Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/11551
DOI URL: http://dx.doi.org/10.21474/IJAR01/11551

RESEARCH ARTICLE

REVIEW ON PATHOGENESIS, PREVENTION AND TREATMENT OF DENTAL CARIES

Isha Bisla

Library dissertation submitted in the Department of Pedodontics and Preventive Dentistry, as a part of MDS research, Swami Devi Dyal Hospital and Dental College, Barwala, Panchkula, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana, India, 132001.

Manuscript Info

Manuscript History
Received: 20 June 2020
Final Accepted: 24 July 2020
Published: August 2020

Abstract

The systemic biological functions and their coordination in a human body are very complicated but perfect to synchronize with circadian external and internal environmental changes. Similar but slightly different to other organs of human body, tooth is a unique conglomeration of soft and mineralized tissue that require controlled mineral deposition. The enamel as a hardest non vital a-cellular and a-vascular mineralized tissue, dentin as a hardest vital tissue and pulp as a specialized connective tissue lined by dentin producing odontoblasts, get self-assembled to form tooth with unique mechanical properties. The Enamel forms a very hard, thin, translucent layer of calcified tissue that covers entire anatomic crown of tooth. Roughly 95% of enamel is calcium and phosphate ions combined to make strong hydroxy-apatite crystals {Ca10(PO4))6OH2},1 to 2% of enamel is made up of organic materials, particularly enamel specific proteins, which have a high affinity for binding hydroxyl apatite crystals and remaining 4% of enamel composition is water. The paramount function of teeth is mastication and preparing food for digestion. Teeth are also essential to symmetric contour of face and agreeable expression of countenance and correct enunciation. When teeth are lost, or become dysfunctional due to some disease, a general derangement of physiologic system follows leading to many systemic and deficiency diseases.

Introduction:-

The systemic biological functions and their coordination in a human body are very complicated but perfect to synchronize with circadian external and internal environmental changes. Similar but slightly different to other organs of human body, tooth is a unique conglomeration of soft and mineralized tissue that require controlled mineral deposition. The enamel as a hardest non vital a-cellular and a-vascular mineralized tissue, dentin as a hardest vital tissue and pulp as a specialized connective tissue lined by dentin producing odontoblasts, get self-assembled to form tooth with unique mechanical properties. The Enamel forms a very hard, thin, translucent layer of calcified tissue that covers entire anatomic crown of tooth. Roughly 95% of enamel is calcium and phosphate ions combined to make strong hydroxy-apatite crystals $\{Ca_{10}(PO_4))_6OH_2\}$,1 to 2% of enamel is made up of organic materials, particularly enamel specific proteins, which have a high affinity for binding hydroxyl apatite crystals and remaining 4% of enamel composition is water. The paramount function of teeth is mastication and preparing food for digestion. Teeth are also essential to symmetric contour of face and agreeable expression of countenance and

Corresponding Author:- Isha Bisla
Address**:-** House Number 349 Sector 8 &9 Part-2 Urban Estate, Karnal 132001, Haryana, INDIA. Library dissertation submitted in the Department of Pedodontics and Preventive Dentistry, as a part of MDS research, Swami Devi Dyal Hospital and Dental College, Barwala, Panchkula, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana, India, 132001.

correct enunciation. When teeth are lost, or become dysfunctional due to some disease, a general derangement of physiologic system follows leading to many systemic and deficiency diseases.

The Enamel often gets dematerialized by acids resulting from microbial fermentation of carbohydrate food residues present in saliva, which later on leads to formation of pits and cavitations on enamel surface. Dental caries in enamel is unique amongst all diseases as enamel is both a-cellular and a-vascular. Thus, in contrast to other tissues, enamel cannot heal itself by a cellular repair mechanism (Zero,1999).[1] Nonetheless, it is now well established that formation of incipient enamel caries is a reversible process where periods of progression alternates with the periods of remineralisation (Silverstone,1977).[2]Given an appropriate change in conditions, remineralisation may even become a predominant process, leading to apparent repair of the lesion (Pearce and Moore, 1985).[3] Caries word is derived from latin, meaning 'rot' or decay and from Greek word 'ker' which means death. Dental caries according to Shafer is defined as "a microbial disease of calcified tissues of teeth, characterized by demineralization of organic portion, and destruction of inorganic portion of tooth". Dental caries is most common totally preventable, modern civilization disease, which is recognized as primary cause of oral pain and tooth loss. It is a bacterially mediated disease characterized by demineralization of tooth surface, which may lead to cavitations, discomfort, pain, and even tooth loss (Rickard *et al*., 2004; Anderson *et al*., 1993; Polydorou *et al*., 2012). [4, 5, 6] It is a major oral health problem that affects 60 to 90% of school children and a vast majority of adults (Rickard *et al*., 2004; Anderson *et al*., 1993; Mu¨ller *et al*., 2007). [4,5,7] According to World Health Organization (WHO) in 2003, dental caries was declared as major public health problem in majority of industrialized countries (Petersen *et al.*, 2003). [8] Worldwide, approximately 2.43 billion people (36% of the population) have dental caries in their permanent teeth. In primary teeth it affects about 620 million people or 9% of population and it is considered as primary pathological cause of tooth loss in children. Owing to its globally high prevalence, dental caries is a 'pandemic' disease characterized by a high percentage of untreated carious cavities causing pain, discomfort and functional limitations. Furthermore, it has a profound impact on general health, social and economic wellbeing of an individual.

Dental caries is a multi-factorial disease caused by interaction of dietary sugars, dental bio-film and the host's dental tissue within oral environment. Bacterial aggregate especially *Streptococcus mutans* and *Lactobacillus*, *Actinomyces spp.* present in dental bio-film act on carbohydrates to produce acids through a process called glycolysis thus lowering the pH of oral cavity. This acid produced by bacteria present in dental plaque leads to chemical dissolution of hard tissue structure of tooth, this process is termed as demineralization. The process of caries formation is a cycle of remineralisation and demineralization with various stages being either reversible or irreversible. A break in equilibrium causes tooth to remineralize or demineralize depending upon concentration of the mineral saturation in oral cavity at that time. Therefore, preventive and therapeutic approach must consider set of these factors. Cariogenic microorganisms colonize tooth surface and form dental bio-film. Reducing the levels of caries associated bacterial species in dental plaque is one of the preventive strategies to inhibit initiation of caries. (Lynch,1996; Johansson *et al*., 2009).[9, 10] Furthermore, to prevent secondary caries that may be related to the presence of residual bacteria under restorations, Meja`re et al.(1979) [11]and Magni et al. (2008),[12] use of an antibacterial treatment after caries removal seems to be meaningful (Polydorou *et al*., 2012). [6] To arrest caries progression, several antibacterial treatments have been proposed in order to mechanically and/or chemically reduce biofilm formation, Mu¨ller et al. (2007), [7] Banerjee et al. (2000), [13] Sbordone and Bortolaia, (2003) [14]and Baehni and Takeuchi, (2003), [15] and reduce amount of residual bacteria after caries removal (Imazato *et al*., 1998; Ozer *et al*., 2005; Wicht *et al*., 2004; Polydorou *et al*., 2006).[16, 17, 18, 19] Nowadays, to treat dental caries, pharmaceutical approaches have gained popularity. Such approaches give opportunity to treat caries without using invasive techniques such as drilling.

