation, and progeria. Through recent sophisticated and advanced technologies such as genomics, proteomics, metabolomics, advances of knowledge in RNA species and new approaches in microscopic analysis methods, researchers have shown that perturbation in the nucleolar structure and function (nucleolar stress) have been associated with human diseases including cancer, viral infection, cardiovascular and neurodegenerative diseases. In this review, we discuss the impact of current research providing new information regarding nucleolar roles and functions in some human diseases and aging.

Keywords: Aging, Biogenesis, Human diseases, Non-ribosomal nucleolar function, Nucleolus

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Introduction

The nucleolus, as an intranuclear component in eukaryotic cells, was first described in the 1830s by two German physiologists; Rudolph Wagner (1835)[1] and Gabriel Valentine (1836)[2] in two separate publications. In 1896, Giuseppe Pianese, an Italian pathologist noticed the importance of its excess volume in malignant cells.[3] The size of the nucleoli and increase in their number, though not a true indicator of malignancy, reflected hyperactivity and proliferative stages of cells.[4,5] The nucleolus can be easily visualized by a conventional light microscope and phase contrast light microscope in living cells. In hematoxylin-eosine stained histopathological sections from routinely processed tissues, the nucleolus is intensely stained (Figure 1).[6]

The nucleolus is specialized and is the most prominent visible nuclear organelle, generally spherical and highly basophilic. The basophilia of the nucleoli is due to the presence of densely concentrated ribosomal RNA (rRNA) but not the result of heterochromatin. rRNA is transcribed, processed and complexes with ribosomal subunits of the nucleolus. Chromosomal regions with genes for rRNA, organize one or more nucleoli in the cell as large and complex nucleoprotein machines, necessitating intense ribosome production for synthesis of proteins during growth or secretion.[7,8]

In the nuclear components, the nucleolus takes up to 25% of the volume of the nucleus. Previously, the nucleus was considered as the 'brain' of the cell, but now it is though that the nucleolus could be the 'brain' of nucleus. Structurally, the nucleolus is made of proteins and ribonucleic acid. At the electron microscope level, the nucleolus at the interphase stage exhibits three major components which are called three classic architecture (tripartite architecture). They are composed of a fibrillar center (FC), appear as roundish structures with various sizes and low electron opacity, surrounded by dense fibrillar components (DFC), intimately associated with FC and embedded in a granular component (GC) composed of granules surrounding the fibrillar components. FC deals with rRNA transcription and has unengaged pools of RNA polymerase I (RNA Pol I) transcription factors. DFC is composed of pre-rRNA processing factor and GC is the site for pre-ribosomal assembly.[6,9-11] Non-ribosomal nucleolar proteins are localized in these components and other sites. Dividing cells often show large nucleoli and the size and the number of the nucleolus correlates with rRNA biogenesis and other non-ribogenic protein synthesis.[12] During cell division; at the prophase stage, the nucleoli are taken apart and at the end of mitosis; during the metaphase stage, they are re-assemble around a specific region known as the nucleolar organizing region (NOR). In this process, some factors remain associated with NOR, whereas some others move to the periphery of chromosome or released.[9,13]

Experts in nucleolar studies found that though the nucleoli are very small, nevertheless, they are extremely intelligent and sometimes have great effects on cell pathology and physiology of human beings. Although it is an integrated

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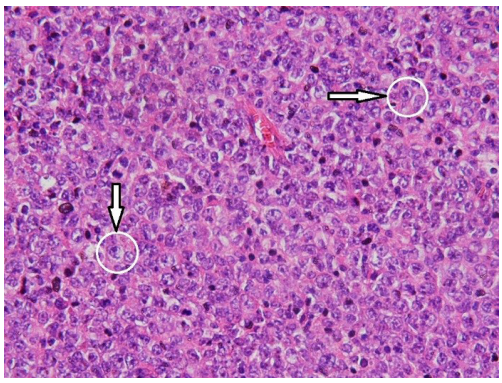

Figure 1. Hematoxylin and Eosin (H&E) Stained X400 of a Case of Malignant Lymphoma. Arrows show the nucleolus (Courtesy of Dr. A. Droodinia, Masih Danshvari Hospital, Tehran, Iran).

