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43rd Sysmex Scientific Seminar

Explanatory Material for Lectures

The Amazing Microbiomes:
Microbes in Healthcare and Medicine

June 5th, 2021(Sat)

Objective of the Seminar

Approximately, tens to hundreds of trillion bacteria coinhabit the human body, outnumbering the human cells which is about 37 trillion. The digestive organ alone is home to several hundred trillion bacteria of more than thousand different species, which amounts to a total weight of about 1kg. In the past, these bacteria were evaluated using culture methods. However, this method did not provide any information on the nature or properties of microorganisms that were unculturable. Since the invention of the next generation sequencing, we have been able to obtain more comprehensive information on the full spectrum of these microorganisms. We have also learned that indigenous microbiota including bacteria may have profound impact on human health and disease, and may even be used as unique and specific treatments. Recently, we have seen outstanding progress in this area. While some relevant information comes to us from the media, there are not many opportunities to obtain truly comprehensive scientific information. In this seminar, four leading experts in this area from Japan will provide us with fully cutting-edge information and insights on this subject. It is our hope that the seminar will stimulate interest in the "amazing" microbiomes that exist inside all of us. I encourage not only healthcare professionals but also as many researchers as possible to attend the seminar.

Prof. **Masato Maekawa**, Planner of the 43rd Seminar

Program (Each lecture is followed by a 15-minute Q&A session.)

*All times are JST

Chairpersons

Kobe : **Masato MAEKAWA**, Professor, Department of Laboratory Medicine, Hamamatsu University School of Medicine

Tokyo: **Tomoki NAOE**, Honorary Director, National Hospital Organization Nagoya Medical Center

Kobe 10:00 ▼ 10:05

Opening Address

Yutaka YATOMI, Chairman of Sysmex Scientific Seminar Planning Committee

Tokyo 10:05 ▼ 11:10

Biological and Medical Impact of Human Microbiome by Metagenomics

Masahira HATTORI, Ph. D.
Professor Emeritus, The University of Tokyo

Kobe 11:10 ▼ 12:15

The Skin Microbiome:
Pathogenic Roles of Bacteria in Inflammatory Skin Diseases

Yuumi MATSUOKA, M.D., Ph. D.
Specially Appointed Associate Professor, Cutaneous Immunology, Osaka University Immunology Frontier Research Center

12:15 – 13:30 Break

Tokyo 13:30 ▼ 14:35

Gastrointestinal Disorders and Intestinal Bacteria:
Advances in Research and Application to Therapy

Toshifumi OHKUSA, M.D., Ph. D.
Special Professor, Department of Microbiota Research, Juntendo University Graduate School of Medicine

14:35 – 14:55 Break

Kobe 14:55 ▼ 16:00

Gut Microbiota and Cardiovascular Diseases:
Gut Commensal Bacteria That Prevent Atherosclerotic Cardiovascular Diseases

Tomoya YAMASHITA, M.D., Ph. D.
Associate Professor, Division of Cardiovascular Medicine, Department of internal Medicine, Kobe University Graduate School of Medicine

Kobe 16:00

Closing address

Masato MAEKAWA, (Planner of the 43rd Seminar) Member of Sysmex Scientific Seminar Planning Committee

Biological and Medical Impact of Human Microbiome by Metagenomics

Masahira HATTORI, Ph. D.
Professor Emeritus, The University of Tokyo

[Summary]

About tens of trillions of microorganisms of more than one thousand distinct species reside in the human body. In recent years, metagenomics (a tool for obtaining collective information on genomes and genetic sequences of an existing microbial community without isolation or cultivation) has been applied in combination with the ultra high-speed next generation sequencing (NGS). This has enabled us to obtain comprehensive genomic information on human microbiota (also known as microbiome) within the intestines, oral cavity, skin and other body areas. Bioinformatic analysis of information obtained by these means has permitted us to discover the overall ecological and biological spectrum of the human microbiome at both genetic and genomic levels. As one example of these findings, we now know that gut microbes are much more closely associated with systemic diseases involving digestive, metabolic, nervous, and other organ systems than was previously understood. Likewise, we understand that ecological and functional diversity of the gut microbiota is affected by various internal and external factors, including foods, medications, age, and the genetic background of the host. In this seminar, I will explain the ecology and function of the human microbiome based on the idea that a human is a superorganism.