In physiologic conditions, the oral fluids (saliva and biofilm) present a higher concentration of calcium (Ca) and phosphate (P) in medium outside of tooth (super saturation) than in hydroxyapatite (HA), which is primary constituent of enamel structure (crystallized form of calcium phosphate). These ions are continually deposited on tooth surfaces and in areas in which demineralization processes occur. This process is known as "natural defense phenomenon" promoted by saliva to promote mineral preservation of tooth enamel (Nidhi and Kunwarjeet, 2012).[20]The loss (demineralization) and gain (remineralisation) of minerals on enamel surface is a dynamic physio-chemical process, which occurs when bacteria present in dental bio-film are exposed to a diet comprising of fermentable carbohydrates, particularly sucrose. Whenever sugar penetrates into cariogenic bio-film, it is converted into acid and bio-film fluid becomes saturated in comparison with mineral component of enamel. In this situation, pH has a low critical value, at which demineralization of enamel occurs. However, after a certain period, when sugar consumption ceases and physiological value of pH is restored, the conditions of super-saturation are re-established. At this time, reposition of a certain quantity of lost mineral occurs, in a process denominated dental remineralisation.

This reposition of lost mineral occurs by means of Ca and P ions present in bio-film fluid and saliva, right after dental bio-film removal by brushing. The quantity of Ca and P replaced is lower than the amount lost, so that small mineral losses occur (Nidhi and Kunwarjeet, 2012).[20] If factors responsible for disease (bio-film and frequent exposure to sugar) are not controlled, with passage of time, mineral loss cannot be impeded. Therefore, events of mineral dissolution will repeatedly occur, and may lead to a degree of demineralization being greater than the remineralising capacity of oral fluids. Therefore, an imbalance in these processes of demineralization and remineralisation may result in small mineral losses, only observed by electronic or optical microscopy as white spots initially and after word formation of cavities on tooth surface. Moreover, it is important to point out that caries disease progresses in a different manner among individuals, as it is a disease in which individual susceptibility is also an important factor for its progression.

For many years, dental profession was influenced by mechanical approach characterized by use of high speed rotary cutting instruments, and dentists predominantly used surgical methods to address dental caries. In the past, once an incipient carious lesion was detected, invasive techniques like "Cut, Drill and Fill" were applied (Albert, 2006). [21] This procedure requires sophisticated equipment and well trained dental personnel, especially when treating apprehensive young children. It is now time that we reconsider the warnings from Gies and G.V Black early in twentieth century that dental profession must not develop its technological base at expense of its biological foundation (Williams,1897 a,b,c; [22,23,24] Black, 1884).[25] Arresting or reversing early carious lesions is major focus of dental community in twenty first century.

Modern Dentistry necessitates a paradigm shift of technique from "cut and drill" to "observe and preserve" (Albert, 2006). [21] Miles Marley, one of several great leaders in preventive dentistry once stated that: loss of even a part of human tooth should be considered as "a serious injury," and that dentistry's goal should be to preserve healthy, natural tooth structure. Over time, dentistry has evolved to a minimal invasive approach, in which dental caries is managed as an infectious disease, deferring operative intervention as long as possible. Dental community still faces a major challenge to develop techniques to promote detection, diagnosis and early interception of the carious process. The phenomenon of reversal of incipient or early enamel caries forms an important part of prevention, leading to apparent repair of lesion. Several investigators have worked towards developing an ideal remineralising agent, which diffuses into the subsurface and promotes reformation of the damaged calcified tissue.

From early 1940s through late 1970, the "Halcyon Days" of fluoride research took place in United States and other parts of the world. Cariostatic action of fluoride was well established and systemic water fluoridation programmes as well as topical fluoride preparations were widely recommended. Fluorides promote formation of fluorapatite in presence of calcium and phosphate ions produced during enamel demineralization (Fusayama and Terachima, 1972). [26] However, for every two fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of Fluorapatite $\{(Ca_{10}(PO_4))_6F_2\}$. Hence availability of calcium and phosphate ions can be a limiting factor for mineralization to occur. Also given the mechanism of remineralisation by topical fluoride, they attempt only to reduce apatite dissolution rather than aiming to promote remineralisation of apatite crystals or replacement of the lost minerals, associated with fluoride use. There is also a risk of fluoride toxicity if administered in high doses or when administered in individuals with inadequate nutrition especially in children (Azarpazhooh *et al.,* 2008). [27]

The limitations and risks associated use of fluoride as a remineralising agent fuelled the need to develop newer non-toxic techniques that deliver calcium and phosphate ions directly into subsurface lesion and boost remineralising properties of saliva. Remineralisation of white spot lesions and carious lesions on tooth are possible with use of currently available formulations such as as tri-calcium phosphate (TCP)-Clinpro^{TM}, di-calcium phosphate dehydrate (DCPD), xylitol carrier, Nano hydroxyapatite- Reminpro^{TM}, amorphous calcium phosphate (ACP) in combination with fluoride, popularly known as Enamelon^{TM}, complexes of casein phospho-peptides in amorphous calcium phosphate (CPP-ACP) popularly known as tooth mousse and Recaldent^{TM}, (CPP-ACPF) as MI paste plus^{TM} which also incorporates the benefits of fluoride (Reynolds, 2008; Mazzaoui *et al.,* 2003). [28,29] The sodium calcium phosphosilicate – bioactive glass or Novamin^{TM}, calcium carbonate carrier-SensiStat^{TM}, and a revolutionary newer technique known as ozone therapy (Healozone^{TM}) are also in use today (Baysan and Lynch, 2005). [30] The current concept of Tissue engineering and Nano technology further bridges traditional gap between preventive non-invasive and surgical procedures, which is need of present day dentistry (Zhao *et al*., 2008). [31] The implementation of these new re-mineralization technologies is faced with many impending challenges such as ingredients compatibility, ability to provide accurate amount of free ions required to promote remineralising process since release of excess calcium ions leads to calculus formation. The newer technology should demonstrate a clear benefit over and above

an established and highly effective agent, namely fluoride (Murray *et al*., 1991). [32]The plea of G.V Black, made in 1909, "to study and understand early carious lesions", should receive our utmost attention. We need to shift our focus from defining dental caries as "cavities" to detecting various stages of the carious process (Shaw, 1954; Navia,1977).[33,34]Purpose of writing this library dissertation is to provide, in-depth knowledge, of different stages of carious deterioration of mineralized tissue of teeth as well as advances made in remineralisation technologies past the use of fluorides. These non – fluoride remineralisation strategies will be of benefit to many since focus currently is shifting from curative to preventive health care. With these alternative remineralisation strategies, we would be able to re-establish the health of oral tissues without being under risk of invasive procedures and toxic side effects.

