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HERBS USED TO TREAT SKIN DISEASES

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ABSTRACT

Skin diseases are numerous and a frequently occurring health problem affecting all ages from the neonates to the elderly and cause harm in number of ways. Maintaining healthy skin is important for a healthy body. Many people may develop skin diseases that affect the skin, including cancer, herpes and cellulitis. Some wild plants and their parts are frequently used to treat these diseases. The use of plants is as old as the mankind. Natural treatment is cheap and claimed to be safe. It is also suitable raw material for production of new synthetic agents. A review of some plants for the treatment of skin diseases is provided that summarizes the recent studies. Natural drugs from the plants are gaining popularity because of several advantages such as often having fewer side-effects, better patient tolerance, being relatively less expensive and acceptable due to a long history of use. Besides herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. For these reasons several plants have been investigated for treatment of skin diseases ranging from itching to skin cancer. So far 31 plants have been reported to be effective in various skin diseases during the past 17 years (1995-2012) of research work, which are mentioned below.

Achyranthes aspera (Common name: Prickly chaff flower, Devil's horsewhip; Family: Amaranthaceae)
Traditionally, the plant is used in boils, scabies and eruptions of skin and other skin diseases. The MeOH extract, alkaloid, non-alkaloid and saponin fractions obtained from the leaves of A. aspera exhibited significant inhibitory effects (concentration 100 µg) on the Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. In this in vitro assay the non-alkaloid fraction containing mainly non-polar compounds showed the most significant inhibitory activity (96.9%; 60% viability). In the in vivo two-stage mouse skin carcinogenesis test the total methanolic extract possessed a pronounced ant carcinogenic effect (76%). The results revealed that leaf extract and the non-alkaloid fraction are valuable antitumor promotors in carcinogenesis.

Allium cepa (Common name: Onion; Family: Liliacea)
A study undertaken in patients with seborrheic keratoses to evaluate the ability of onion extract gel to improve the appearance of scars following excision, has shown that this extract gel improved scar softness, redness, texture and global appearance at the excision site at study weeks 4, 6 and 10 as assessed by the blinded investigator. In another study, the antifungal activity of aqueous extracts prepared from A. cepa (onion; AOE) and Allium sativum (garlic; AGE) were evaluated against Malassezia furfur (25 strains), Candida albicans (18 strains), other Candida sp. (12 strains) as well as 35 strains of various dermatophyte species. The results indicated that onion and garlic might be promising in treatment of fungal Pharmacognosy Reviews

A. sativum (Common name: Garlic; Family: Liliaceae)
In a study conducted on Swiss albino mice in whom cancer was induced by 7,12-dimethylbenz(a)anthracene (DMBA) revealed that best chemo preventive action of garlic was observed in mice in which garlic treatment was performed before and after the induction of skin carcinogenesis. Garlic ingestion delayed formation of skin papillomas in animals and simultaneously decreased the size and number of papillomas, which was also reflected in the skin histology of the treated mice. The protective effect against skin cancer elicited by garlic in mice is believed to be due at least in part to the induction of cellular defense systems.

Aloe vera (Common name: Barbados aloe; Family: Xanthorrhoeaceae)
Aloe vera has shown very good results in skin diseases and it is often taken as health drink. It is also found effective in treating wrinkles, stretch marks and pigmentations. It also seems to be able to speed wound healing by improving blood circulation through the area and preventing cell death around a wound. One of the studies conducted on mice to investigate the effects of Scutellariae radix and Aloe vera gel (AV), in spontaneous atopic dermatitis (AD)-like skin lesions revealed that the group receiving only AV in a dose of 0.8 mg/kg p.o provided relief in AD due to reduction of interleukin (IL)-5 and IL-10 levels.[10]
The gel has properties that are harmful to certain types of bacteria and fungi. A cream containing 0.5% aloe for 4 weeks reduced the skin “plaques” associated with psoriasis.[3] Application of gel helped in the improvement of partial thickness burns.[4] When applied to the skin, the gel seems to help skin survive frostbite injury.[5] It might delay the appearance of skin damage during and after radiation treatment.[6]
Azadirachta indica (Common name: Neem; Family: Meliaceae)

Leaf extract is applied externally on boils and blisters.[7] In one study, skin tumors were induced in mice by topical application of DMBA (500 nmol/100 µl for 2 weeks) followed by TPA (1.7 nmol/100 µl of acetone, twice weekly) as a promoter. The test group received aqueous Azadirachta indica leaf extract (AAILE) orally at a dose level of 300 mg/kg body weight three times a week for 20 weeks. The results of this study revealed the chemopreventive potential of A. indica against murine skin carcinogenesis.[8] Study designed to determine the modulatory effect of aqueous AAILE on cell cycle-associated proteins during two-stage skin carcinogenesis in mice in which skin tumors were induced by topical application of DMBA as a carcinogen followed by the repetitive application of TPA as a promoter. Skin tumors obtained in the DMBA/TPA group exhibited enhanced expression of proliferating cell nuclear antigen (PCNA, index of proliferation), p21 and cyclin D1, with no alterations in p53 expression in comparison to the control group. Tumors in AAILE + DMBA/TPA group exhibited low PCNA and cyclin D1 expression and enhanced expression of p53 and p21 in comparison to the DMBA/TPA group. The skin tumors obtained in the AAILE + DMBA/TPA group exhibited high lipid peroxidation levels in comparison to the tumors obtained in the DMBA/TPA group. The observations of the study suggested that AAILE behaves as a pro-oxidant in the tumors, thereby rendering them susceptible to damage, which eventually culminates into its anti-neoplastic action. Also, cell cycle regulatory proteins may be modulated by AAILE and could affect the progression of cells through the cell cycle.Another study, conducted on an anti-acne moisturizer formulated from herbal crude extracts and investigated for the physico-chemical parameters as well as antibacterial activity of the formulation, revealed that ethanol extract of Andrographis paniculata, Glycyrrhiza glabra, Ocimum sanctum, A. indica and Green tea possessed the potential for inhibiting acne. It was observed that the optimal formula of anti-acne moisturizer was satisfactorily effective to control acne inducing bacteria i.e., Staphylococcus epidermis and Propionibacterium. Herbals have great potential to cure different kinds of skin diseases. More than 80% of people in India depend on traditional health care and use different plant based products for curing skin related problems.

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PLANT VARIATIONS IN MANAGEMENT OF DERMAL DISORDERS

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ABSTRACT

Maintaining healthy skin is important for a healthy body. Many people may develop skin diseases that affect the skin, including cancer, herpes and cellulitis. Some wild plants and their parts are frequently used to treat these diseases. It is also suitable raw material for production of new synthetic agents. A review of some plants for the treatment of skin diseases is provided that summarizes the recent technical advancements.
The common medications for topical use include[7]: Antibacterials: These medicines, like bactroban or cleocin, are often used to treat or prevent infection, Anthralin (drithocreme, micanol and others): Although not often used, these help to reduce inflammation and can help treat psoriasis, Antifungal agents: Lamisil, lotrimin and nizoral are few examples of common topical antifungal drugs used to treat skin conditions such as ringworm and athlete's foot Benzoyl peroxide: Creams and other products containing benzoyl peroxide are used to treat acne Coal tar: This topical treatment is available with and without a prescription, in strengths ranging from 0.5% to 5%. Coal tar is used to treat conditions including seborrheic dermatitis (usually in shampoos) or psoriasis. Currently, coal tar is seldom used because it can be slow acting and can cause severe staining of personal clothing and bedding Corticosteroids: These are used to treat skin conditions including eczema and come in many forms including foams, lotions, ointments and creams Retinoids: These medications (such as retin-A and tazorac) are gels or creams derived from vitamin A and are used to treat conditions including acne Salicylic acid: This medication is available in the form of lotions, gels, soaps, shampoos and patches. It should be used sparingly as putting too much on one's body at once can cause toxicity. Salicylic acid is the active ingredient in many skin care products for the treatment of acne and warts.

Oral treatments for skin conditions include: Antibiotics: Oral antibiotics like erythromycin, tetracycline and dicloxacillin are used to treat many skin conditions, Antifungal agents: Common oral antifungal drugs such as ketoconaole and diflucan can be used to treat more severe fungal infections, Antiviral agents: Common antiviral agents include valtrex, acyclovir and famavir. Antiviral treatments are used for skin conditions including those related to herpes, Corticosteroids: These medications, including prednisone can be helpful in treating skin conditions linked to autoimmune diseases including vasculitis and inflammatory diseases such as eczema and psoriasis. Dermatologists prefer topical steroids to avoid side-effects; however, short-term use of prednisone is sometimes necessary Immunosuppressants: Immunosuppressants, such as azathioprine and methotrexate, can be used to treat conditions including severe cases of psoriasis and eczema Biologics: These new therapies are the latest methods being utilized to treat psoriasis and other conditions. Examples of biologics include enbrel, humira, remicade, stelara and amevive.

Cannabis sativus (Common name: Charas, Ganja; Family: Cannabinaceae)
The powder of the leaves serves as a dressing for wounds and sores. Ganja is externally applied to relieve pain in itchy skin diseases. Hemp seed oil is useful for treatment of eczema and host of other skin diseases like dermatitis, seborrhoeic dermatitis/cradle cap, varicose eczema, psoriasis, lichen planus and acne roseacea. By using hemp seed oil, the skin is strengthened and made better able to resist bacterial, viral and fungal infections. Crushed leaves are rubbed on the affected areas to control scabies.