〈Explanatory material〉

The Microbiome

The human body is coinhabited by tens of trillions of microbiomes of about 1000 different species. These indigenous microorganisms can be differentiated from pathogens that invade the body and generate infections. The living organisms that compose the indigenous microbial community (the microbiota) are mainly bacteria, although bacteriophages and fungi have also been detected. The genes and full genomes of individual microbes, their products, and metabolites are collectively defined as the microbiome. Recent research has found that the gut microbiome is more closely associated with the pathogenesis of disease than was previously understood.

History of human microbiome analysis

Systematic research directed toward understanding the human gut microbiome began as early as the 1960s. At that time, researchers conducted bacteriological research by isolating and culturing fecal bacteria. By the mid-1980s, molecular biology had advanced to a point where researchers could begin using DNA sequences and genetic information (e.g., the 16S rRNA gene) as a means to identify specific bacterial species and strains. Many previously unidentified bacteria were revealed by 16S rRNA analysis methods which do not require isolation or *ex vivo* culture. This research also revealed that many human gut bacteria cannot be cultured with methods currently available. However, one of the disadvantages of the 16S rRNA analysis is that it provides no immediate information on bacterial growth or function. This disadvantage was overcome in 2004 with the introduction of metagenomics, an analysis method involving comprehensive collection and analysis of full genomes from species identified within a given microbiome. Once a genome has been identified, we can perform bioinformatics analysis in order to determine the specific nature and types of bacteria that are present in a given tissue as well as functional analysis based on specific genetic sequences. In the 2010's, the advent of next generation sequencing (NGS) (with >10 million times greater sensitivity than conventional methods) has made it possible to generate sequence data from a substantially larger bacteria and genes. This means that a given microbiome could be completely characterized in a short time. Most recently, nuclear magnetic resonance and mass spectrometric technologies have been developed, which permit comprehensive evaluation of a given proteome (i.e., the set of proteins detected in a specific population), and the metabolome (i.e., the complete set of metabolites in a specific population). Research that applies "-omics" methods, which integrate genomes, genes, proteins, and metabolites, is already on the horizon.

Physiological effects of the human microbiome and its impact on health and disease

The recent worldwide expansion of human microbiome research was initiated by Dr. Jeffrey Gordon (USA) and his colleagues, who published a report in 2009 demonstrating that gut microbial flora from obese subjects induced obesity. Likewise, his group followed up with another publication in 2013 on fecal microbiota transplant. In this report, microbes from the gastrointestinal tract of a healthy person were transplanted into a patient with refractory enteritis to achieve complete cure. These reports suggest that gut microbial flora can play a critical role in both maintaining health and promoting disease. Results from many other studies, notably those featuring next generation sequencing (NGS) have provided insight into the association of gut microbial flora with virtually all of the characterized "lifestyle diseases", not only those related to the digestive system but also the immune, nervous, and metabolic systems as well as cancer. Gut microbes promote various physiologic effects and are capable of unique interactions with the human host. Efforts to reveal the molecular mechanisms underlying these observations are ongoing.

Future outlook

As mentioned above, human gut microbiome is known to contribute to development of diseases, and efforts to specify bacteria of their product that cause various diseases is also underway. Therefore, we may expect to see, on a global basis, development of novel diagnostic and treatment methods utilizing or targeting specific bacteria, and new lifestyle or diet for disease prevention. Furthermore, research on microbes in the oral cavity and on the skin will most certainly follow. Human microbiome research is based on the idea that humans are superorganisms composed of genomes and microbiomes. This concept will certainly promote new discoveries in this field.

[Reference materials and websites] as of June 30, 2020

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The Skin Microbiome:
Pathogenic Roles of Bacteria in Inflammatory Skin Diseases

Yuumi MATSUOKA, M.D., Ph. D.
Specially Appointed Associate Professor, Cutaneous Immunology, Osaka University Immunology Frontier Research Center

[Summary]

The skin promotes protection by physically and immunologically differentiating the host from its external environment. There are many microorganisms that reside on the skin surface, including bacteria, fungi, and viruses; these microorganisms coexist and interact with host cells and play critical roles in supporting host immune function. Among microbes that are typically harmless, some become pathogenic under aberrant conditions. For example, *Staphylococcus aureus* can be either benign or pathogenic. Studies suggest that the balance of indigenous microorganisms present on the skin surface is a critical factor toward preventing both infectious and chronic inflammatory skin diseases (e.g., atopic dermatitis). In this seminar, I will outline the recent progress in research on the skin microbiome and will focus on our current studies that feature *S. aureus* in the pathogenesis of atopic dermatitis.