part of the cell's eukaryotic nucleus, it plays by its own rules in many respects.[14,15] Ahmad and colleague reported an updated 2008 proteome database of the nucleoli. They identified more than 50 000 peptides composing over 4500 human proteins. The data was detected from purified nucleoli, providing intense constituents of the nucleolar proteins.[16]

Pappu and Harmon, the researchers at the Washington University School of Engineering and Applied Science with collaboration of Brangwynne's Laboratory at the School of Chemical and Biological Engineering of Princeton University, developed a computational modeling of molecular architectural key proteins and RNA molecules which are encoded in the nucleolus. They have interactions which are sufficient to explain the spatially organized substructures of the nucleolus. By this model, they found a new understanding into the form and function of a cell's nucleolus which may lead to new ways to treat disease, suggesting that the nucleolus machinery is like a factory.[17]

More awareness on RNA species, recent development on genomics, proteomics, metabolomics technologies, high resolution electron microscopy and functional proteomic studies have affirmed many activities of cells and their components.[7,18-20] For more than two decades, direct proteomic analysis of the human nucleolar proteome has been introduced by investigators.[21] As a result of these new investigations, more information has been gathered on nucleolus functions; particularly about its regulatory processes which are unrelated to ribosome biogenesis.[22-24]

In this review, we searched Google scholar, Scopus and Medline databases and briefly discuss these established activities of the nucleolus in some diseases. At the beginning, we focus on the classic activity of ribosomal biogenesis.

Protein Synthesis and Ribosome Biogenesis of the Nucleolus

The crucial role of the nucleolus is ribosome biogenesis and production of protein. This process, which is regulated at different stages by many factors, starts with transcription of rRNAs in the nucleolus through action of RNA polymerase I to generate 47S pre-rRNA. This in turn becomes activated, modified and cleaved to form derivatives of rRNA including 28S, 18S and 5.8 S rRNAs. The species of rRNAs, by specialized protein processing complexes, are further processed to their maturation state; mature 28S, 18S and 5.8S rRNA. Through transcription by RNA polymerase II (RNA pol II), the species are assembled with ribosomal proteins to 5S rRNA and then in the nucleus are transcribed by RNA polymerase III (RNA Pol III) and finally exported from the nucleus to the cytoplasm to give rise to cytoplasmic ribosomes which acts as the central players of translation of mRNA into proteins. In this manner, due to the vital role of nucleolus in protein synthesis, it actively determines the metabolomic state of a cell.[10,24,25]

Interestingly, there are also non-conventional biogenic roles of the nucleolus in physiological conditions. Boisvert and colleagues reported the nucleolar mechanisms to control embryonic development, stem cell differentiation, nucleolar transport of the cell cycle regulators and transcription factors dictating cell linkage to the nucleoloplasm. These mechanisms have great role on accomplishment of the differentiation program.[26]

The Nucleolar Functions in Cell Homeostasis and Disease

The nucleolus is a complex molecular machine with multidimensional roles which are actively involved in the production of many important and prominent proteins of the ribonucleoloprotein family.[27,28] Analysis, through proteomics, has shown that only around 30% of the nucleolar proteomes deal with conventional ribosome biogenesis.[9,23,28] This fact revealed that the remainder of molecular products are involved in a number of cellular processes.

Many nucleolar proteins such as nucleolin (Ncl), nucleostemin (NS), nucleophosmin (NPM) and fibrillarin (Fbl) are engaged in both cell homeostasis and disease. Stem cell pluripotency maintenance, telomere length regulation, inhibition of stem cell differentiation, senescence and cell death are among the pivotal roles played by NS protein.[29,30] Several studies performed in the past two decades indicated that both quantitative and qualitative structural functions of the nucleolus have significant roles in various cellular functions and homeostasis. For example, diseases associated with deregulated ribosomal biogenesis are the result of functional mutation in the nucleolar constituent of the ribosome or factors closely associated with polymerase I (Pol I) transcription and processing, which are collectively named ribosopathies.[31,32]