Crocus sativus (Common name: Saffron; Family: Iridaceae)
Saffron is a naturally derived plant product that acts as an antispasmodic, diaphoretic, carminative, emmenagogic and sedative. The chemopreventive effect of aqueous saffron on chemically induced skin carcinogenesis using a histopathological approach was studied. Its ingestion inhibited the formation of skin papillomas in animals and simultaneously reduced their size. Saffron inhibited DMBA-induced skin carcinoma in mice when treated early. This may be due, at least in part, to the induction of cellular defense systems.[8] It has also been found useful in treatment of psoriasis.[9]

Curcuma longa (Common name: Turmeric; Family: Zingiberaceae)
A study conducted on male Swiss albino mice in whom skin cancer was induced by topical application of DMBA, revealed a significant reduction in number of tumors per mouse in the group receiving 1% curcumin obtained from rhizomes of C. longa.

Euphorbia walachii, Euphorbia hirta, Euphorbia tirucalli (Common name: Wallich spurge; Fam. Euphorbiaceae)
Juice of E. walachii is used to treat warts and skin infections.[6] A study, conducted on various species of Euphorbia, E. hirta, exhibited best antioxidant activity. The plant extracts showed more activity against Gram-positive bacteria and fungi. The best antimicrobial activity was shown by E. tirucalli. The study supported the folkloric use of E. hirta and E. tirucalli against some skin diseases caused by oxidative stress or by microorganisms.

Ficus carica, Ficus racemosa, Ficus bengaalensis (Common name: Fig; Family: Moraceae)
In some rural areas of Iran, a traditional method for the treatment of warts comprises the use of fig tree (F. carica) latex. A study conducted in patients with warts has revealed that this therapy of warts offers several beneficial effects including short-duration therapy, no reports of any side-effects, ease-of-use, patient compliance and a low recurrence rate. Although, exact mechanism of the antiwart activity of fig tree latex is unclear it is likely to be the result of the proteolytic activity of the latex enzymes.[3] F. recemosa L. bark powder is used externally in case of pimples, itches and scabies and F. bengaalensis L. bark powder is also used externally to cure scabies.[7]

Lavendula officinalis (Common name: Lavender; Family: Labiatae)
The effects of lavender oil (1:500, 1:100, 1:10, 1:1, 1:0) on mast cell-mediated immediate-type allergic reactions in mice and rats have been studied. It has been reported to inhibit concentration-dependently the histamine release from the peritoneal mast cells. It also inhibits immediate-type allergic reactions by inhibition of mast cell degranulation in vivo and in vitro when tested on mice and rats

Lawsonia inermis (Common name: Henna; Family: Lythraceae)
Henna is a traditionally used plant of Middle-East that is applied on hands and feet. In the traditional system of medicine, leaf paste is applied twice a day, on the affected parts to cure impetigo.[4] In a study, clinical improvement in the patients suffering from hand and foot disease due to use of capecitabine, an anti-cancer drug, with use of henna revealed anti-inflammatory, antipyretic and analgesic effects of henna.[10]

Mangifera indica (Common name: Mango; Family: Anacardiaceae)
The gum is used in dressings for cracked feet and for scabies. Latex is applied to cure ulcers.[7] Aqueous extract of stem-bark (MIE, 50-800 mg/kg i.p.) produced a dose-dependent and significant ($P < 0.05$-0.001) anti-inflammatory effect against fresh egg albumin-induced paw edema in rats.

Prunus persica (Common name: Peach; Family: Rosaceae)
Ethanolic extract of the flowers (Ku-35) (50-200 µg/ml) were found to inhibit UVB and UVC induced deoxyribonucleic acid (DNA) damage by the COMET assay in the skin fibroblast cell (NIH/3T3). In addition, Ku-35 inhibited UVB-or UVC-induced lipid peroxidation, especially against UVB-induced peroxidation at higher than 10 µg/ml.

Thyme vulgaris (Common name: Thyme; Family: Lamiaceae)
It may relieve the symptoms of cellulitis, an infection of the skin caused by bacteria which can lead to pain, tenderness, edema, fever, chills and reddening of the skin. It may also offer anti-fungal and antibacterial benefits. However, the University of Maryland Medical Center cautions that thyme has not been proven to specifically benefit cellulitis. In addition, this herb may raise the risk of bleeding.
Herbals have great potential to cure different kinds of skin diseases. More than 80% of people in India depend on traditional health care and use different plant based products for curing skin related problems. More than 50% of plant species useful for treatment of skin diseases appear to be restricted to forests, so activities such as deforestation, habitat destruction, urbanization etc., may pose a serious threat to these species.

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SKIN PROTECTANT CELLULAR AND INTRACELLULAR EFFECTS OF MELATONIN

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ABSTRACT

The environmental factors as radiation, physical injuries, chemicals, pollution, and microorganisms, the skin requires protective chemical molecules and pathways. Melatonin, a highly conserved ancient molecule, plays a crucial role in the maintenance of skin. As human skin has functional melatonin receptors and also acts as a complete system that is capable of producing and regulating melatonin synthesis, melatonin is a promising candidate for its maintenance and protection. Below, we review the studies of new metabolic pathways involved in the protective functions of melatonin in dermal cells. We also discuss the advantages of the topical use of melatonin for therapeutic purposes and skin protection. In our view, endogenous intracutaneous melatonin production, together with topically-applied exogenous melatonin and its metabolites, represent two of the most potent defense systems against external damage to the skin.

The pineal gland secretes melatonin into the blood circulation to exert a range of well-documented physiological functions.

Classical chronobiology considers melatonin exclusively a hormone that regulates the circadian day–night rhythm and seasonal biorhythms. At least in part, these effects of melatonin are indirectly mediated by coupling to other endocrine systems, whose output/signalling activity is modulated by the photoperiod-dependent pineal secretion of melatonin. Additionally, currently recognized physiological melatonin activities in the mammalian system include the modulation of immune defense responses, body weight and reproduction, tumor growth inhibitory and anti-jet-lag effects[1].

Independent of these effects, melatonin exerts many direct, receptor-independent activities, acting for example as a potent direct antioxidant, as a chemotoxicity reducing agent and a putative anti-aging substance Melatonin is a highly lipophilic substance that easily penetrates organic membranes and therefore is able to protect important intracellular structures including mitochondria and DNA from oxidative damage directly at the sites where such damage occurs.

Intriguingly, melatonin also up-regulates gene expression and activity of several antioxidative enzymes such as Cu/Zn-superoxide dismutase (CuZn-SOD), Mn-superoxide dismutase (Mn-SOD), catalase and glutathione peroxidase (GPx)[2]. Thus, melatonin not only acts as a potent antioxidant itself, but also is capable of activating an entire endogenous enzymatic protective system against oxidative stress t is now evident that the physiological level of melatonin has to be defined individually for each tissue, since the body liquids, tissues or organs mentioned above reveal melatonin levels which are 10- to 1000-fold higher than plasma melatonin concentrations which formerly might have been considered as ‘pharmacological’. However, this observation throughout several completely different body compartments is highly suggestive for local tissue-specific melatonin synthesis since plasma levels would be too low to build this high tissue levels[3]. Therefore, the presence of tissue-specific, local melatoninergic systems have been suggested that would have the biological role of counteracting specific, tissue-related regional stressors exactly at the place where they occur.

In fact, such a melatoninergic antioxidative system (MAS) has been discovered recently in a highly differentiated manner in the skin Since changes in skin and coat phenotype/function represent a major form of mammalian adaptation to changing environmental challenges, it is not surprising that melatonin – the major neuroendocrine regulator that couples photoperiod changes to complex endocrine responses – impacts on mammalian skin physiology. In fact, indications that melatonin is involved in the regulation of seasonal hair growth and pigmentation can already be traced back several decades[4]. For example, in several mammalian species, melatonin can alter wool and cashmere production, the development and frequency of pelage cycling and seasonal moulting as well as coat colour.

While the effects of melatonin on hair follicle biology have long been most obvious, yet are still insufficiently understood. This should not detract from the accumulating body of evidence that melatonin’s functions in skin biology and skin pathology extend far beyond the modulation of hair growth and/or pigmentation. A few examples may suffice to illustrate this wide range of – at times, seemingly contradictory – functions.

Melatonin suppresses apoptosis and stimulates growth in both serum-starving HaCaT keratinocytes and serum-free-cultured fibroblasts. In contrast, the growth of serum-supplemented HaCaT keratinocytes is inhibited by melatonin at low concentrations, whereas very high concentrations of melatonin (4–20 × 10^{-6} MOL) were found to stimulate cell growth under the same serum-supplemented culture condition.

Strikingly, pinealectomized (i.e. melatonin-deficient) rats have been reported to show markedly reduced back, abdominal and thoracic skin thickness, along with an increase of lipid peroxidation and a decrease in the number of dermal papillae and hair follicles as well as of antioxidative enzymes (CAT, GPx). Melatonin substitution to these rats reportedly restored skin thickness, reduced lipid peroxidation and enhanced antioxidative enzyme activity These results were later supplemented by the same group by ultrastructural evidence: compared to unsubstituted animals, melatonin-treated,

pinealectomized rats showed reduced cytological atypia, decrease of nuclear irregularity, normalization of tonofilament distribution and mitochondrial integrity as well as of dermal collagen fibre structure[4].