〈Explanatory material〉

Skin microbial flora

Microorganisms inhabit the skin much in the same way as they do in the gastrointestinal tract and the oral cavity. Microbial flora detected on the skin can be differentiated from those found at other sites. Skin microbial flora typically includes bacteria of the genera *Corynebacterium*, *Cutibacterium*, *Staphylococcus*, and *Micrococcus* as well as fungi (i.e. *Candida* and the dermatophytes) and viruses. It has recently been discovered that the nature and content of skin microbial flora depends on exposure to ultraviolet light, acid-base balance, temparature, water content, sebum content and general topography. The skin microbiome, especially bacteria, has been the subject of study for many years. In early studies, bacterial samples were collected by scraping the skin with a sterile swab and cultured on a nutrient agar to identify the properties of bacteria that grew on it. However, as this method only allowed investigation of culturable bacteria, the diversity of the bacterial flora had been underestimated. New methods based on advanced gene sequencing techniques now permit us to identify the full diversity of bacterial flora. Today, these methods have been applied not only to bacteria but also to fungi and viruses, enabling us to determine the full spectrum of skin microbial flora.

Sequencing technology in research on skin microbial flora

Novel sequencing methods based on specific microbial gene sequences are now used widely to analyze the composition of the microbial flora at the skin as well as other sites. As the conventional Sanger sequencing method was too time-consuming and incapable of this type of analysis, 21st century applications of next generation sequencing (NGS) have facilitated analyses of the composition of the microbial flora. Until quite recently, NGS methodology utilized primarily amplicon sequencing. With this method, the microbial flora were first divided into kingdoms, such as bacteria and fungi, and compositions were determined to the genus level, although not to the species level. Since the development of the shotgun metagenomic sequencing technology, which can perform simultaneous analyses of all the genetic material in a single sample, microorganisms can be identified at the species level. The accuracy of the information provided on the composition of the microbial flora continues to improve.

Skin microbial flora and its relationship with human disease

The composition of skin microbial flora in a healthy human adult remains stable over a long period of time regardless of changes in the environment. The skin microbial flora is believed to prevent pathogen invasion and likewise has a significant impact on the immune response of the host. Changes in the skin microbial flora may be associated with the development of skin disorders. Among several examples, a gram-positive microorganism, *Cutibacterium acnes* characterized with a unique expression profile may be associated with the common adolescent skin condition, acne vulgaris (acne). Likewise, the skin microbial flora in primary immunodeficiency patients may have

a high ratio of favoring microorganisms associated with opportunistic infections, These include *Candida, Aspergillus* spp., *Serratia,* and *Acinetobacter*. Such microorganisms may not only have a direct impact on the onset of skin infections, but also trigger various skin diseases or its progression by inducing physiological or immunological change in the host skin. These microorganisms may have an impact on the incidence of skin infections and may relate directly to the immune responses of the immunocompetent host, which may result in clinical deterioration. This has been noted particularly in the case of *Staphylococcus aureus* and its impact on the occurrence and recurrence of atopic dermatitis. This topic has been the subject of extensive investigation and some elucidation has emerged.

〈Glossary〉

Staphylococcus aureus

Staphylococcus aureus is a facultative anaerobic gram-positive bacterium, that may be among the most pathogenic of the organisms in the genus *Staphylococcus*. This microbe may be detected on the skin and in the nasal cavities of a healthy adult, but can also cause suppurative diseases (e.g., cellulitis and impetigo). It is also a major source of food poisoning and serious systemic diseases, including pneumonia, osteomyelitis, arthritis, endocarditis, sepsis, and toxic shock syndrome. *S. aureus* produces and releases numerous exotoxins, including epidermolytic toxins A and B, toxic shock syndrome toxin-1 (TSS-1), and *S. aureus* enterotoxins (SEs), all of which contribute to disease pathogenesis. *S. aureus* has become resistant to many antibiotics; methicillin-resistant *S. aureus* (MRSA) is one of the most common of the antibiotic-resistant strains.