Historical review:

Dental caries is the most common, preventable global oral health problem in the world today. Dental caries results from an ecological imbalance in the equilibrium between tooth minerals and oral bio-films. It is characterized by microbial activity, resulting in fluctuations in oral pH due to bacterial acid production which causes chemical dissolution of the hard tissue structure of the tooth. The microbial community of caries is diverse and contains many facultative and obligate anaerobic bacteria. Dental caries can affect the human day to day life in numerous ways i.e. presence of tooth pain, infection of dysfunction of the stomatognathic system which can limit the necessary ingestion of energetic foods, affecting growth in children and adults as well as learning, communicational skills and recreational activities. Caries is a common human disease that only attacks vital teeth in an environment under certain oral conditions—conversely—caries does not infect a tooth once the host is dead. Studies by 19th century clinicians (Abbott. 1879 a,b,c.; Black, 1884; Williams, 1897 a,b,c.; Webb, 1883.; Miller, 1890; Dexter, 1876). [35,36,37,22,23,24,38,39,40] The paper considers the caries literature and analyzes its timeline; erudite articles by Mandel (1983), [41] Newbrun (1989), [42] Nikiforuk (1985), [43] Tanzer (1995) [44] and Zero (1999).[1] Twentieth century scientists have clarified the intriguing complexity of the caries mosaic as an infectious disease (Leme *et al*., 2006).[45] The dental community realizes that the failure of the patient to remove or disrupt dental plaque biofilms or minimize frequent consumption of dietary sugars permits cariogenic bacteria to establish a dominant parasitic community.

Hominids and Early Human Era:

Skeletal remains are an excellent historical kymograph of human conditions. Lufkin reported that a 500,000 year old Pleistocene skull from a human ancestor from Java had severely worn teeth; however, no decay was evident. Neanderthal skull from Paleothic era (40,000 to 25,000 year ago) was found with major alveolar bone loss, missing teeth, and various level of decay in the remaining teeth. (Lufkin, 1938). [46]

Hippocrates (460-357 BC) suggested that medicine should be dissociated from magic and witchcraft—his doctrine of disease based on humoral pathology exerted its influence on medical thought for many centuries. Stagnation of depraved juices in teeth caused dental pain (Guerini, 1909). [47] The affections of the teeth to depend on natural predisposition and accumulated filth and corroding action of same (Prinz, 1945).[48] Moreover, Aristotle (384–322 BC) observed a relationship of eating sweets with dental caries and proposed the question, "Why do figs, when they are soft and sweet, produce damage to teeth (Pickerill, 1924). [49]

Galen (131 AD) considered that lack of proper nutrition caused "weak, thin and brittle teeth while excessive nutrition caused inflammation to produce soft tissue and that loose teeth were the result of excess moisture that impaired the nerves" and caries is the result of the internal accumulation of corroding humors. Guerini wrote that during the reign of Hammurabi (circa 2100 B.C.) a "Code of Laws", was left on clay tablets with judicial dictates defining fees and demanding skillful medical treatment of patients against unscrupulous mystics (Guerini, 1909). [47] Before then, Ruffer discussed that most disease was attributed to the presence of unseen demons in the body or to an insult that was caused against a particular god (Ruffer, 1921). [50] Cuneiform tablets from that age served as the medical reference that defined special incantations to request the Babylonian god, Ea to "get hold of the worm and pull it from the offending tooth (Guerini, 1909). [47]

Pre-Restorative Era (up to 1850 A.D.)

While dentistry is a young profession that emerged in the mid nineteenth century as a separate discipline that focused on treatment of diseases associated with teeth and their supporting tissues but dental and oral health problems had afflicted humans throughout history. Until the eighteenth century, dental treatment was rather simple and was based on extraction of teeth, use of traditional remedies. In the pre restorative era, many observations were made about the causes of dental caries, its causes and treatment did not advance much until the 18th century.

The first full text on dental diseases and their treatment was published in 1728 when Pierre Fauchard, a French surgeon, wrote "*Le Chirurgien Dentiste*." Fauchard rejected the tooth worm theory of dental caries. He described enamel hypoplasia as "an erosion of the enamel" and recommended that hypo plastic areas be smoothed using files. Fauchard recommended total excavation of carious cavities and filling them with lead, tin, or gold foil.

With the beginning of the second industrial revolution in 1875 (including the advent of the telegraph, transatlantic cable, telephone, incandescent light bulb, diesel engine, wireless communication, and airplane flight), dentistry was on the verge of experiencing a new revolution that focused on conserving teeth rather than extracting them.

The Restorative Era (1850 - Present)
Late in the nineteenth century, dentists were faced with an increasing demand to conserve teeth from the ravages of dental caries. Amalgam was first used in Europe, but in 1855, Dr. W. M. Hunter and Dr. E. Townsend in the United States published a formula of amalgam that consisted of tin, silver, and mercury. While amalgam was initially criticized because of its mercury content and poor physical properties, it had improved to become the material of choice by the end of the nineteenth century. In 1883, a battery-powered electric dental engine was developed. The mechanical improvement continued to ease the practice of dentistry with the introduction of faster dental engines and hand pieces during the late nineteenth century.

Historical Review of Etiology :
Tooth Worm Theory:
A Sumerian text from 5000 BC describes a "tooth worm" as the cause of dental caries. Perhaps the Guinea worm, *Druncunculus medinensis*, that came from infected drinking water is the tooth worm. In *Dracunculiasis*, the gravid female can expel over 500,000 juvenile worms in the presence of cool water, which facilitates the release process (Schmidt and Roberts, 1989). [51] It could be that exposed vital pulps, which are periodically exposed to cool drinking water and attract gravid females with their release of thousands of Guinea worms. This could have occurred in the ancient world where drinking water was often obtained from deep cool wells—the natural reservoir for the intermediate host of *Druncunculus medinensis*, a cyclopoid crustacean (Schmidt and Roberts, 1989). [51] Evidence of this belief has also been found in ancient India, Egypt, Japan, and China. The legend of the worm is also found in the writings of Home, and as late as the 1300s AD the surgeon *Guy de Chauliac* still promoted the belief that worms cause tooth decay. Many cultures worldwide left oral and written accounts about such observations. One of the common treatments for the tooth worm at that era was to place a few drops of Oil of Vitriol (sulphuric acid) into the cavity (Ring, 1971).[52] It is not surprising the ancient tooth worm theory as reported by Guy de Chauliac (1300–1368) continued into so many cultures (Mandel, 1983). [41]

The Internal Theory of Caries Formation:
In France, Ambrose Pare, known as the father of Surgery, published his *"Computer Works"* in 1575. This included practical information about dentistry, such as tooth extraction and the treatment of tooth decay and jaw fractures. The Frenchmen pare (1510-1590) moved away from tooth worm theory, declaring that a toothache was due to internal forces of hot and cold humors that resulted in caries, he stated that "teeth organs alter the manner of bones, suffer inflammation and quickly suppurate to become rotten.