At the beginning of this century, Raska et al disclosed that the nucleoli, besides being ribosome factories, have other multipurpose functions. They found that among these are its involvement in mRNA export or degradation, biosynthesis of the signal recognition particles (SRP), biogenesis of some snRNAs and tRNAs, the sequestration of regulatory proteins, control of the cell cycle in aging and stress sensing. Intensive proteomics research on the nucleolus in yeast was the major source of this progress.[7]

Nucleoli form around the NORs on acrocentric short arm of the chromosomes 13, 14, 15, 21, and 22. Each contains

clusters of approximately 300 ribosomal DNAs (rDNAs), tandemly repeated genes for rDNAs, mostly arranged in head to tail manner.[33,34] Several NORs can be found within a species and each is located in a separate chromosome. By light microscopy and silver staining, we can detect these molecules as silver positive NORs particles (AgNOR). Their number and size correlate with cell proliferation activities and tumors[33-37] and accordingly can be used as an indicator for detection of malignant tumors. Human cells have AgNOR on the acrocentric region of the short arms of the five mentioned chromosomes.[7,34,35]

In some human diseases, using proteomics mass spectrometry based analysis have shown that many proteins set in different compartments of the nucleolus play various roles in cells performances.[22,38]

Nucleolar micro RNA (miRNA)

The RNA constituent's analysis of the nucleolus also showed a novel finding: the identification of micro RNA in the nucleolus. microRNAs (small short non–coding RNAs) are genome encoded small RNAs, around 22 nucleotides, generated via canonical and non-canonical pathways.[39] The finding of the nuclear and nucleolar targeted miRNA species and demonstration of RNA interference (RNAi) and other species of miRNAs, which can function in the nucleolus, have been newly discovered. It was previously thought that microRNAs are only acting in the cytoplasm and have cytoplasmic role in mRNA transcription. Today, it has been discovered that in addition to cytoplasmic action, microRNAs have great role in the nucleolus. Different species of miRNAs have been found in the nucleolus and functional RNAi has been shown to be active in the nucleoli. A significant number of small non-coding RNAs (ncRNAs), regulating mRNA translation, are situated in the GC of the nucleolus.[9,11,40-42]

Nucleolar Promoting Genome Stability

In response to adverse growth conditions, metabolic deficiency, and oxidative stress, rRNA production is down regulated by mechanisms involving transcription factors and epigenetic modification. This perturbation of the nucleolar action and integrity has been called 'nucleolar stress'.[43] The repeating structure of rDNAs with its high rate of transcription makes rDNAs highly susceptible to nucleolar stress, genomic instability and DNA damages.[10,43] The nucleolus reacts against these DNA damages by activating diverse signaling pathways.[23,43] DNA-damaging response (DDR) proteins which operate in the nucleus also play some nucleolar repairing role as well. Upon DNA damage, a number of DDR accumulate in the intranucleolar body (INB) and the nucleolar cap structure, providing a platform for employing specific factors that sense rDNA damages and repair them in the nucleolus and perform genome stability.[44]

Nucleolus Connection to the Cell Cycle and Lifespan

Numerous studies have shown connection of the nucleolus to cell cycle regulation. Perturbing ribosomal synthesis and nucleolar homeostasis triggers a prompt arrest of cell cycle progression.[27,45] Activation of P53 can be achieved through inhibition of RNA pol I activity produced by DNA damage in the human cells, which has significant influence on activation of P53. The p53 pathway also can be activated by a direct disturbance of the nucleolar architecture.[46-48] Nucleolar stress condition caused by perturbation of ribosome biogenesis or interruption of the nucleolar architecture, elicits a surveillance system, leading to rapid activation of p53 and cell cycle arrest. This may trigger G1/S or G2/M arrest.[27] Effects of other subunit proteins which have links with nucleosomal cell cycle regulation, such as 5S rRNA and C-MYC, have been reported. Hannan and his team reviewed mutations in those proteins that have interaction with Pol l complex and regulate rRNA transcription and their roles in human disease.[31]