[Reference materials and websites] as of June 30, 2020

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5) Importance of the skin bacterial flora in immune response. Nature Digest; Nature research (https://www.natureasia.com/ja-jp/ndigest/v9/n10/). Importance of the skin bacterial flora in immune response/39041)

Gastrointestinal Disorders and Intestinal Bacteria:
Advances in Research and Application to Therapy

Toshifumi OHKUSA, M.D., Ph. D.
Special Professor, Department of Microbiota Research, Juntendo University Graduate School of Medicine

[Summary]

Having a range of biological functions including suppression of pathogen invasion into the body, the production of short-chain fatty acids, the production of vitamins, and control of the immune system, gut bacteria are reported to cohabit with human beings, and their research had long focused on bacterial species that could be cultured using ordinary techniques. In the 1990s, however, meta-genome analysis of bacterial genes became possible, showing that unculturable bacteria account for a large portion of gut microbiota which is made up of approximately 40 trillion bacteria of 1000 species. Furthermore, microbiota composition varies among patients with different diseases (dysbiosis) compared with healthy subjects. Therefore, dysbiosis has been increasingly attracting attention as a cause of disease. With regard to gastrointestinal diseases, dysbiosis was reported to be involved in inflammatory bowel disease, irritable bowel syndrome, and non-alcoholic fatty hepatitis, and has recently been said to be of relevance to colorectal cancer, liver cancer, pancreatic cancer, esophageal cancer, and other diseases. In this lecture, I would like to summarize the relationship between gut microbiota and gastrointestinal diseases, and describe new treatments that target gut bacteria.

〈Explanatory material〉

Diagnosis of gastrointestinal diseases and changes in major diseases

Following the discovery of *Helicobacter pylori*, causal factors for cancer, gastric ulcers, duodenal ulcers, and other diseases have been identified, leading to the development of eradication therapies for the bacteria. The prevalence of gastric ulcers, duodenal ulcers, and gastric cancer in Japanese people have thereby been decreasing steadily[1,2]. In contrast, diseases such as inflammatory bowel disease, reflux esophagitis, and colorectal cancer have been increasing. With regard to liver diseases, the discovery of the hepatitis C virus led to the identification of the cause of non-A non-B hepatitis and the development of antiviral drugs for the disease; hence hepatitis C is now curable. As for hepatitis B, antiviral drugs have been developed to enable us to suppress hepatitis progression, making it possible to conquer hepatitis B and C, as well as associated liver cancer. Due to overnutrition, on the other hand, the incidence of non-alcoholic fatty hepatitis has been increasing. In addition, the incidences of biliary and pancreatic diseases such as gallstones, biliary carcinoma and pancreatic cancer have been increasing with recent trends toward an aging society. Given the fact that the genetic background of the Japanese people has remained nearly constant, this change in major gastrointestinal diseases is attributed largely to environmental factors, of which gut microbiota changes associated with dietary lifestyle changes, such as westernization of dietary habits, are drawing attention.

Relationship between Inflammatory Bowel Disease (IBD) and Gut Bacteria

1. In inflammatory bowel disease (IBD), the gut mucosal membrane loses its protective capacity due to leaky gut

It has been brought to attention that in inflammatory bowel disease (IBD), especially ulcerative colitis (UC), increased mucosal permeability leads to leaky gut syndrome. According to a British multicenter study[3], UC is characterized by single-nucleotide polymorphism (SNP) in genes including: HNF4A, which is involved in cell-cell junctions, including tight junctions and adherence junctions; CDH1, which encodes E-cadherin (involved in the formation and maintenance of cell-cell adhesion); and LAMB1, which encodes the laminin β1 subunit (expressed in the gut basal membrane and playing a key role in the immobilization of single-layer epithelium). This SNP reduces the epithelial barrier function, resulting in leaky gut. In addition, many studies have reported that high-fat diets increase mucosal permeability[4], and the recently increasing IBD in Japan is likely to be associated with leaky gut due to a high-fat diet. Therefore, it can be presumed that the leaky gut allows cohabiting gut bacteria to enter the mucosal membrane to cause inflammation and ulcers, manifesting in IBD.