Collagen synthesis is controlled by proline hydroxylase which uses superoxide anion radical as the specific substrate together with L-proline yielding hydroxyproline on the precollagens. The removal of the ROS superoxide anion radical by melatonin would therefore prevent collagen synthesis. This corresponds well to the finding that melatonin also protects against pressure-induced ulcer formation in rat skin, as reflected by reduced lipid peroxidation, tissue neutrophil infiltration, along with increased glutathione (GSH) levels and reduced degenerative skin changes. One of many arguments that advocate the administration of melatonin as a therapeutic adjuvant in burns patients is that skin damage induced by thermal injury is reduced by melatonin, likely by limiting oxidative damage[5]. Oxidative damage is also a key pathogeneic element in skin flap necrosis after plastic surgery: in pinealectomized rats, skin flaps of melatonin-treated animals exhibited reduced lipid peroxidation, nitric oxide formation and ratio of skin flap necrosis, along with increasing levels of GSH, GPx and superoxide dismutase (SOD) compared to non-melatonin-treated rats.

Clinically, topically applied 0.5% melatonin reduces UV-erythema when administered before, but not when applied after UV-irradiation. This was confirmed by another group showing that not only melatonin but also other antioxidants (vitamin E and vitamin C) have no effect on UV-erythema when administered after UV-irradiation, irrespective of the time course of application.

Associated immunological skin responses, as exemplified by UV-induced suppression of the Mantoux response, are also not inhibited by melatonin when applied after UV-exposure. This indicates that the UV-induced free radical formation in skin is an immediate event which can only be antagonized by antioxidants that are already present at the target sites and at the time point of UV-exposure.

The antioxidant and DNA repair properties of melatonin raise the theoretical possibility that it may also prevent or reduce cutaneous photo-aging. In healthy skin, melatonin reduces the collagen accumulation, an indicator of skin aging. Melatonin also inhibits chemically induced carcinogenesis in rat skin, represented by reduction of the number of benzo(a)pyrene-induced papillomas; this is paralleled by attenuated lipid peroxidation and prevention of the binding of benzo(a)pyrene or its metabolites to DNA [6].Indeed, melatonin treatment reportedly reduced benzo(a)pyrene-induced tumor frequency by 30% in mice. Melatonin may also play a role in the thermoregulatory control of human skin blood flow, at least in healthy males.

A few selected aspects of melatonin's proposed role as a major skin protectant deserve to be discussed in more detail, since they are of particular clinical and/or pharmaceutical interest. The photo-induced melatonin metabolism leading to the generation of antioxidant melatonin metabolites in human keratinocytes represents an antioxidative cascade which has been described earlier for chemical or other tissue homogenate systems and has now been identified in the skin to protect this important barrier organ against UVR-induced oxidative stress-mediated damaging events on DNA subcellular, protein and cell morphology level. This newly identified MAS of the skin likely extends to skin compartments beyond the epidermis, namely to the dermis and the hair follicle, and may have evolved as a defense mechanism against the multi-facetted threats of environmental stress, especially UVR, to which the skin is life-long exposed.

The UV-induced melatonin metabolites, especially AFMK, are themselves potent antioxidants . ROS – mainly the hydroxyl radical – occurring under UV-irradiation in the skin react directly with melatonin. The latter is either autonomously produced in epidermal and/or hair follicle keratinocytes where it engages in intracrine signalling/interactions or released into the extracellular space to regulate auto-, para- or endocrine signalling. The reaction of melatonin with hydroxyl radicals induces the formation of 2-OH-melatonin and 4-OH-melatonin which are then further metabolized to AFMK and by arylamine formamidase or catalase to AMK[7]. During this process, hydroxyl radicals are scavenged, and resulting damaging events are either indirectly or directly reduced via decrease of lipid peroxidation, protein oxidation, mitochondrial damage and DNA damage.

For application in clinical dermatology, exogenous melatonin should rather be used topically than orally, since orally administered melatonin appears in rather low levels in the blood due to prominent first-pass degradation in the liver, thus limiting skin access.

Topical administration circumvents this problem. In addition, as we could show in our own investigations, melatonin can penetrate into the stratum corneum and build there a depot due to its distinct lipophilic chemical structure.

Therefore, endogenous intracutaneous melatonin production, together with topically applied exogenous melatonin, can be expected to provide the most potent defense system against cutaneous photodamage and multiple other pathologic conditions that produce oxidative stress (e.g. in chronic skin inflammation, such as atopic dermatitis)

In chemotherapy-induced damage, melatonin significantly reduces cisplatin-induced testicular toxicity in rats. Also, amicacin- or cisplatin-induced nephrotoxicity in rats is prevented by melatonin through enhancement of the GSH (reduced glutathione)/GSSG (oxidized glutathione) ratio, reduction of lipid peroxidation and restoration of the enzymatic antioxidant GPx. In primary rat renal tubular cisplatin-treated epithelial cells, melatonin exerts its protective effects via scavenging ROS and reducing DNA fragmentation, much stronger than its precursors or metabolites such as tryptophan, serotonin or 6-hydroxymelatonin[8]. Melatonin also protects against doxorubicin-induced cardiotoxicity in rats by stimulating the activity of antioxidative enzymes (CAT, GSH), reducing lipid peroxidation and protecting against mitochondrial damage.

This suggests that melatonin can potently protect against chemotherapy-induced damage through different biological mechanisms in a number of organs. Unfortunately, this has not yet been investigated in a dermatological context.

Melatonin may even protect the skin against the highly destructive effects of IR. The skin ranks among the chief target tissues for the well-recognized undesired effects of IR (years, while SCC development is strongly correlated with IR in combination with cumulative UV-irradiation exposure[9], with basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) representing the most common IR-induced skin malignancies. BCC specially develops when IR occurs before the age of 20). The molecular precondition for IR-induced skin cancer development is severe and widespread DNA damage, predominantly due to IR-mediated hydroxyl radical generation.

Hydroxyl radicals are a result of IR-induced radiolysis of water, leading to formation of oxidized bases, DNA–DNA intrastrand adducts, DNA single- and double-strand breaks and DNA–protein cross-linking which all lead to genomic instability, a prerequisite for tumor promotion and development .

Since melatonin is a highly efficient hydroxyl radical scavenger, it is not unexpected that it acts highly protective against IR-induced damage at a single time point or from lymphocytes which were preincubated).

Melatonin markedly inhibited formation of chromosome aberrations and micronuclei in IR-exposed lymphocytes separated before IR from healthy volunteers who orally took melatonin (300 *in vitro* m with melatonin at the concentration of 2MOL (Gy of IR, the cell survival rate was reduced to 37%, whereas preincubation with melatonin.

When cultured human fibroblasts were exposed to 8μMOL) led to an increased survival rate of 68%. These survival enhancing effects of melatonin correlated with reduced lipid peroxidation of the cell membranes (represented by lowered malondialdehyde levels) and decreased apoptotic pre-G1 peak. Of note, the pathways influenced by melatonin were not p53- nor p21-dependent. Interestingly, the use of different antioxidants (including trolox, the water-soluble analogue of α-tocopherol) has shown that the antioxidant must be applied before IR-exposure in order to effectively scavenge ROS formed during IR, just as it is true for the antioxidant effects of melatonin in connection with UVR.

Since the discovery of the strong antioxidant properties of melatonin, which until then had exclusively been appreciated as a circadian and seasonal biorhythm regulator, a tremendously wide spectrum of targets and effects of melatonin has evolved in a great variety of tissues and organisms.

The predominant feature of melatonin that has surfaced in consequence is that of a potent cytoprotective substance on multiple different levels of cell damage, both in physiological and pharmacological concentrations.

The presence of specific and functionally active membrane, cytosolic and nuclear melatonin receptors in mammalian (including human) skin and its appendages suggests the skin to be a major melatonin target. Parallely the demonstration of AANAT activity in hamster skin of transcripts for melatonin-synthesizing enzymes in human skin and hair follicle cells as well as in cutaneous tissues and of inducible melatonin synthesis and metabolism in keratinocytes and hair follicles identifies mammalian skin and its appendages as major extrapineal sites of melatonin synthesis and metabolism[10]. A steadily growing body of evidence now supports that the functional role of melatonin and its metabolites fully extends to skin and hair biology/pathology including the effects of melatonin on heat- and pressure-induced skin injury, ulcer formation, apoptosis, necrosis, melanogenesis, hair shaft growth and hair follicle receptor modulation as well as tumor growth suppression. Finally, the main environmental skin stressors (UVR, IR) are effectively counteracted by melatonin in the context of a complex intracutaneous MAS.In fact, in human biology, the skin may be unrivalled as a model organ for elucidating the full range of melatonin functions, targets, metabolism, receptors and regulation in health and disease. Moreover, growing evidence suggests that ligands of membrane, nuclear and cytosolic melatonin receptors (including antioxidant melatonin photoproducts) may be recruited as adjuvant therapy in a wide range of problems in clinical dermatology, ranging from wound healing via vitiligo, atopic eczema, sarcoidosis, diabetic foot syndrome and pruritus to carcinoma and melanoma.