Studies on the detailed function of the nucleolar role in lifespan and aging have contributed to new insight to the nucleolar mechanisms behind longevity, senescence and progeria.[49-52] It has been shown that aging is regulated by nutrient-sensing pathways through activation of the insulin/insulin-like growth factor signaling pathway. RNAi splicing factor leads to partial loss of this signaling pathway.[10] Researchers involved in aging phenomenon have long been investigating for a biomarker factor of aging to predict health and longevity. Varnesh Tiku, a scientist at the Max Planck Institute, Division of Biology of Aging in Germany, has recently, by studying long lived mutant of a roundworm "Caenohabditis elegans", discovered that those roundworms with mutant genes had smaller nucleoli than their shorter lived relatives. The same researchers in another experiment on humans analyzed muscle biopsies from individuals older than sixty years old. They underwent modest dietary restriction coupled with exercise, a common way for prolonging lifespan and increasing health. They found that cells in their muscle biopsy had smaller nucleolus after intervention than before.[51] The same researchers and other investigators reported that the molecular disarrangement of the nucleolar proteins cause several pathophysiologic impacts including aging and lifespan.[51-55] *Nature Communication News* also published reports entitled, "nucleoli, a cellular hallmark of longevity" in three consequent years (Nature Common News 8:2016, 2017 and 2018).

Many articles published by various investigators dealing with molecular function of the nucleolus reported nucleolar functions in regards to lifespan, aging, senescence, premature aging, damage sensing pathways and nucleolar stress.[10,53] Thus, the nucleolus is not solely a housekeeping factor for ribosome biogenesis, but it also induces specific pathophysiological events more than its conventional effects as it was thought before. Alteration of the nucleolar activities is a main regulator of development, aging and disease processes.[56,57]

A list of 166 DNA damage (DDR) proteins with their nucleolar location has been declared in a human database. Ogawa and Baserga assembled this database and listed 166 DNA repair proteins that were located in the nucleolus.

The crosstalk between DDR response and the nucleolar function of making ribosomes have been discussed and a greater appreciation for the overlap of this function has been reported[58] though it should be answered whether the nucleolus is only a storage depot for DDR proteins or it actively plays specific nucleolar roles. Altogether, it has been shown that functional proteomic and genomic studies indicate that the nucleolar damage has an active role in promoting genome instability.[23,59]

Inhibition of the activity of RNA Pol I in human cells has profound effects on p53 activation.[43,48,60] As mentioned before, the p53 pathway and its great functional impacts can be also activated by a direct disturbance of the nucleolar architecture.[48] Mechanisms involved in disturbing ribosome biogenesis or disruption of the nucleolar architecture cause nucleolar stress which triggers surveillance system, leading to p53 activation and cell cycle arrest.[27] In addition, a p53-independent pathway regulates nucleolar segregation and antigen translocation in response to rDNA damage and repair.[61]

The Nucleolus Functions in Cancer

Regarding the relationship between cancer transformation and nucleolar function, two major questions arise:

1- Whether the nucleolar alternation is the result of neoplastic transformation? or
2- The up-regulation and molecular changes of the nucleolus are serving as a risk factor in cancer development?[6]

Recently, following findings are in favor of the nucleolar function as a risk factor:

The nucleolus, via its functional dysregulation, can lead to the malignant phenotype[12,25,45,56] These dysregulations include DDR, derangement in maintaining genome stability and its spatial organization, epigenetic regulation, cell cycle control, stress responses, senescence, and global gene expression.[23] Systematic and directed proteomics and genomics, including various proteome databases, showed that there are many DDRs involved in nucleolar rDNA genome stability and is the reason for their alteration in activities.[16,23] During malignant transformation, rDNA transcription rates, as a result of activation by oncogenic signaling or release from repression by tumor suppressor pathways, are up-regulated.[21,59,62,63]

Researchers have provided new insight into how deregulation in RNA Pol I activity, which may lead to tumor genesis, and suggested new drugs targeting rDNA transcription and RNA Pol 1 which may be great promise for treatment of cancer.[64,65]

The following can be highlighted regarding nucleolar function/dysregulation in cancer:

1. Protein synthesis is consistently increased in cellular neoplastic transformation.[23]
2. It produces oncogenic proteins and tumor suppressor proteins (P53, C-MYC etc.).[64-66]
3. Polymerase I transcription of rRNA genes is negatively regulated by tumor oncogenes and suppressor genes.[67,68]
4. In cancer, basal RNA polymerase transcription apparatus becomes deregulated.[69,70]
5. The nucleolus, by inhibiting polymerase transcription of rRNA gene, activates p53, pRB, C- MYC and changes the cell cycle. The nucleolus may directly regulate p53 export and cause degradation.[69,71] Expression of p53 in cancers can be detected by immunohistochemistry staining procedure.[66]
6. Performing extra-ribosomal function that contribute to malignancy[62]
7. Up-regulation of ribosome production and alterations in the ribosome structure and defect in nucleolar function in keeping homeostasis all contribute to neoplastic transformation.[6, 63,65]
8. Inappropriate rDNA repair causing chromosomal rearrangements may lead to cancer.[63]

Almost all cancer cells display large size and/or increased number of nucleoli. Indeed, the nucleolar size in some cancer cells can be used as a parameter for predicting biologic behavior of the tumor, with increasing size corresponding to worse prognosis, or to be used in classifying the tumors.[3,4,64] Several publications are dealing with tumor markers.[64] Using fluorescence microscopy, Su and his team identified a new marker protein to detect nucleoli upon severe stress and during drug treatment.[72] Inhibition of numerous nucleolar proteins and depriving them of their intrinsic dynamic nature is another research question for tumor markers and therapeutic agents.

Anticancer Activities by Targeting Nucleolar Biogenesis

Contemporary research infers that the nucleoli have a much broader role in malignant tumor transformation.[6] This action is more prevalent for extra-ribosomal functions of the nucleolus.[7] As increases in rDNA transcription is a common feature of human cancer and it depends on nucleolar suppressors and oncogene activities, thus it has become a research plan to expect new anticancer therapies which could be based on inhibition of many nucleolar tumorigenic activities including polymerase I activity.[65,62,71] Trying to develop drugs targeting RNA Pol II has produced candidates such as CX-3543 and CX-5461 which act as small molecule inhibitors.[9] Recent advances into how nucleolar malfunctioning in cellular processes become a powerful driving force for human cancers is a purpose to develop new therapeutic drugs including nanoparticles for treatment of cancer. A long list of reports targeting various small nucleolar molecules involved in tumorigenic process has been published.[23,59,62,73]

The Nucleolus and Neurodegenerative Diseases

The nucleolar stress, as a result of the cellular and molecular change accompanying impaired nucleolar activity, have also been implicated in the nervous system taking advantage of newly animal models.[11] Neuronal nucleolar stress, as a cause of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson disease, Huntington disease and many others have been among disorders which have been

investigated by researchers.[7,9,11,74] Of interest, number of studies on the nucleolar stress in neurodegenerative disease is rapidly growing.[74] Pietrzak's article showed that silencing of rDNA may occur during early stage of AD and play a role in AD-related ribosomal deficit and finally lead to dementia.[75] As a mechanism to decrease rDNA gene expression in AD patients, differential methylation activity of the rDNA has been proposed and specific methylation pattern could be used as a marker for AD and its progression.[74,75] Post mortem brain tissue revealed disruption of dopaminergic neuron in Parkinson's disease.[76]

Numerous reports recently published account for nucleolar function in various neurological disorders with particular organ involvement.[11,77]

The Nucleolus in Cardiovascular Disease

Enlargement and multiplication of the nucleolus is revealing that increased protein synthesis and growth is one of the early changes observed in hypertrophic cardiomyopathy.[30] Activity of cardiac NOR, which can be visualized by silver staining (Ag NOR), positively correlates with weight of myocardium, thickness of the left ventricular wall and maximal diastolic pressure in hypertensive heart disease, suggestive of increased NOR and nucleolar activity.[30] Nucleolar proteins such as Ncl or C23, which is a multifunctional major nucleolar phosphoprotein, NS, and has a critical role in pre-rRNA processing, NPM or B23 and FbI with important role in biogenesis, nucleolar stress and nucleolar regulatory function are mainly associated with cardiac pathophysiology.[24,30]