Pierre Fauchard (1678-1761) proposed that caries was caused by a tumor of osseous fibers that displaced parts of teeth causing its destruction. The Frenchman Paré (1510 to 1590) is credited to have almost singlehandedly elevated the respect of the dentist to a position of valued recognition in the public eyes. Paré moved away from the tooth worm theory, declaring that a toothache was due to internal forces of hot or cold humors that resulted in caries, he stated that "teeth organs alter the manner of bones, suffer inflammation and quickly suppurate to become rotten"—hence the concept of inflammation from within the tooth (Paré, 1634).[53] Kirk wrote that Pierre Fauchard (1678–1761) discredited the tooth worm theory, and was one of the first to prefer the more technical term of caries, which he thought was caused by a tumor of osseous fibers that displaced parts of the teeth causing its destruction (Kirk, 1923).[54] Lufkin discussed the writings of Bondett and Jourdain who preferred the term of dental gangrene to caries (Lufkin, 1938).[46] Lufkin wrote that the common thought of many in the 1700s was that tooth decay was caused by death of bone and soft tissues from around or within the teeth. Hunter of London expressed dissatisfaction with the term caries and preferred the term mortification, and held to the concept of the inflammation theory from internal decay, but he did not offer an alternative opinion of any substance (Hunter, 1778).[55] In 1806, Fox was among the first of his contemporaries to use the term dental caries and opinioned that caries was the result of inflammation of the inner membrane along the pulp dentin wall (Fox, 1806). [56] In 1831, Bell of England adhered to the concept of

inner inflammation, but he felt caries had a hereditary factor; he preferred the term dental gangrene to decay or caries; thinking that gangrene was a consequence of thermal change (cold to hot), which immediately penetrated to the enamel-dentin junction, resulting in decay. Bell wrote that when dental gangrene first occurred in the bone surrounding the tooth, necrosis resulted in gangrene of the pulp causing its destruction and then penetrated through the dentin, and eventually to the enamel (Bell,1831). [57] In contrast to this Koecker in 1842 observed that decay first began on the outer enamel surface then penetrated to the enamel – dentin junction and invaded the tubules to eventually infect the pulp tissues (Köecker, 1842). [58]

The External Chemical Theory of Caries Formation:

In the late 1700s into the early 1800s, a number of scientists from different countries made parallel observations that caries was caused by external chemical agents instead of internal factors. Professor Harris of Baltimore Maryland, Robertson of England, Hope of Edinburgh, and Dr. Wescott and Dr. Dalyrymple had collectively studied histological preparations of extracted human teeth and noted that caries could not have been caused by the mechanism of internal inflammation or from physiological changes inside the tooth (Robertson, 1835),[59] and suggested that caries was caused by chemical disintegration of the tooth denouncing the theory of inflammation from inside the tooth. He postulated that gastric acids acted upon particles of food lodged in pits and fissures and began their destruction.

A parallel publication by Rognard of Paris in 1838 noted that caries began on the tooth surface where its effects were first seen. Rognard's clinical observations demonstrated that when extracted non-carious teeth were fixed in place of missing human teeth, caries occurred in the pits and fissures of the fixed tooth-within a few weeks (Rognard, 1838).[60] Abbott described enamel caries in its earliest stage as a chemical process that dissolved the minerals causing the breaking apart of crystals, followed by the organization of a protoplasmic mass that invaded the dentin. Abbott wrote that caries consisted of chemical demineralization and the dissolution of dentin into a "glue-giving basis-substance" around and between the tubules that breaks apart into medullary elements associated with secondary formations of micrococci and leptothrix (Abbott, 1879 a,b,c). [35,36,37]

D'esirabode, the Surgeon Dentist to Louis Phillippe, King of France from 1830 to 1848, differed with the period's collective writings on inflammation. He designated seven varieties of decay that were based on age, color, texture, damage, and other effects. During those years, a great deal of confusion surrounded the idea that caries was the cause of mingling of gastric acids with mouth fluids. (Dèsirabode,1841). [61] Dr. Black was one of the first academics to assemble the complete pieces of the puzzle regarding the cause of caries. Black wrote that tooth caries could occur when oral fluids were habitually acidic or alkaline, and that initiation of caries was directly dependent upon lodging of food particles and gelatinous debris at pits and fissures of the tooth, followed by the fermentation of the debris with the production of acids that cause the demineralization process (Black, 1884). [25]

Chemo Parasitic Theory of Caries Formation:

At the International Medical Congress held in London in 1881, Dr. Miles and Dr. Underwood proposed that dental caries development was dependent on the presence and proliferation of "organisms." They claimed that dental caries was caused by direct action of microorganisms that penetrated the dental tubules and destroyed the organic component of the dentin leaving the inorganic parts to be broken down and washed away in fluids of his mouth (Brock, 1961).[62]

In 1840, the theory of fermentation had been fully explained by Von Liebig—an unlikely non dental scientist whose chemistry research was first presented as an oral report to the British Association for the Advancement of Science, with their full acceptance (von Liebig, 1847).[63] The mechanics of fermentation had been used for centuries, but it required the genius of Professor Von Liebig to present it to the scientific world in a meaningful form. Until Von Liebig, there was no understanding of fermentation in terms of chemical processes. In that era, an acceptable theory of dental caries required something more than the simple hypothesis of chemical dissolution of enamel by an acid. The acid theory was close to the true cause of caries, but the level of science of the preceding decades simply failed to understand the missing equation—bacteria. In retrospect, due to the absence of available fermentation science before Von Liebig, it is easy to understand that until the work of Louis Pasteur from 1857 to 1876 demonstrating the necessity of microbes in fermentation (Brock, 1961).[62] just why the scientific understanding of bacterial fermentation causing caries was never completely understood. When we project a few decades ahead in our scientific understanding of bacterial fermentation, we can see that Miller presented the chemo-parasitic nature of bacteria within the oral cavity and their importance in the initial cause of acid demineralization of enamel and

invasion through the enamel-dentin junction to infect the tubule complex leading to destruction of collagen and other proteins (Miller, 1890).[39] It seems the actual person who might be credited with actually "FIRST" describing the exact science of caries may be left to other writers. It simply appears that its "discovery" was a collective effort by several individuals.