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STEM CELLS OF THE SKIN

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ABSTRACT

Stem cells are undifferentiated cells capable of generating, sustaining, and replacing terminally differentiated cells and tissues. They can be isolated from embryonic as well as almost all adult tissues including skin, but are also generated through genetic reprogramming of differentiated cells. Preclinical and clinical research has recently tremendously improved stem cell therapy, being a promising treatment option for various diseases in which current medical therapies fail to cure, prevent progression or relieve symptoms. With the main goal of regeneration or sustained genetic correction of damaged tissue, advanced tissue-engineering techniques are especially applicable for many dermatological diseases including wound healing, genodermatoses (like the severe blistering disorder epidermolysis bullosa) and chronic (auto-)inflammatory diseases. This review summarizes general aspects as well as current and future perspectives of stem cell therapy in dermatology.

Stem cells (SCs), common to all multicellular organisms, are specified as undifferentiated self-replicating cells possessing the ability to generate, sustain and replace terminally differentiated cells. They show two key features: self-renewal (cell divisions with maintenance of the undifferentiated state), and capability of in vivo and in vitro reconstitution of a given tissue via differentiation into specialized cell types1. SCs are commonly subdivided into two main entities, embryonic stem cells (ESCs) (pluripotent) and adult SCs (multipotent or unipotent). A third category of “embryonic-like” cells, so-called induced pluripotent cells (iPSCs), has been added in the last years. iPSCs are developed through genetic manipulation of differentiated cells. Classical hierachial model of stem cell differentiation. ESC: embryonic stem cell, iPSC: induced pluripotent stem cell, NSC: neural stem cell, EpSC: epidermal stem cell, HSC: hematopoietic stem cell, MSC: mesenchymal stem cell.The attributes “pluri-, multi- and unipotent” describe the SC's potential to yield a range of cell lineages. While pluripotent SCs are able to give rise to all cell types in an organism, multipotent and unipotent SCs remain restricted to specific tissue(s) or lineages. The level of potency is linked to the developmental stage of the organism and is evaluated by functional assays and assessment of various cellular/molecular markersContinuous exposure of the skin to environmental mechanical and chemical stress requires permanent self-renewal of the epidermis, dermis and adnexa (hair follicles [HFs], sebaceous glands, and sweat glands), even into adulthood, to maintain its diverse functions (e.g., as a barrier). This self-renew ability is contributed to the skin own SCs, which are slow cycling multipotent cells located in the epidermis, dermis and the HFs. In response to external stimuli like wounding, they start to proliferate in order to regenerate the skin tissue2.

When primary cultures of keratinocytes are grown in vitro, three types of colony cell growth develop, i.e., holo-, mero- and paraclones. They represent the proliferative compartment of human squamous epithelia. However, only the holoclone-forming cells possess full self-renewing capabilities and long-term regenerative potential, harbouring the features of epidermal SC. Notably, the term holoclone only describes the proliferative capacity of a keratinocyte in vitro. Nevertheless the progeny of a single epidermal holoclone can regenerate a fully functional epidermis in vivo. Their decendents, i.e., meroclone- and paraclone-forming cells, instead show a gradual loss of SC function with only limited proliferative capacity and self-renewal. Paraclone-forming cells are defined by a short replicative lifespan (up to 15 cell generations) after which they terminally differentiate, whereas meroclone-forming cells represent a transitional stage between the holoclone and the paraclone. The latter possesproliferative properties expected from transient-amplifying cells, which are an undifferentiated population in transition between SCs and differentiated cells, The process by which adult epidermal SCs renew themselves and yield daughter cells depend on the tissue type and various other conditions, to include developmental stage, environmental injury, steady tissue turn-over and remodeling. Two models of epidermal differentiation and regeneration (hierarchical versus stochastical) have been described, in order to elucidate the nature and behaviour of interfollicular epidermal SCs, lying within the basal layer. The conceptual framework for these SC niches, their structure, compositions and operating process is steadily being updatedSkin SCs reside in specialized morphological and functional units with a specific microenvironment. These so-called niches may contain various SCs as well as supportive cells providing framework or signaling to the SCs. Within human skin, at least five different niches have been delineated (basal layer of the epidermis, HF bulge, base of sebaceous gland, dermal papillae and dermis), that harbor different types of skin SCs5:

a) Interfollicular epidermal SCs are scattered singly across the dermal-epidermal junction. In the mucosa and on the palms and soles, SCs are located at the baseof the rete ridges. They constitute about 1%~7% of epidermal basal cells. Several human SC markers have been described, including high surface expression of α6 and β1 integrins that may be relevant for sustaining the attachment of epidermal SC to their basement membrane through hemidesmosomes. Progenies from epidermal SCs that withdraw from the cell cycle3, show a suppression of integrin α6 expression, before they start

differentiating and moving towards the skin surface, where they slough off along terminal differentiation after approximately 4 weeks. Furthermore, p63 (a homologue of tumor suppressor p53), a low expression of transferring receptor (CD71) and desmoglein 3 as well as LRIG1, the scaffold protein FERM domain-containing protein 4A (FRMD4A), and CD46 have been established as interfollicular SC markers6.

b) Beside tissue regeneration interfollicular SCs have been shown to be invested with the ability of generating hairs3. In HFs, several distinct SC-types have been identified. One multipotent SC population resides in the bulge located at the base of the HF (during telogene phase of hair development) or beneath the HF-associated sebaceous gland (in anagen phase). This follicular component is established during embryonic hair morphogenesis and resists periodic degeneration during the hair growth cycle4. Stimulation of these SC to exit their niche as well as their proliferation and differentiation to form mature HFs is closely linked to the hair growth cycle. HF bulge SC show expression of the molecular markers such as cluster of differentiation 200 (CD200), keratin 15 (K15), Lgr5+ and pleckstrin homology-like domain family A, member 1 (PHLDA1) as well as transcription factors Sox9+, Lhx2+ and NFATc Beside these epidermal SCs, another multipotent precursor cell population resides in HFs and dermal papillae that originate from the embryonic neural crest. These epidermal neural crest SCs (EPI-NCSCs) hold clonal multipotency that can give rise to melanocytic, neuronal and myogenic cell lineages in vitro and show differentiation potential toward mesenchymal lineages, as they are able to give rise to adipocyte, chondrocyte, and osteocyte progeny7. Because of their advantageous physiological plasticity, multipotency, simple accessibilityand non-controversial ethical issues, these EPI-NCSCs are considered promising donor cells for the repair of nervous system injuries.

a) Sebaceous glands, attached to the HFs, are supposed to descend from different follicle SC populations, including Krt15+ bulge cells, LGR6+ and junctional zone SCs. Other studies describe the existence of periglandular Blump1-expressing sebaceous progenitors and a SC population within the gland itself. Progenitors give rise to terminally differentiated sebocytes that degenerate along holocrine secretion, releasing lipid-rich sebum into the hair canal that maintain an adequate lubrication of the skin surface8.

b) Melanocyte SCs derive from the neural crest and permanently reside in the HF bulge, basal epidermis and probably also in the dermis. They give rise to pigment-producing melanocytes in the epidermis and the hair matrix. The fate of the melanocytes within the follicle is connected to the HF phases, where melanocytes proliferate and differentiate during anagen, and diminish through apoptosis in catagen. Dysfunction of this SC population results in pigmentation defects that phenotypically manifest as hair graying. The latter underlies an increased apoptosis of melanocyte SCs due to higher oxidative stress subsequent to the deficiency of anti-apoptotic Bcl2 protein that occurs with aging9.

c) The steady remodeling of the dermis and fibroblasts as their primary cellular component is managed via mesenchymal SCs10. They are located in the connective tissue within the dermis, surround HFs (especially in the follicular sheath and papillae) or are found among pericytes around blood vessels. Beside fibroblasts, dermal mesenchymal SCs generate myofibroblasts, endothelial cells, nerves, blood vessels, osteoblasts, chondrocytes and adipocytes. Morevoer, they are crucial for the coordination of the complex process of wound healing by attracting other host cells, growth factors and extracellular matrix (ECM) secretory proteins. Dermal SCs lack uniform distinctive markers but adhere to plastic in contrary to other SC11s.