2. Is inflammatory bowel disease caused by gut bacteria?

It has recently been considered that in IBD, collapse of the gut microbiota balance causes dysbiosis, reduces the abundance of anti-inflammatory bacteria, and allows inflammatory bacteria to proliferate, resulting in the development of mucosal inflammation and ulcers[5]. Gut bacteria believed to cause UC include sulphate-reducing bacteria that produce hydrogen sulfide[6], *Fusobacterium varium* (which we have discovered)[7], *Bacteroides vulgatus*[8], and *Escherichia coli*[9]. Suspected causal bacteria of CD include *Mycobacterium paratuberculosis* (*Mycobacterium avium* subspecies *paratuberculosis*)[10], adherent-invasive *Escherichia coli*[11], and more recently, *Fusobacterium nucleatum*[12] and *Klebsiella pneumoniae*[13]. Of course, it seems that these bacterial species alone are not the causal factors, and that their combinations and groups in the same genus may also be causative.

New treatments of gastrointestinal diseases targeting gut bacteria

In addition to IBD, gastrointestinal diseases such as irritable bowel syndrome, chronic constipation, small-intestine ulcers caused by non-steroidal anti-inflammatory drugs, liver diseases including non-alcoholic fatty hepatitis, esophageal cancer, pancreatic cancer and colorectal cancer, have all been shown to share the leaky gut due to increased mucosal permeability and gut microbiota dysbiosis. Accordingly, new treatments targeting gut bacteria have been developed[14], namely antibacterial antibiotics, probiotics with live cells of *lactobacilli* and *bifidobacteria*, prebiotics with *oligosaccharides* for growth of *lactobacilli* and *bifidobacteria*, synbiotics based on a combination of probiotics and prebiotics, and fecal microbiota transplant (FMT). While these treatments have traditionally been used since old times, they have been reevaluated because various diseases were found to be attributable to gut microbiota dysbiosis as stated above, and they are currently administered for the purposes of eradicating causal bacterial groups and correcting gut dysbiosis. Although these new treatments have been somewhat successfully administered in patients who have not responded to conventional treatments, there have been cases of non-responders and a few unwanted effects, keeping the treatments at the pilot study stage. Even so, they are subject to high expectations as groundbreaking cause-specific treatments over conventional symptomatic approaches.

Future outlook

A wide variety of diseases have been attributed to the loss of microbiota diversity, or dysbiosis. Some bacteria increase and others decrease, and causal bacteria are likely to be among the increasing species and groups. We should endeavor to identify causal species and groups, rather than merely attributing the diseases to dysbiosis. The present status of microbiota analysis remains at the genus level, rather than the species level. Therefore, it will take some more time to realize cause-specific treatment. I hope the further progress of research will reach the species level and lead to the development of innovative treatments for the diseases mentioned above. The pathway to the goal is short.

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Gut Microbiota and Cardiovascular Diseases:
Gut Commensal Bacteria That Prevent Atherosclerotic Cardiovascular Diseases

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[Summary]

Recent findings have revealed that commensal gut microbiota is associated with the onset and progression of various diseases. These observations have promoted worldwide research into the use of commensal enteric bacteria as biomarkers to predict disease and also as therapeutic targets. In the cardiology field, studies have suggested that the incidence of cardiovascular events was significantly elevated among those with relatively high blood concentration of trimethylamine-N-oxide (TMAO), a bacterial metabolite derived from the biomolecule, choline.

In our clinical research, we identified two specific commensal species, *Bacteroides vulgatus* and *Bacteroides dorei*, that were represented in enteric microbial flora at significantly lower levels among patients with coronary artery disease compared to levels detected in individuals with other lifestyle-related conditions. We explored this observation using mouse models of arteriosclerosis. We found that mice responded to oral administration of these strains with diminished levels of the proinflammatory agent, lipopolysaccharide (LPS) in both blood and feces. Overall, we concluded that these bacterial species promoted anti-inflammatory effects as well as controlling arteriosclerosis. Similar correlations, were identified in human subjects, i.e., low levels of LPS activity in the feces corresponding with high levels of the two *Bacteroides* species. We are examining the possibility that administration of these bacteria may result in prevention of arteriosclerotic disease. In this seminar, I will present results from these studies.