In 1881, Dr. W. D. Miller suggested that the acid produced by microorganisms in the mouth caused caries of the enamel, and caries in dentin resulted from acidic decalcification. Bacteria produced the acids that led to the demineralization of enamel and dentin. He also noted that bacteria did not need to be present in enamel or dentin to initiate demineralization. Miller's research led to a storm of debate and controversy, following the dissemination of Miller's findings, dietary and nutritional factors received extensive study for most of the last 150 year.

The nutrition-caries hypothesis was partially discredited by the finding that populations who were malnourished had lower caries prevalence than those who were well nourished. Also discrediting that hypothesis were findings from the pivotal studies ever reported in the dental literature, the Vipeholm Dental Caries Study, which found that frequent consumption of sugar increases the risk of developing dental caries. Throughout the twentieth century, many researchers and dentists recognized that dental caries is a product of the interplay of many factor. In his article on "dental caries redefined." Dr. Keyes explained the interplay between the local cariogenic bacteria in plaque, fermentable carbohydrates, "constitutional factors" related to "species and strains," and the tooth structure. The work of Dr. Keyes and Dr. Fitzgerald proved that dental caries is an "infectious process" of the teeth. Their work led to the definition of dental caries as multi-factorial disease with interplay of three principal factors: the host, the micro flora, and diet.

In small Berlin laboratory the Robert Koch, Miller observed certain bacteria could convert starch by ptyalin (amylase) to form sugar that was fermented to lactic acid (Miller, 1890). [39] Miller cited the work of Milles and Underwood who wrote that caries most likely caused decalcification as a consequence of acids secreted by oral bacteria (Milles and Underwood, 1881). [64] Miller's experiments supported studies that implicated caries due to the corrosive action of lactic acid from bacteria that demineralize the mineral of enamel and dentin (Miller, 1890). [39] In hindsight, it seems that Miller's failure to recognize the true relationship of plaque bacteria to localized dental caries may have been due to his lack of clinical experience compared to that of Black (Black, 1884). [25] Professor Black wrote in his 1884 paper Formations of Poisons by Micro-organisms "That fermentation is the result of the life-processes of certain forms of micro-organisms may now be accepted as a truism, and will not be argued". He realized that fermentation was a chemical process and that a number of substances may be formed naturally by "true processes". Having read Miller's publications and studies Black wrote "what is called fermentation by an organized fermentable agent is but the first step in true fermentation (Black, 1884). [25] Until that time, Miller's observations of fermentation had been mainly to study the digested agent (dentin) by lactic acid (Miller, 1890). [39] Miller had asked of the microorganisms of decay "what is its food, and in what chemical form is it delivered back after having served the purposes of the organism". It now seems that Black was able to piece together the complex puzzle of the cause of human caries by his own and other colleague's research data.

Professor Davis wrote in his textbook "the most rapid caries was of a light or white color and that the hypersensitive nature of this substrate is very high, whereas moderately colored yellow and brown varieties are less sensitive and that the darker brown to black that represents the slow progressing form is much less sensitive when compared to normal." Davis identified two levels of carious dentin—a superficial zone—located towards the oral surface and called infected dentin was caused by the action of lactic acid and proteases from certain bacteria that left a soft leathery substrate. The deeper zone, located towards the pulp, was called affected dentin, often referred to as secondary caries, being composed of fewer bacteria and demineralized dentin (Davis, 1923). [65] Black's use of references is an indication of his erudite nature. It was obvious his depth of reading, understanding, knowledge, and forward thinking about the cause of caries for that era surpassed many others (Black, 1886 a). [66] He understood that caries disintegration always begins on the enamel surface of the tooth in some pit or irregularity and that acid was formed at the very spot where caries begins. His clinical experience showed him that certain foods were associated with higher levels of caries. He grasped the importance of bacteria feeding upon lodged food particles and fermenting them to organic acids. Black had made certain personal histological observations. Caries penetration of dentin occurs by following the tubules to the pulp; his extended observations showed that pulp exposures occurred with the least destruction of dentin; "exposure of the pulp will occur that is to say, the more perfect the development, the more complete the penetration is confined to the direction of the tubules." He demonstrated that carious softening tended to be in isolated tubules, whereas softening of a ground section of dentin in a mineral acid

was seen at its whole entirety; their appearances are distinctly different. Black also observed that in the initial carious invasion, the internal diameter of the tubules became enlarged and using an aniline dye stain, he demonstrated the tubules were occupied with bacteria.

Regarding enamel caries, Black's laboratory studies demonstrated that enamel rods fell apart at the periphery and not in the rod center. His 1884 article summarized many of previous observations, "Decay of the teeth is certainly a specific disease, running a specific course, and evidently arising from a specific cause, but this cause is not yet certainly known While there is no decay without the presence of an acid, there is not necessarily decay because of the presence of an acid (Black, 1886 b). [67] It is important to realize that J. Leon Williams, a colleague of G. V. Black, also observed dental caries as an in situ phenomenon in teeth associated with an overlying "thick felt-like mass of acid-forming microorganisms" otherwise known as dental plaque (Williams, 1897a,b,c). [22,23,24]

Using various microscopic techniques, Furrier illustrated six-zones of carious dentin: bacteria-rich, bacteria-few, pioneer-bacteria, turbid-layer, transparent and a vital reaction layer. However, from a clinical point of view, tactile discrimination of caries varied from clinician to clinician due to its softness (Furrier, 1922). [68] The issue of caries discrimination was solved by Professor Fusayama and Terachima, using an in vivo stain. They demonstrated that softened carious dentin is composed of two layers (Fusayama and Terachima, 1972). [26] Their research demonstrated an outer infected carious zone just below the enamel-dentin junction densely populated with facultative and anaerobic bacteria that secrete organic acids capable of dissolving hydroxyapatite proteases that degrade collagen and other proteins causing detachment of apatite crystals leaving the once solid substrate to simply collapse on itself. This outer infected caries is completely dead, with no capacity to register any sensitivity to tactile or thermal stimuli and is not physiologically capable of remineralisation. This fact makes its removal clinically painless as no anesthesia is necessary.

The deeper affected carious dentin is generally 1,000 to 2,500 m thick and generally contains only a few pioneer bacteria. It is somewhat softened due to organic acids dissolving the mineral rich crystals without proteases damaging the organic proteins (Fusayama and Terachima, 1972). [26] This deeper carious zone is vital with a sensory capacity to respond to various stimuli. Once the clinician reaches this vital layer with minimally invasive instrumentation, they realize when to stop instrumentation as the underlying affected tubule complex is physiologically capable of remineralisation with crystals that fill the lumen of dentinal tubules to become sclerotic (Massler, 1978; Kidd and Fejerskov, 2004).[69,70] Importantly, the application of these principles has evolved into the therapeutic use of indirect pulp capping (Law and Lewis, 1961; Hawes et al., 1964 and Coll, 2008). [71,72,73] and stepwise excavation (Ridell et al., 1996; Bjørndal, 2008 and Ricketts et al., (2009) [75- 76] for the conservative preservation of the vital dental pulp during clinical caries removal as long as a "bacteriometic" seal can be maintained (Brannstrom, 1981; Mertz-Fairhurst et al., 1998; Fejerskov and Kidd, 2008 and Cox et al., 2002). [77, 78, 79, 80]

Historical review of Preventive Management:

As described earlier, many interventions were suggested based upon observations that had not been rigorously evaluated. One of the most scientifically untested slogans in dentistry has been and still is, to some extent, the concept of "extension for prevention." Dr. M. H. Webb proposed this concept more than one hundred years ago. Despite the lack of scientific documentation supporting the concept of extension for prevention, its liberal use in cavity preparation continued for most of the twentieth century.