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THE USE OF ADIPOSE-DERIVED STEM CELLS IN SKIN DISEASES (NONHEALING WOUNDS)

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ABSTRACT

The promising and encouraging results derived from the use of adipose-derived stem cells (ADSCs) in many diseases are a subject of observation in preclinical studies. ADSCs seem to be the ideal cell population for the use in regenerative medicine due to their easy isolation, nonimmunogenic properties, multipotential nature, possibilities for differentiation into various cell lines, and potential for angiogenesis. This article reviews the current data on the use of ADSCs in the treatment of healing of chronic wounds.
Keywords: nonimmunogenic properties, multipotential nature, possibilities for differentiation

The damage to the skin leads to debilitating effects forming wounds. A wound is defined as a disruption of the normal anatomic structure and functional integrity of the skin. Chronic or nonhealing wounds are wounds that do not progress through the normal wound healing process, resulting in an open laceration of varying degrees of severity [1, 5]. Impaired healing is often associated with ischemia, diabetes mellitus, tumor, venous and pressure ulcers, and severe infections, and it can be the cause of reduced quality of life, disability, and even death [3]. Therefore, wound healing remains a major challenge, and there is a need to develop treatments for improved therapy. Among the various strategies, the most promising seems to be the use of stem cells. This process remains a challenge to date and causes debilitating effects with tremendous suffering. Recent advances in tissue engineering approaches in the area of cell therapy have provided promising treatment options to meet the challenges of impaired skin wound healing [2]. Wound healing is a complex process, covering four mutually overlapping phases: hemostasis, inflammation, proliferation, and remodeling. For the proper process to proceed, all steps must occur in the correct order and time [4.7]. In many chronic wounds, the elongation inflammatory phase leads to the damage of normal tissues, the production of an excessive amount of proinflammatory cytokines, and the prolonged presence of neutrophils, which causes the degradation of the extracellular matrix (ECM) due to an increase in the secretion of matrix metalloproteinases (MMPs) [9,] Restoring the integrity of the skin involves several cell types, extracellular matrix components, and cytokines [57]. It is believed that what is physiologically responsible for the renewal of epidermal stem cells is located only in the basal layer of the epidermis. However, after damage to the skin, stem cells “bulge” in the region of the hair follicle and take additional responsibility for skin regeneration, particularly in the initial stage [4,8]. Cell cultures enriched with stem and progenitor cells can be administered to patients via various methods: a direct application on the wound (e.g., as a suspension), injectable (arteriography), intravenous administration, or application of the culture on the appropriate biological scaffold. The most populous cells are the autologous progenitor cells of the epidermis. Current research is focused on bone marrow and adipose-derived stem cells being used in wound healing [4, 5]. ADSCs are involved in the process of healing indirectly by secreting a number of growth factors (IGF, TGF-β1, VEGF, HGF, and FGF2) with a paracrine action that activates keratinocytes and fibroblasts of the skin by stimulating the processes of neovascularization through the generation of anti-inflammatory cytokines, as well as having antioxidant and antiapoptotic effects. ADSCs release wound healing factors and can stimulate recruitment, migration, and proliferation of endogenous cells in the wound environment. The studies suggest that ASCs can affect other cell types specifically in skin tissue via the paracrine method [9]. They may also be directly transformed into fibroblasts and keratinocytes. Human ADSCs can be converted to epithelial cells expressing the characteristics of cytokeratins 5, 14, 19, and α6; integrins; and even desmoglein 3. They can also differentiate into fibroblast cells, demonstrating not only their morphological similarity but also their ability to also express cell surface proteins including vimentin and fibronectin [60]. An important feature of the ADSCs is that they produce an antioxidant that protects fibroblasts from oxidative stress [36]. One of the factors that induce ADSCs to increase secretion of growth factors and antiapoptotic factors is hypoxia at the wound site. It has been shown that culturing the ADSCs under hypoxic conditions improves their ability to bind to the adhesion molecules (ICAM-1, VCAM-1), which leads to faster neovascularization, increased production of bFGF, and increased ADSC proliferation [9]. A recent study provides evidence that stromal cell-derived factor-1 (SDF-1) can increase the therapeutic effect of ADSCs in cutaneous chronic wounds. It may protect against cell apoptosis in hypoxic and serum-free conditions through activation of the caspase signaling pathway in ADSCs.The first attempts at healing chronic wounds were performed using ADSCs from lipoaspirate, even without culturing in vitro [9]. This technique is commonly used in aesthetic medicine, avoiding the manipulation that might influence their biological functioning. The simplest method is the application of a component of the adipose tissue-derived multicellular stromal vascular fraction (SVF), after enzymatic digestion and centrifugation of lipoaspirate. SVF is a heterogeneous population of MNCs that include ADSCs of the mesenchymal phenotypes (analogous

to MSCs), endothelial progenitor cells (EPCs), hemopoietic progenitors, monocytes, leukocytes, and pericytes. Pericytes are the most important for angiogenesis, and they stabilize nascent blood vessels. The administration of wound single-cell suspensions often leads to the formation of aggregates and islet necrosis which can occur after cell injection. Monolayer-cultured cells are poorly retained in local transplantations, nullifying the therapeutic intent or resulting in unexpected stem cell behaviors. Then, the low cell engraftment efficiency by injection approach has significantly limited the clinical translation of stem cell therapies [7]. Some techniques use matrices like atelocollagen or the scaffolding of silk fibroin-chitosan suspension ADSCs. Sivan et al. used fibrin and fibronectin to construct an in vitro niche and the mimicking of an in vivo provisional matrix, which plays a dual role in the support of hemostasis, accelerates cell attachment and growth, and is responsible for the increased survival of differentiated cells. Now, researchers are focused on the three-dimensional (3D) culture systems of ADSCs to build multicellular constructs with an extracellular matrix (ECM) and to demonstrate better therapeutic efficacy. The study by Cerqueira et al. used human ADSCs with an extracellular matrix (ECM) as a natural tissue glue that was applied to three layers to form a 3D structure (these are known as “technique sheets”). Then, they were transferred to wounds in mice, obtained by the complete excision of the skin. Restoration of the skin was observed with the formation of new hair follicles and vessels. This resulted in a greater stability of transplanted ADSCs, through cell-cell and cell-ECM interactions. The sheet technique greatly improves the efficiency of transplanted ADSCs. Feng et al. describe a simple method for the 3D culture of adipose-derived stem/stromal cells (ADSCs) which prepares them into a ready-to-use injectable. They transferred suspensions of monolayer-cultured ASCs to a syringe containing hyaluronic acid gel (a naturally derived ECM component) and then incubated the syringe as a 3D culture vessel (microspheroids of human ADSCs). They confirmed high therapeutic efficacy in pathological wound repair in vivo. However, central necrosis was reported when spheroids of mesenchymal stem cells reached a diameter of 200 μm in a suspension-rocking culture system. There are high hopes for a new technology that uses a semi-interpenetrated polymer network (semi-IPN) structure. It was developed by combining this polymer with hyaluronic acid (HA), leading to an in situ cross-linkable hydrogel with significantly increased porosity, enhanced swelling behavior, and improved cell adhesion and viability in both 2D and 3D cell culture models.

Next step in the current research is looking for additional materials that may resemble a physiological niche for stem cells to enhance cell retention. Conditioned media for ADSCs have been reported to enhance angiogenesis, enhance epithelialization, and affect recruitment or proliferation of macrophages and endothelial progenitor cells during the healing process. Dong et al. have developed a method of using an injectable poly(ethylene glycol) (PEG)-gelatin hydrogel with highly tunable properties. Murine ADSCs can be easily encapsulated into the hydrogel, which supports ADSC growth and maintains their stemness. This method significantly improves cell retention, enhances angiogenesis, and accelerates wound closure using a murine wound healing model. Then, the injectable PEG-gelatin hydrogel can be used for regulating stem cell behaviors in 3D culture or to deliver cells for wound healing and other tissue regeneration applications [10]. It was found that long-term cell viability could be achieved for both in vitro (21 days) and in vivo (14 days) studies. With ADSCs, this hydrogel system showed potential as a bioactive hydrogel dressing for wound healing [7]. In accordance of the basis of many studies, the best wound healing is achieved by using ADSCs with platelet-rich plasma (PRP). Their presence has caused more rapid proliferation of fibroblasts and keratinocytes in vitro. PRP is a source of growth factors, necessary for healing, such as PDGF, TGF, IGF, and EGF. They are concentrated in the platelets. In addition, PRP can act as a scaffold for other types of cells such as mesenchymal stem cells. On the other hand, higher concentrations of PRP in vitro culture can slow down the rate of regeneration due to proteolytic enzymes (PRP-collagenase, elastase, and cathepsin) which inhibit cell growth. The best results have been achieved after using a maximum 10% PRP. Healing of chronic cutaneous wounds and ulcers is troublesome and may require the use of skin substitutes. Adipose-derived stem cells have immense potential as an autologous cell source for treating wounds and regenerating skin, in conclusion DSCs appear as the ideal cell population for the use in regenerative medicine:they are unlimited in supply and easily obtainable from adipose tissue; they are autologous, nonimmunogenic cells; they have a multipotential nature and easily differentiable into various cell lines; they have a significant potential of angiogenesis; they can be easily cultured and have high affinity for 3D scaffolds.

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RELATİONSHİP BETWEEN ANDROGENETİC ALOPECİA AND CARDİOVASCULAR COMORBİDİTY MARKERS İN WOMEN

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ABSTRACT

Background
Although androgenetic alopecia is a cosmetic problem for lots of men and women, it's also investigated about relations with systemic diseases. Severity of androgenetic alopecia was found related to coronary artery diseases in some groups.

Methods
Twenty seven female volunteers with androgenetic alopecia older 18 years old were enrolled to the study. Androgenetic alopecia were scored by Ludwig classification. Blood biochemical markers which are related to cardiac diseases were studied. These were serum total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, dehydroepiandrosterone sulphate,total testosterone, C reactive protein levels. As imaging carotid ultrasonography was used. Right and left common carotid arteries' intima media thickness were measured.

Results
No corelation with other serum low-density lipoprotein, total chlolesterol , triglyserides , C reactive protein and androgenetic alopecia severity could be found. A negative corelation between serum high-density lipoprotein level and Ludwig severity was investigated.