Autoimmune Disease and Nucleolus

Autoimmune disease is a complicated disease related to various factors including genetic, epigenetic and environmental hazards. Currently, more than 80 autoimmune diseases or related disorders have been reported, but many of these illnesses share common triggers, symptoms and pattern.[78] Several hypotheses have been proposed, and it has been discussed how the disease develop and what could be the outcome. Many explanations have been proposed for the pathophysiology of autoimmune disease. Though studies showed that they are not mutually exclusive and a combination of these hypotheses could provide a more comprehensive explanation of autoimmune diseases. Reimer and Raska proposed four distinct proteins in RNA protein complexes of the nucleolus as target of human autoimmune antibodies.[79]

As many autoimmune disease patients are female, recently Brook proposed one possible explanation for this female bias that is due to disruption of the inactive X chromosome. Brook proposed a new hypothesis of autoimmune disease based on this inactive X chromosome[78,80] as one of the two X chromosomes in each female cell become inactivated and appears as heterochromatic body. Usually it is located between the nuclear membrane and nucleolus, but it has close association with the nucleolus. The nucleoli help to maintain their inactive state. In abnormal conditions, such as cellular/nucleolar stress, the nucleolus becomes very active and can expand dramatically, engulfing the inactive silent X chromosome and thereby, a polyamine constituent present in the nucleolus could stabilize auto antigenic complexes including those arising from disruption of the inactive X chromosome. In fact, at least transiently, proteomic studies showed that many auto antigens are components of the nucleolus.[81]

The Nucleolus and Viral Disease

Viruses interact with the nucleolus and its antigen. Viral proteins co-localize with nucleolar protein factors such as Ncl, B23, 7-2 RNP, .PM-Scl and fibrillin and during infection can cause viral redistribution. During viral infection, numerous viral components localize in the nucleolus while different host components are distributed around or are modified.[82]

Nucleoli become involved in adenovirus infection and the virus affects the host cell nucleoli. Proteomics studies analyzing the adenovirus infected cells in the cell culture identified 351 proteins with 24 proteins showing at least two fold changes after infection.[83]

Arizola reported that potential therapeutic intervention in HIV-1 infection involve nucleolar trafficking small nucleolar RNA (snoRNA) which include U16. Functional inhibition of HIV-1 mediated infection by snoRNA (U16) has been reported for therapeutic purposes. It has been demonstrated that the HIV-1 rev- protein localizes to the nucleolus and interacts with nucleolar protein.[84] Targeting the nucleolus and displacing nucleolar antigen autoimmunity to Ncl and fibrillin have been associated with a number of diseases including viral infections and might play a role in the initiation of these conditions and also can be used for therapeutic purposes.[85-87]

Conclusion

Up to the year 2000, the nucleolus had been looked at as an organelle solely involved in ribosome biosynthesis. However, due to discovery of more nucleolar functional activities and a better understanding of the nucleolus molecular composition, attraction of researchers in this field was promoted.

A tremendous amount of research on the nucleolar structure, in addition to appreciation of pathological effects of the nucleolar molecules, placed the nucleolus at the top of pathological and physiological investigations. Thus, recent approaches through advanced technological methods of assessment of the molecular behavior, composition, structure and functional activities and maintenance of the nucleolus and pathological implication of their disturbed function became interesting research questions. Researchers all over the world have provided new insights into the nucleolar role as a multidimensional signaling hub which plays important role in keeping cellular homeostasis, lifespan and causes human diseases. Over or under activity of the nucleoli and nucleolar stress lead to pathologic conditions, senescence and disease.

Association of nucleolar stress, cellular dysfunction and human diseases including cancer, cardiovascular, neurodegenerative, autoimmune disorders, infectious and metabolic disorders became important research topics. In addition, identifying potentially new risk factors may also help to develop novel therapeutic modalities. It is speculated that battling diseases caused by targeting ribosome synthesis, conventional or nonconventional, and proper dynamic control of the nucleolar activity is essential for health and new therapeutic approaches for treatment of diseases.

Further Reading

We recommend referring to the following articles: Raska I, 2006, Montanaro L et al. 2008, Tiku V 2018, Lam Y, 2015, and Lindstrom MS, 2018.

Authors' Contribution

MB introduced the idea, collected data, prepared a draft of the manuscript. MHA finalized the draft by correcting the manuscript and checking the references. ShD critically read the manuscript and made revisions.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

Not applicable.

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