Around the time of Miller's death in 1907, a young dentist unknowingly ignited a new revolution through his keen observations in preventive dentistry. Dr. F. S. McKay, who was practicing in Colorado Springs, Colorado, noticed that many of his patients had "mottled enamel." He pinpointed the problem, after several field investigations, as related to the drinking water. In 1930, H.V. Churchill, a chemist with the Aluminium Company of America, discovered that fluoride may be the cause for the enamel mottling, the condition that we now refer to as "Fluorosis". In 1931, Dr. H. T. Dean was assigned by the United States Public Health Service to study the association between fluoride and "mottled enamel," which led to associating fluoride with reduced dental caries prevalence and severity. Dean led a series of field investigations that confirmed the association between fluoride in the drinking water and fluorosis and the potential beneficial effect of fluoride in caries prevention. In a classic research program, it was found that adjusting the fluoride concentration to around 1.0 ppm (parts per million) could lead to a significant reduction in dental caries prevalence and severity in children with minimal cosmetic side effects.

From the early 1960s through the late 1970s, the "Halcyon Days" of fluoride research took place in the United States and other parts of the world. By the 1950s, topical applications of fluoride were widely provided by dentists in the United States. While the mechanism of action of fluoride as a cariostatic agent is still being debated, there is strong evidence that fluoride, provided systemically in drinking water or applied topically through exposure to fluoridated water, tooth brushing, or other means, is effective in reducing the burden of dental caries Although fluoride is effective in preventing cares on all tooth surfaces, occlusal surfaces remain the most vulnerable to caries attack. A major breakthrough in resolving this problem came when it was discovered that creating small tags or roughness on the enamel surface by the application of a weak acid (phosphoric acid) significantly enhanced the retention of an acrylic filling material. This discovery led to a series of research initiatives on acid etching, adhesion, and sealant materials for preventing dental caries in pits and fissures. During the twentieth century, other approaches to prevent dental caries have been proposed and tested.

Historical review of Restorative process of Dental Caries:

For most of the nineteenth and twentieth century, dental caries was managed through removal of de-mineralized enamel of dentin and placing synthetic materials to restore anatomical form, function, and aesthetics. In addition to the technological advances in anesthesia and tissue-cutting instruments and devices (hand pieces), the field of restorative dentistry has benefited significantly from the revolutionary advances in new dental material and the intention of the dental air turbine head piece in 1946. These advances have led to the provision of sophisticated restorative care and, consequently, the saving of the teeth. However, failure to deal with the problem of diagnosis and clinical decision-making may have led to over-restorative as well as under-restoration of decayed teeth. The conservative management of dental caries has picked up momentum in the last ten years of the twentieths century with the advent of new materials and tools. Various studies have reported the outcome of sealing in dental caries, even when it is in dentin. There have been calls for changing the paradigm from drilling and filling to managing the disease an infection. An Ounce Of Prevention Is Worth A Pound of Cure.

This expression from Benjamin Franklin (1706–1790) means it is better to avoid problems in the first place, rather than trying to fix them once they arise. In a 1886 lecture to students, G. V. Black stated "The day is surely coming, and perhaps within the lifetime of you young men before me, when we will be engaged in practicing preventive, rather than reparative, dentistry" (Dr. Samuel D. Harris 1998). [81] We wonder what Black would think if he realized that most of today's dental schools throughout the world still teach a restorative focused curriculum; rather than a series of preventive courses. Since the 1970s, our profession has witnessed the introduction of caries detectors, acid etchants, glass ionomers and composites that seem more suited to minimal intervention than Black's extension for prevention concepts of amalgam placement. The addition of fluoride to public water has proved effective to reduce caries in human dentitions, post developmental use of fluoride is known to cause a significant reduction in caries through topical interaction with surface enamel and dentin throughout life (Koulourides and Cameron, 1980; Reynolds, 2009; Featherstone, 2000) [82, 83, 84] Other measures have shown that an alteration or reduction of dietary sugars also results in a major decrease of caries in experimental animal models (Shaw, 1954; Navia, 1977). [33, 34]

It is interesting to pause and reflect on dental research since mid-1800. Once caries was known to begin on the external tooth surface and proceed inwards, the dental profession gained recognition amongst the worldwide populace. As the science of caries prevailed, the tooth worm faded into oblivion. New devices and technologies emerged in parallel fashion and became used in the laboratories of clinicians who were searching for answers to the biology of the tooth and caries. North American notables such as Harris (1806–1860), Black (1836–1915), Webb (1844–1883), Williams (1852–1932), and Miller (1853–1907) all shared very common childhood experiences (Black, 1884; Webb, 1883; Miller, 1890). [25, 38, 39] They were not born of nobility or gentry, but grew up in humble rural surroundings and learned of life by spending long hours in the pursuit of Nature. American cultural history records that almost every home contained the popular textbook of the day of Comstock's Philosophy for family reading and group discussions after dinner time in the evening (Comstock, 1844). [85] Each of these individuals had a similar introduction to dentistry and study, they used their own personal finances; no governmental agency dispensed research funds for their research. They pursued answers to questions that had evaded other colleagues and published their findings because they wanted to make sure new knowledge was available to colleagues worldwide. There was no academic pressure to publish or perish.

Today and Future

Looking at the progress in the field of dentistry throughout the second millennium, we have achieved unprecedented successes using the scientific method to improve not only the understanding of the caries process but also its

prevention and treatment. In the twenty first century, there is a need to re-establish new research programs on dental caries. These programs should have a more preventive approach.

Recent advances in biology and engineering should open new doors for the prevention and management of dental caries and, most importantly, a better understanding of the etiology of the disease. These new research programs should develop new methods that can validly and reliably assess the caries actively in enamel and dentine. Advances in micro-Electro Mechanical Systems (MEMS), nanotechnology, sub-micron fiber-optic biochemical sensor, energy transducer super molecules (artificial photosynthetic antennae) and in-vivo Nano sensors open the door for new research initiatives to develop novel approaches to diagnose and prevent dental caries. Development of newer materials aims at re-mineralizing the decayed tooth structure. Advances in tissue regeneration may open the door for methods to restore damaged or delayed tooth structure.