〈Explanatory material〉

The current state of cardiovascular disease

Heart disease is the second leading cause of death in Japan following cancer[1]. In the aging Japanese society, we observe increasing rates of heart disease, including arteriosclerosis, valvular heart disease, atrial fibrillation, and chronic heart failure. Risk factors for arteriosclerosis include smoking and lifestyle-related disorders, including diabetes, hypertension, and hyperlipidemia. Arteriosclerosis can ultimately result in serious cardiovascular events including acute myocardial infarction and stroke. Even if these risk factors are controlled, cardiovascular events cannot be prevented as effectively as one would hope. There are likely to be other risk factors that have not been identified. Among the risk factors which are yet to be identified are called the "residual risk", for which researches are ongoing to see if their treatment could lead to prevention of cardiovascular events. Such researches have suggested that commensal gut bacteria could be one of the residual risk factors that could both contribute to and prevent cardiovascular diseases such as srteriosclerosis.

Relationship between lifestyle-related diseases and enteric bacteria

Several studies have provided evidence for altered gut microbiota among patients with diabetes. Among these findings, reduced numbers of *Clostridium* spp. and *Akkermansia muciniphila* were detected in the gut microbiomes of diabetic patients compared to those that were non-diabetic. *Clostridium* spp. produce butyric acid, a short-chain fatty acid that has a direct impact on host immune responses and metabolism[2]. Similarly, diabetic mice receiving oral doses of *Akkermansia* spp. responded with improved glucose metabolism and evidence of suppression of arteriosclerotic disease. These findings raised expectations for the possibility of preventing lifestyle-related diseases by altering the gut microbiome[3]. Results from a clinical study conducted in Belgium, in which patients with obesity and glucose metabolism disorders were treated with *Akkermansia* spp., also showed definitive improvements[4].

Metabolites of enteric bacteria that promote cardiovascular events

Choline is found in relatively high concentrations in eggs, dairy products, and shrimp; carnitine, a

biomolecule with similar chemical structure, can be found in high amounts in red meat. These biochemicals are metabolized into trimethylamine (TMA) by enzymes produced by enteric bacteria in the digestive tract. TMA is absorbed in the intestinal tract and metabolized into trimethylamine-*N*-oxide (TMAO) by liver enzymes. There is one report demonstrating that high blood levels of TMAO are associated with an increase in cardiovascular events[5]. This result suggests that specific enteric bacteria may contribute to deteriorating arteriosclerotic disease by producing metabolites that are associated with the occurrence of cardiovascular events. Efforts are currently underway to develop drugs to prevent arteriosclerosis by inhibiting enzymes that result in the production of TMA[6].

Components of bacteria that promote inflammation

Lipopolysaccharide (LPS), a cell membrane component from Gram-negative bacteria, activates immune cells via Toll-like receptors and causes serious inflammation. This inflammation further leads to progression of infectious diseases such as sepsis. Gram-negative bacteria themselves often initiate infections. However, the level of LPS's inflammation-evoking power varies depending on the bacteria. The kinetics of LPS *in vivo*, including transit from the intestinal tract to the blood, has not been entirely revealed.

Bacteroides species

Bacteroides species have been reported to decrease in obese people. There are also reports in Japan and China that they reduced in patients with coronary artery disease[7,8]. Recently, it has been reported that data in Japanese dementia patients showed a decrease in *Bacteroides*, to which attention has been attracted. Another report[9] presented a result from an animal experiment indicating that, in addition to the fact that *Bacteroides* are abundant in people without coronary artery disease, they prevent arteriosclerosis. In response, we have been studying the possibility of using *Bacteroides* to prevent arteriosclerosis.

Future outlook

As the association between the gut microbiota and the pathogenesis of human disease has been recognized, gut bacteria have been identified as potentially new therapeutic targets. The composition of the gut microbiota differs between individuals, and can have a direct and personalized impact on physical constitution. An improved understanding of the gut microbiota will help us to design personalized and tailored therapeutic strategies. In the future, evaluation of the gut microbiome may become a feature of a routine medical checkup.

[Reference materials and websites] as of June 30, 2020

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