Conclusions
Patients of androgenetic alopecia must be also concerned about cardiac diseases. Measurement of carotid arteries' intima media thickness in people with androgenetic alopecia measurement can be helpful in determining risk of premature cardiovascular heart diseases. There is a need for more clinical studies of relationship between serum lipids especially high-density lipoprotein and androgenetic alopecia severity in women.
Keywords: female type androgenetic alopecia, DHEA-S, carotid intima media thickness, cardiovascular heart diseases,HDL

Introduction
Androgenetic alopecia (AGA) is one of the most common aesthetic problem of men and women. AGA usually has different phenotypes in men and women. In men usually there is a diffuse regression of frontal hair line and baldness is seen clearly. Norwood-Hamilton classification is used for male type AGA . In women usually only central thinning is observed but baldness is not clear and thiny hair is still preserved. Telogen hair and shedding is more clear than men. Ludwig classification is used in women's AGA (1-4).
But It also must be cared about the co-existing systemic diseases. AGA was also correlated with the coronary artery disease severity.In a lot of clinical studies, relations between the blood lipid levels and AGA was also shown before. In some studies CRP was related with subclinic atherosclerosis (3).
AGA was also named as a determinar of heart diseases. Common carotid artery intimate thickness (CIMT) was related with coronary artery disease and premature atherosclerosis. In many clinical studies measurement of CIMT was found directly related to symptomatic coronary artery diseases. These kinds of studies are done usually among male patients with AGA. Womens' statistics are limited about AGA and cardiac ischemic disease markers. So in this study we investigated the correlations between some blood biochemical markers' related to cardiac ischemic diseases and carotid intima media thickness values in women with AGA.

Methods
This study was designed as a prospective single-center study, and it was conducted under the ethical principles reported in the Declaration of Helsinki. It was approved by the University of Health Sciences, Izmir Tepecik Training and Research Hospital Ethical Review Committee. Female patients diagnosed with female type androgenetic alopecia at our center between February 2019 and February 2020 constituted the target population. 54 over 18 aged volunter women with AGA were accepted to the study. Mean of age was 40.93 (22-66). AGA of patients were scored according to Ludwig score. 10 patients were at Ludwig 1 Grade, 22 of them were at Ludwig 2 Grade, and 22 of them were Ludwig 3 Grade.

Blood biochemical markers which are related to cardiac diseases and AGA were studied. These were total cholesterol, high density lipoprotein (HDL);low density lipoprotein (LDL), triglycerides, dehydroepiandrosterone sulphate (DHEAS),total testosterone, C-reactive protein (CRP) levels (Table 1).

Table 1. Normal values of serum parameters

Parameter	Normal Value	
TG	0 - 150	mg/dL
Total cholesterol	0-200	mg/dL
LDL	0-130	mg/dL
HDL	35-55	mg/dL
DHEAS	211-492	ug/dL
CRP	<0.5	mg/L
Testosteron	2.49-8.36	ng/ml

TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, DHEAS: dehydroepiandrosterone sulphate, CRP: C reactive protein (CRP)

Carotid ultrasonography was used for imaging CIMT. Right and left common carotid arteries' intima media thickness were measured.These parameters were independent variables so their relations were investigated by Pearson correlations statistics.
One skilled radiologist investigated the CIMT. A B-mode Ultrasound system with linear probe was used (Philips Affiniti 70 G). The participitants were requested to lie on supine position with slightly hyperextension to get optimal imaging. Posterior wall IMT were measured.

Results
No corelation with other serum LDL, total chlolesterol , triglyserides ,CRP and AGA severity could be found. A negative corelation between serum HDL level and Ludwig severity was investigated (Table 2).

Table 2. Correlations between all parameters

P	Total cholesterol	TG	HDL	LDL	CRP	Total testosterone	DHEAS	Right CCA	Left CCA	Ludwig
Age	0.79	0.82	0.38	0.73	0.15	0.38	0.17	0.14	0.053	0.97
Total cholesterol	-	0.01*	0.82	0.0001*		0.73	0.64	0.96	0.67	0.23
TG	0.01*	-	0.48	0.26	0.41	0.34	0.73	0.55	0.49	0.77
HDL	0.82	0.48	-	0.17	0.14	0.87	0.06	0.28	0.39	0.049* r=-0.38
LDL	0.0001*	0.26	0.17	-	0.33	0.4	0.54	0.85	0.62	0.23
CRP	0.64	0.41	0.14	0.33	-	0.03*	0.005* r=-0.52	0.11	0.14	0.64
Total testosterone	0.73	0.34	0.87	0.4	0.03*	-	0.13	0.69	0.65	0.96
DHEAS	0.64	0.73	0.06	0.54	0.005* r=-0.52	0.13	-	0.9	0.73	0.039* r=-0.4
Right CCA	0.96	0.55	0.28	0.85	0.11	0.69	0.9	-	0.0001*	0.27
Left CCA	0.67	0.49	0.39	0.62	0.14	0.65	0.73	0.0001*	-	0.34

TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, DHEAS: dehydroepiandrosterone sulphate, CRP: C reactive protein (CRP), CCA: common carotid artery. *$*p \leq 0.05$*

The mean age of patients was 40.93±10.86. Ten patients were at Ludwig grade 1, 22 patients were at Ludwig grade 2 and 22 of them were at Ludwig grade 3. If we look at blood lipid levels, the mean value of total cholesterol was 200.99±32.16, triglyceride mean value was 117.95±64.68, HDL mean value was 56.41±13.1, LDL mean value was 119.96±28.12. We

determined the mean value of CRP as 0.23±0.23, of total testosterone as 4.9±20.2, of DHEAS as 169.68±95.85. Mean value of right common carotid artery (CCA) was 0.88±0.55 mm and mean value of left CCA was 0.93±0.6 (Table 3).

Table 3. Correlations between serum lipid levels, CRP and androgenetic hormones, CCA measurements

P	Total testosterone	DHEAS	Right CCA	Left CCA
Total cholesterol	0.73	0.64	0.96	0.67
TG	0.34	0.73	0.55	0.49
HDL	0.87	0.06	0.28	0.39
LDL	0.4	0.54	0.85	0.62
CRP	0.03*	0.005* r=-0.52	0.11	0.14

TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, DHEAS: dehydroepiandrosterone sulphate, CRP: C reactive protein (CRP), CCA: common carotid artery. **p ≤ 0.05*

There was significantly negative correlation between HDL and Ludwig (P = 0.049). We determined significant correlation between CRP and total testosterone and negative correlation between CRP and DHEAS, in addition between DHEAS and Ludwig (respectively P = 0.03, P = 0.005, P = 0.039). There were no correlations between other parameters (Table 4).

Table 4. Correlations between Ludwig and other parameters

P	Total cholesterol	TG	HDL	LDL	CRP	Total testosterone	DHEAS	Right CCA	Left CCA
Age	0.79	0.82	0.38	0.73	0.15	0.38	0.17	0.14	0.053
Ludwig	0.23	0.77	0.049	0.23	0.64	0.96	0.039* r=-0.4	0.27	0.34

TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, DHEAS: dehydroepiandrosterone sulphate, CRP: C reactive protein (CRP), CCA: common carotid artery. **p ≤ 0.05*

DİSCUSSİON

Androgenetic alopecia (AGA) must be cared about the co-existing systemic diseases. AGA was also correlated with the coronary artery disease severity.In a lot of clinical studies (5-7) increased risk of atherosclerosis related cardiovascular events with vertex baldness was reported (8).
Relation between blood lipid levels and AGA was also shown before (9-12). Dyslipidemia was found more common in people with AGA (13). Clinical studies among women are limited (14-16).
14 of the 54 CIMT were equal or over 1 cm . 1 cm is usually accepted as normal uplimit of CIMT. Common CIMT was related with coronary artery disease and premature atherosclerosis in many studies. Although we couldn't find any association with AGA and CIMT this could be our limited patient number (17). Also hypertension could be another factor effecting this relation (18). None of our patients had hypertension problem.
In some studies CRP was related with subclinic atherosclerosis. Especially, high sensitive CRP has been associated with increased CIMT in both risk populations and in population-based samples. It is clear that elevated serum high sensitive CRP is causal to the initiation and progression of atherosclerosis. Recent evidence has suggested a possible direct pathogenic role (17). These results were incompatible with our study, we didn't find any relationship between CRP and CIMT.
Although androgenetic alopecia is a cosmetic problem for lots of men and women, it should also be mentioned about systemic diseases. Early start of AGA was found related to coronary artery diseases (12). AGA was also named as a determinar of heart diseases. Cardiac investigation of people with coronary artery diseases can be helpful. In many clinical studies measurement of carotid artery intima media thickness was found directly related to symtomatic coronary artery diseases. These kind of studies are done usually among male patients with AGA. Womens' statistics are limited about AGA and cardiac ischemic disease markers. In this study we investigated the correlations of some blood biochemical markers related to cardiac ischemic diseases and carotid intima media thickness values.

HDL protects the vascular wall from aggressive factors (endothelial adhesion, migration of monocytes, etc.) and facilitates the reverse transport of cholesterol. The ratio of TGs to HDL was a strong predictor of myocardial infarction and atherosclerosis (19). A negative corelation between HDL and Ludwig severity was observed in our study. This is compatible with the literature (8,20).