References:-

1. Zero, D. T. (1999). Dental caries process Dent. Clin. North Am.43: 635–64.
2. Silverstone, L.M. (1977). Remineralization phenomena. Caries Res. 11 (1): 59–7
3. Pearce, E. I. F., Moore, A. J. (1985). Remineralization of softened bovine enamel following treatment of overlying plaque with a mineral enriching solution. J. Dent. Res. 64 416–21.
4. Rickard, G.D., Richardson, R., Johnson, T., McColl, D., Hooper, L. (2004). Ozone therapy for the treatment of dental caries. Cochrane Database Syst Rev.3. CD004153.
5. Anderson, M.H., Bales, D.J., Omnell, K.A. (1993). Modern management of dental caries: the cutting edge is not the dental bur. J Am Dent Assoc. 124:36e44.
6. Polydorou, O., Halili, A., Wittmer, A., Pelz, K., Hahn, P. (2012). The antibacterial effect of gas ozone after 2 months of in vitro evaluation. Clin. Oral Investig. 16:545e50.
7. Mu¨ller, P., Guggenheim, B., Schmidlin, P.R. (2007). Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur. J. Oral Sci. 115:77e80.
8. Petersen, P.E. (2003). Continuous improvement of oral health in the 21st century. The approach of the WHO Global Oral Health Programme. Community Dent. Oral Epidemiol.31:3e23.
9. Lynch, E. (1996). Antimicrobial management of primary root carious lesions: A review. Gerodontology. 13:118e29.
10. Johansson, E., Claesson, R., van Dijken, J.W. (2009). Antibacterial effect of ozone on cariogenic bacterial species. J. Dent. 37: 449e53.
11. Meja`re, B., Meja`re, I., Edwardsson, S. (1979). Bacteria beneath composite restorationsda culturing and histobacteriological study. Acta Odontol. Scand. 37:267e75.
12. Magni, E., Ferrari, M., Hickel, R., Huth, K.C., Ilie, N. (2008). Effect of ozone gas application on the mechanical properties of dental adhesives bonded to dentin. Dent. Mater. 24:1428e34.
13. Banerjee, A., Watson, T.F., Kidd, E.A. (2000) Dentine caries excavation: a review of current clinical techniques. Braz. Dent. J. 188: 476e82.
14. Sbordone, L., Bortolaia, C. (2003). Oral microbial biofilms and plaquerelated diseases: microbial communities and their role in the shift from oral health to disease. Clin. Oral Investig. 7:181e8.
15. Baehni, P.C., Takeuchi, Y. (2003). Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis. 9:23e9.
16. Imazato, S., Imai, T., Ebisu, S. (1998). Antibacterial activity of proprietary self-etching primers. Am. J. Dent. 11:106e8.
17. Ozer, F., Unlu, N., Karakaya, S., Ergani, O., Hadimli, H.H. (2005). Antibacterial activities of MDPB and fluoride in dentin bonding agents. Eur. J. Prosthodont. Restor. Dent. 13:139e42.
18. Wicht, M.J., Haak, R., Schutt-Gerowitt, H., Kneist, S., Noack, M.J. (2004). Suppression of caries-related microorganisms in dentine lesions after short-term chlorhexidine or antibiotic treatment. Caries Res. 38:436e41.
19. Polydorou, O., Pelz, K., Hahn, P. (2006). Antibacterial effect of an ozone device and its comparison with two dentin-bonding systems. Eur. J. Oral Sci. 114:349e53.
20. Nidhi, G., Kunwarjeet, S. (2012). Try to believe it-amazing Remineralizing technologies. J. Pharm. Biomed. Sci. 24(24): 79-82.
21. Albert, M.P. (2006). Minimal intervention dentistry. The Hong Kong Medical Diary. 11(8):2
22. Williams, J. L. (1897a). A contribution to the study of pathology of enamel. Dental Cosmos. 39 (3): 169–196.
23. Williams, J. L. (1897b). A contribution to the study of pathology of enamel. Dental Cosmos. 39 (4): 269–301.
24. Williams, J. L. (1897c). A contribution to the study of pathology of enamel. Dental Cosmos. 39 (5): 353–374.