Women with androgenetic alopecia usually don't have higher levels of circulating androgens. They have been found to have higher levels of 5-alpha reductase which converts testosterone to dihydrotestosterone, more androgen receptors, and lower levels of cytochrome P450 which converts testosterone to estrogen. Most women with androgenetic alopecia have normal menses and normal endocrine function, including gender-appropriate levels of circulating androgens (21,22).

CONCLUSİONS

Although androgenetic alopecia is a cosmetic problem for lots of men and women, it should also be mentioned about systemic diseases AGA can be named as a determiner of ischemic heart diseases. Cardiac investigation of people with AGA can be helpful.. Measurement of biochemical cardiac risk factors and CIMT as a routine in people with AGA can be helpful in determining risk of premature cardiovascular heart diseases. We found a negative relationship between AGA and serum HDL lipid levels. There is a need for more clinical studies of relationships between serum lipids especially HDL and AGA severity in women.

Authors' contribution: All authors took part at every stage of study. All authors read and approved the final version of the manuscript

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MICROBIAL CHARACTERISTICS OF POST-TRAUMATIC OSTEOMYELITIS

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ABSTRACT

OBJECTIVE: The one of the most important issues in traumatology is prevention and treatment of purulent-septic complications of traumatic diseases.
The aim of our study was to establish correlations between osteomyelitis caused by bacterial flora and immunological factors.

METHODS: On the basis of a comprehensive study of bacteriological and immunological data in 100 patients with various etiologies osteomyelitis, using correlation analysis was determined: that the types of microbial complications following trauma and the date of the body's immune system depends on etiological factors. The frequency of microbes is different and depends on the localization of the injury and the surgical intervention.

RESULTS: frequency of the etiological factors in the contingent we studied, was distributed as follows: S. aureus-36,9%, S.Epidermidis-52,4, Ps. aeruginosa 27,4%, E. coli- 13,1%, Proteus- 27,4%. Associated infection (or co-infection e.g. S. aureus+S. Epidermidis, or St.Epidermidisis+Ps. Aeruginosa and etc) occurred in 22.6% of cases. A significantly high correlation coefficient was observed in patients who came to the clinics spontaneously or with delay, as well as with the early onset of the infectious process (up to two weeks) and surgical treatment. It also correlates positively with a decrease of following immunological parameters: NK, CD4+, CD8+ and CD 19+, the leucocytes phagocytic index is reduced and the blast transformation reaction of lymphocytes rate was increased. There is a significant correlation with benign outcome of treatment, which indicates that, the patient was sent for outpatient treatment (R=0, 79).
Keywords: Trauma, Osteomyelitis, Microorganism, Etiological factor, Immune system data.

The one of the most important issues in traumatology is prevention and treatment of purulent-septic complications of traumatic diseases. According to the received data the frequency of purulent complications depends on the severity of the injury and on the body's immune system data, as well as the etiological factors.
Osteomyelitis is a bacterial infection of the bone or bone marrow often resulting in hospital admission. Osteomyelitis may result from contiguous spread of infection from adjacent soft tissues and joints, hematogenous seeding, or direct inoculation of bacteria into the bone as a result of trauma or surgery [1].
The particular importance in this regard is the determination of the interaction of etiological factors with immunological data of the disease.
Conceivably, there may have been major changes in the microbiology, types of osteoarticular infections, and the characteristics of patients at risk. For example, the widespread use of medical devices in orthopaedic surgery and increased life expectancy of the population are all factors related to the increased rates of some osteoarticular infection [2].
Recent changes in the epidemiology, pathogenesis, diagnosis, treatment, and prognosis of this disease have varied according to population [3].
The annual incidence was higher for men than for women and increased with age ($p < 0.001$) [4].
The aim of our study was to establish correlations between osteomyelitis caused by bacterial flora and immunological factors.

Material and methods
We studied 100 patients with chronic osteomyelitis who were in different clinics. Their age was determined from 16 to 80 years. including 79 men, 21 women.We used immunological, bacteriological, clinical-laboratory research methods. We determined of anti-microbial antibodies titer, and etiological factors. The material was processed by correlation analysis method. The correlation was considered reliable If $r>0.25$ ($p<0.05$).
In the study of immune status, we measured immune cells, and used standard flow cytometric methods using two-colors fluorescence on samples of whole blood, it is possible to establish the ranges of absolute numbers of NK, CD3+, CD4+ and CD8+ , CD19+ (T and B- lymphocytes), by the cytofluorometry of "FACSCount" using monoclonal antibodies of the company Becton Dickinson.subsets in the routine laboratory.

NK cell-activating receptors play an important role in the recognition of targets, which transduce the signals necessary for cellular machinery to induce target injury and cytokine production. NK cells (NKT) are cells with natural non-immune killer activity that have signs of T-lymphocytes.

Immunoregulatory index CD4+/CD8+ as the name implies, reflects the ratio of CD4 + cells (T-helper cells) to CD8 + cells (T-cytotoxic cells).It is a relative indicator that has an approximate value, Its slight increase or decrease does not have an independent diagnostic value. Nevertheless, changes in the index force the clinician to focus on the reasons for the deviation of the indicated index.

For measure sensitive lymphocytes we used the method of lymphocytes blast-transformation reaction (RBLT), which is necessary for patient's inspection with immunologic infringements. It is applied in the different fields of medicine to identification of a sensitization to antigens and may be used for assessing the functional state of human lymphocytes. During this reaction, we used bacterial polysaccharide as an activator of lymphocytes. That is ensured by assessing 72-hour phytohemmagutinin-stimulated lymphocyte blast-transformation reaction (LBTR) by immunocytochemical method in a luminescence microscope in indirect immunofluorescence test. A Ki-67 nuclear antigen marker is Ki-67 monoclonal antibody. The functional capability of lymphocytes is described by a quantity of visualized (light-producing) Ki-67-positive cells having light emission foci in the cell nucleus visualized after the artificial stimulation to PHA proliferation. If the antigen-positive cell value is less than 49%, the lower functional activity of lymphocytes is stated in the individuals suffering the disordered immune status, using the given method enables the early immune diagnosis.

Antimicrobial antibodies were detected by the passive hemagglutination reaction (RPHA) , modified by the Boyden (1952).RPHA is placed in plastic plates or in test tubes with dilutions of the patient's blood serum, to which erythrocyte antigenic diagnosticum is added. Serum dilution occurs as follows1:20, 1:40, 1: 80, 1: 160, 1: 360 and etc.

Phagocytes index of neutrophils. A leukocyte suspension is obtained from the blood in a certain way, which is mixed with the exact number of microorganisms (1 billion microbes in 1 ml). After 30 and 120 minutes, smears are prepared from this mixture and stained according to Romanovsky-Giemsa.

About 200 cells are examined under a microscope and the number of phagocytes that have absorbed bacteria, the intensity of their capture and destruction is determined. The phagocytic index is the percentage of phagocytes that have swallowed bacteria after 30 and 120 minutes to the total number of cells examined. Phagocytic index - the average number of bacteria in the phagocyte after 30 and 120 minutes (produce a mathematical division of the total number of bacteria absorbed by phagocytes by the phagocytic index. Phagocytosis completeness index - calculated by dividing the number of killed bacteria in phagocytes by the total number of absorbed bacteria and multiplying by 100. Phagocytic index: after 30 minutes - 94.2 ± 1.5, after 120 minutes - 92.0 ± 2.5

We studied the following groups of factors: age, localization of trauma, forms of fractures, methods of treatment, indicators of cellular and humoral immunity, etiological factors and etc. The material was processed by Speamen correlation analysis method, what was considered reliable if $r>0.25$ ($p<0.05$). Data were analyzed using the SPSS 23.

Results

Frequencies of etiological factors in patients with osteomyelitis are given in Figure 1.

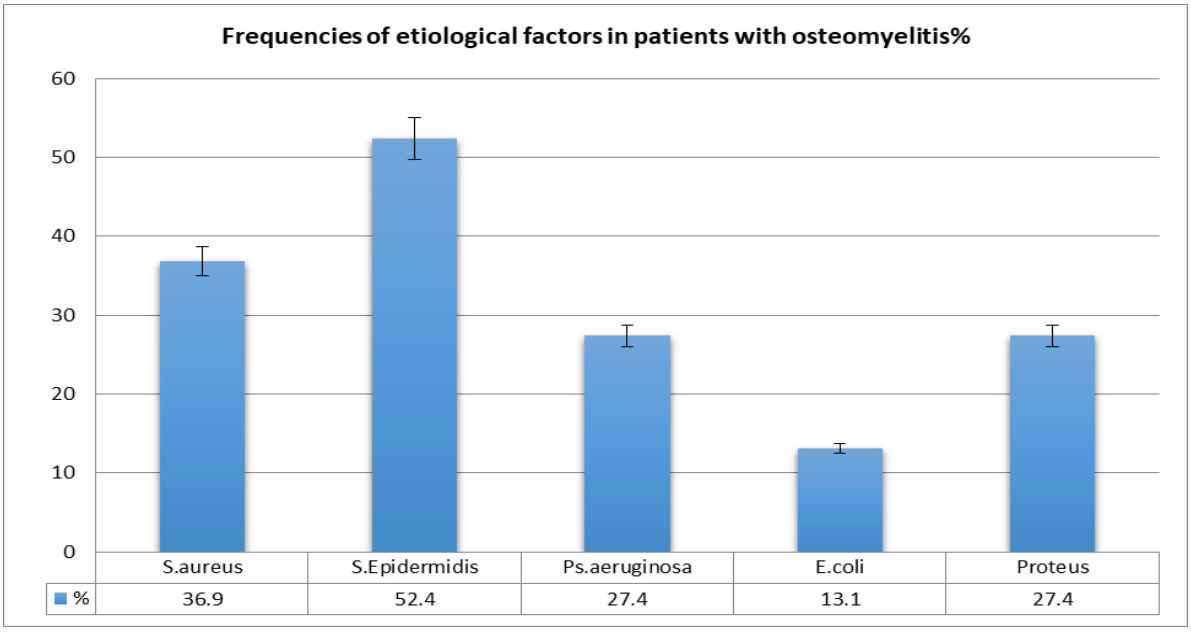

Figure 1. Frequencies of etiological factors in patients with osteomyelitis%

The frequency of the etiological factor in the contingent we studied, was distributed as follows: S. aureus-36,9%, S.Epidermidis-52,4, Ps. aeruginosa 27,4%, E. coli- 13,1%, Proteus- 27,4%.Associated infection (or co-infection e.g. S. aureus+S. Epidermidis, or St.Epidermidisis+Ps. Aeruginosa and etc) occurred in 22.6% of cases.