25. Black, G. V. (1884). The Formation of Poisons by Microorganisms: A Biological Study of the Germ Theory of Disease, P. Blakiston's & Son, Philadelphia, Pa, USA,.
26. Fusayama, T., Terachima, S. (1972). Differentiation of two layers of carious dentin by staining. J.Dent.Res.51(3) :866
27. Azarpazhooh, A., Limeback, H. (2008). Clinical efficacy of casein derivatives: a systematic review of the literature. J. Am. Dent. Assoc. 139(7):915-24.
28. Reynolds. (2008) Calcium phosphate-based remineralization systems: Scientific evidence? Aust. Dent. J. 53(3):268-73.
29. Mazzaoui et al. (2003). Incorporation of CPP–ACP into glass ionomer cement. J. Dent. Res. 82 (11):914-918.
30. Baysan, A., Lynch, E. (2005). The use of ozone in dentistry and medicine. Prim Dent. Care.12:47-52.
31. Zhao, H., He, W., Wang, Y., Zhang, X., Li, Z., Yan, S., Zhou, W., Wang, G. (2008). Biomineralization of large hydroxyapatite particles using ovalbumin as biosurfactant Mater. Lett.62: 3063–5.
32. Murray et al. (1991). Murray J J., Rugg-Gunn A J and Jenkins G N. Fluoride in Caries Prevention 3rd edn (Oxford: Butterworth-Heinemann).
33. Shaw, H. (1954). The effect of carbohydrate-free and carbohydrate-low diets on the incidence of dental caries in white rats," J. Nutr. 53: 151–162.
34. Navia, J. (1977). Animal Models in Dental Research, U. Alabama Press, Birmingham, Ala, USA.
35. Abbott, F. (1879a). Caries of human teeth. Dental Cosmos. 21(2): 57–64.
36. Abbott, F. (1879b). Caries of human teeth. Dental Cosmos. 21(3): 113–119.
37. Abbott, F. (1879c). Caries of human teeth. Dental Cosmos. 21(4): 177–184.
38. Webb, M. H. (1883). Notes on Operative Dentistry. The S. S. White Dental Manufacturing, Philadelphia, Pa, USA.
39. Miller, W. D. (1890). Micro-Organisms of the Human Mouth. The S. S. White Dental Manufacturing, Philadelphia, Pa, USA.
40. Dexter, J. E. (1876). A History of Dental and Oral Science in America, American Academy of Dental Science, Samuel S. White, Philadelphia, Pa, USA.
41. Mandel, D. (1983). Caries through the ages: a worm's eye view. J. Dent. Res. 62 (8): 926–929.
42. Newbrun, E. (1989). Cariology, Quintessence Publishing, Chicago, Ill, USA, 3rd edition.
43. Nikiforuk, G. (1985). Understanding Dental Caries, vol. 1, Karger, Basel, Switzerland.
44. Tanzer, J. M. (1995). Dental caries is a transmissible infectious disease: the Keyes and Fitzgerald revolution. J. Dent. Res. 74 (9): 1536–1542.
45. Leme, F. P., Koo, H., Bellato, C. M., Bedi, G., Cury, J. A. (2006). The role of sucrose in cariogenic dental biofilm formation—new insight. J. Dent. Res. 85(10): 878–887.
46. Lufkin, W. (1938). A History of Dentistry, Lea & Febiger, Philadelphia, Pa, USA.
47. Guerini, V. (1909). A History of Dentistry from the Most Ancient of Times until the End of the Eighteenth Century, Lea & Febiger, Philadelphia, Pa, USA.
48. Prinz, H. (1945). Dental Chronology, Lea and Febiger, Philadelphia, Pa, USA.
49. Pickerill, H. P. (1924). The Prevention of Dental Caries and Oral Sepsis, The MacMillan, Toronto, Canada.
50. Ruffer, M. A. (1921). Studies on the Paleopathology of Egypt, University of Chicago Press, Chicago, Ill, USA.
51. Schmidt, G. D., Roberts, L. S. (1989). Foundations of Parasitology, Times Mirror/Mosby, St. Louis, Mo, USA, 4th edition.
52. Ring, M. E. (1971). Anton van Leeuwenhoek and the tooth-worm. J. American Dent. Assoc. 83 (5): 999–1001.
53. Paré. (1634) The Works of that Famous Chirurgion, Ambroise Pare, Coates & Young, London, UK, translated from Latin by Johnson.
54. Kirk, E. C. (1923). Pierre fauchard. Dental Cosmos. 65: 881–884.
55. Hunter, J. (1778). Practical Treatise on the Diseases of the Teeth, and the Consequences of them, Treatise Upon the Human Teeth (Historia Naturalis Dentium Humanorum), Den Hague, The Netherlands.
56. Fox, J. (1806). The History and Treatment of the Diseases of the Teeth and Gums, London, UK,
57. Bell, T. (1831). Anatomy, Physiology, and Diseases of the Teeth, Highley, London, UK.
58. Köecker, L. (1842). Principles of Dental Surgery, Baltimore, Md, USA.
59. Robertson,W. (1835). A Practical Treatise on the Human Teeth, Showing their Causes of Their Destruction and the Means of Their Preservation, Old Square, Birmingham, UK.
60. Rognard, M. (1838). Oral Microbiology and Infectious Disease: A Textbook, Gazette des Hospital, Paris, France.
61. Dèsirabode, M. (1841). Surgeon dentist to the king: complete elements of the science and art of dentistry. Am. J. Dent. Sci. 160 (1).

62. Brock, T. (1961). Milestones in Microbiology, Prentice-Hall, Englewood Cliffs, NJ, USA.
63. von Liebig, J. (1847). Part II, on the Chemical Processes of Fermentation Decay and Putrefaction, Chemistry in Its Application to Agriculture and Physiology, T.B. Peterson, Philadelphia, Pa, USA.
64. Milles, G. A., Underwood, A. S. (1881). Cause and treatment of dental caries, in Communication to the Dental Section of the International Medical Congress, Transactions of the International Medical Congress, London, UK, .
65. Davis, W. C. (1923). Essentials of Operative Dentistry, C.V. Mosby, St. Louis, Mo, USA, 4th edition.
66. Black, V. (1886a). American System of Dentistry, Lea Brothers & Co, Philadelphia, Pa, USA.
67. Black, V. (1886b) General and Dental Pathology Vol I, Part IV, Predisposing Causes of Caries, Philadelphia, Pa, USA.
68. Furrier (1922). Die Verkalkungazonen bei der Dentinkaries, Schwez, Mschr ZHK, 21: 182–358.
69. Massler, M. (1978). Preserving the exposed pulp: a review. J. Pediatr. Dent. 2(3): 217–227, 1978.
70. Kidd, A. M., Fejerskov, O. (2004). What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J. Dent. Res. 83: C35–C38.
71. Law, B., Lewis, T. M. (1961). "The effect of calcium hydroxide on deep carious lesions," Oral Surgery, Oral Medicine, Oral Pathology, 14 (9): 1130–1137.
72. Hawes, R., DiMaggio, J., Sayegh, F. (1964). Evaluation of direct and indirect pulp capping. J. Dent. Res. 43: 808.
73. Coll, A. (2008). Indirect pulp capping and primary teeth: is the primary tooth pulpotomy out of date?" Pediatric Dent. 30 (3): 230–236.
74. Ridell, L.K., Cvek, M., Mejàre, I. (1996). Pulp exposure after stepwise versus direct complete excavation of deep carious lesions in young posterior permanent teeth. Endodont. Dent. Traumatol. 12 (4): 192–196.
75. Bjørndal, L. (2008). Indirect pulp therapy and stepwise excavation. Pediatr. Dent. 30 (3): 225–229.
76. Ricketts, D., Kidd, E. A. M., Innes, N., Clarkson, J. (2009). Complete or ultraconservative removal of decayed tissue in unfilled teeth (review) The Cochrane Collaboration, no. 3, 1–17.
77. Brannstrom, M. (1981). Dentin and Pulp in Restorative Dentistry, Wolf Medical Publications, London, UK.
78. Mertz-Fairhurst, J., Curtis J. W., Ergle, Jr., J. W., Rueggeberg, F. A., Adair, S. M.(1998). Ultraconservative and cariostatic sealed restorations: results at year 10. J. American Dent. Assoc. 129 (1): 55–66.
79. Fejerskov, O., Kidd, E. A. (2008). Dental Caries the Disease and Its Clinical Management, Blackwell Munksgaard, Oxford, UK, 2nd edition.
80. Cox, C. F., Bogen, G., Kopel, J., Ruby, J. D. (2002). Repair of pulpal injury by dental materials in Seltzer and Bender's Dental Pulp, K. M. Hargreaves and H. E. Goodis, Eds., Quintessence Publishing, Chicago, III, USA.
81. The Dr. Samuel D. Harris National Museum of Dentistry, Baltimore, Md, USA, 1998.
82. Koulourides, T., Cameron, B. (1980). Enamel remineralization as a factor in the pathogenesis of dental caries. J. Oral Path. 9 (5): 255–269.
83. Reynolds, C. (2009). Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence. Advances Dent. Res. 21: 25–29.
84. Featherstone, D. B. (2000).The science and practice of caries prevention. J. American Dent. Assoc. 131(7): 887–899.
85. Comstock, J. L. (1844). A System of Natural Philosophy, Pratt Woodford, New York, NY, USA.