The table 1 below shows the correlations between S.Aureus frequency and immunological data during osteomyelitis. S. Aureus predominantly found in 16-30 year old patients, and shows a credible positive correlation with a gunshot and open fractures.

Table 1. Correlations between S. aureus frequencyand immunological parameters during osteomyelitis

N	Name the factors	Correlation - r	N	Name the factors	Correlation - r
1	Age 16-30 years	0.32		General anesthesia	0.90
2	Fracture of femoral neck	-0.40		Anti-microbial antibody titer varied from 1:160 to 1:320 (at norm 1:20)	0.50
3	Fracture of femur	0.252		NK cells >	-0.59
4	Malleolar fractures	-0.33		NK <	0.56
5	Displaced fracture	0.29		CD 3+>	-0.78
6	Open fracture	0.87		CD 3+<	0.81
7	Closed fracture	-0.78		CD 4+>	-0.56
8	Gunshot fracture	0.55		CD 4+<	0.32
9	Fracture of ribs and chest bone	-0.40		CD 8+>	-0.58
10	Entering spontaneously into a clinic	0.74		CD 8+<	0.38
11	Entering toa clinic by ambulance	-0.61		Immunoregulatory index CD4+/CD8+ >	-0.55
12	Entering to a clinic within 24 hours to 2 weeks	0.29		Reaction of blast- transformation of lymphocytes (RBLT) rate (N 6-15%) <	0.41
13	Surgical treatment. due to the secondary purulent process	0.90		Reaction of blast- transformation of lymphocytes (RBLT) rate (N 6-15%) > 15%	0.29
14	Surgical treatment	0.63		CD 19>	-0.78
15	Conservative treatment	-0.81		CD 19<	0.74
16	Local analgesia	-0.72		Factocytic index of neutrophils <	0.51

r>0.25, p<0.05

Table N 2- shows the correlations between the frequency of S. epidermidys and immunological parameters during osteomyelitis.
The frequency of S. epidermis is reliably positively correlated with this localization of trauma: femoral neck fracture, malleolar fractures, rib fracture, brought to the clinic by ambulance, conservative treatment, and local anesthesia.

Table 2. Correlations between S. epidermis and the cells of the immune system during osteomyelitis.

N	Name the factors	Correlation - r	N	Name the factors	Correlation - r
1.	Fracture of femoral neck	0.32	15.	Anti-microbial antibody titer was measured from: 1:160 to 1:320:160-1:320	-0.29
2.	Malleolar fractures	0.44	16.	NK cells >	0.61
3.	Displaced fracture	-0.34	17.	NK cells <	-0.43
4.	Open fracture	-0.62	18.	CD 3 >	0.71

5.	Closed fracture	0.72	19.	CD 3 <	-0.54
6.	Gunshot fracture	-0.47	20.	CD 4>	0.48
7.	Fracture of ribs and chest bone	0.32	21.	CD 4<	0.57
8.	Entering spontaneously into a clinic	-0.62	22.	immunoregulatory index CD4+/CD8	0.53
9.	Entering to a clinic by ambulance	0.64	23.	CD 19 >	0.71
10.	Surgical treatment	-0.41	24.	CD 19 <	-0.52
11.	Conservative treatment	0.73	25.	Factocytic index of leukocytes	0.29
12.	Surgical treatment. due to the secondary purulent process	-0.65	26.	The patients were sending tooutpatient treatment	-0.61
13.	Local analgesia	0.67	27.	Complicated by sepsis	0.66
14.	General anesthesia	-0.65			

r>0.25, p<0.05

A significantly high correlation coefficient was observed in patients who came to the clinics spontaneously or with delay, as well as with the early onset of the infectious process (up to two weeks) and surgical treatment. It also correlates positively with a decrease of following immunological parameters: NK, CD4+, CD8+ and CD 19+, the leucocytes phagocyte index is reduced and the blast transformation reaction of lymphocytes rate was increased. There is a significant correlation with benign outcome of treatment, which indicates that, the patient was sent for outpatient treatment(r=0.79).
Such a correlation is manifested on the part of immune cells: CD3+, CD4+, CD8+ and CD19 are activated, the immunoregulatory index is also elevated, while the phagocyte index of leukocytes is reduced. The presence of S. epidermis correlates with the development of sepsis r=0,66.
Frequency of purulent process caused by Ps. Aeruginosa shows a positive correlation with open fracture(r=0.34.), as well as with femoral and gunshot fractures (r=0.351 and r=0.35 respectively). Bringing the patient to the clinics by ambulance(r=0,64), general anesthesia(r=0,33), age>60(r=0,34).
E. coli reveals a reliable correlation with lung damage (r=0,27). Proteus Reveals a significant correlation with a fracture of the shin bones (r=0,31), foreign body (bullet, bone fragment or other dense body left in a tissue)(r=0,28), as well as surgical treatment (r=0,28).

Discussion

Osteomyelitis is a bone marrow inflammation, usually caused by an infectious agent. It has a heterogeneous pathophysiology [5].
Chronic bone infections are more often linked with diverse bacterial biofilms [6]. According our study, Frequency of purulent process caused by Ps. Aeruginosa shows a positive correlation with open fracture, as well as with femoral and gunshot fractures. Bringing the patient to the clinics by ambulance, general anesthesia.
The annual incidence was higher for men than for women and increased with age ($p < 0.001$) [7]. According to our data, the majority of patients are male, although **S. aureus** correlates with young age and by Ps. Aeruginosa age >60.
Hypervirulent *K.pneumoniae* linked to osteomyelitys [8]. According this data, E. coli reveals a reliable correlation with lung damage.
Proteus reveals a significant correlation with a fracture of the shin bones, foreign body (bullet, bone fragment or other dense body left in a tissue), as well as surgical treatment.
According to the literature, Staphylococcus aurous was the most common bacteria (64.36%, n=97) followed by Pseudomonas Aeruginosa (17.10%,n=26), E. Coli (11.84%,n=18) and Proteus Mirabilis (7.24%,n=11) [9].
Thus, our research showed that the forms of post-traumatic complications and the reactivity of the organism depend on the etiological factors. The frequency of different etiological factors varies according to the location of the injury and the surgical intervention. The conditions in which the patient enters the clinic have a certain value. High frequency is observed, the risk of complication with S. Epidermidis and P. Aeruginosa in patients who are admitted by ambulance, wile S. Aureus shows a reliable correlation in spontaneously admitted patients.

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BIOLOGICAL AGENTS DURING ATOPIC DERMATITIS THERAPEUTICAL MANAGEMENT

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ABSTRACT

The complicated form of atopic dermatitis, which affects adults so children, is a debilitating disease with a significant decline of patients' quality of life. Etiological factors are currently a topic of study and interpretation, the main features of atopic eczema are skin barrier disturbance and immune dysregulation. Severe refractory disease that fails to improve with conventional therapy may benefit from biologic therapy. Progress in understanding immunopathology of atopic dermatitis have allowed identification of therapeutic molecular targets in the field of biological therapy. Our reviewe is about different biological treatments with a focus on novel targeted agents: Systemic immunotherapy (Omalizumab, Dupilumab, Lebrikizumab, Tralokinumab, Nemolizumab, Ustekinumab, Fezakinumab, Tezepelumab, Apremilast, allergen specific immunotherapy), and topical agents (Tofacitinib, Crisaborole).
Keywords: Dermatitis, atopic; Biological agents.

Introduction

Atopic dermatitis (AD), also known as atopic eczema is a common chronic or recurrent inflammatory skin disorder with a significant social and economic impact worldwide, affecting 2.1–4.9% of adult population, and 15–20% of children (1,2). An increasing prevalence of AD has been reported, especially in low-income countries (3). Furthermore, the past decades brought a 2–3-fold increase in prevalence in industrialized countries (3). Generally AD onset is in early childhood, as one of the first steps of the 'atopic march', which describes the natural history of atopic manifestations, and it is characterized by xerotic skin and acute flare-ups of intensely pruritic eczematous lesions (4). Recent studies recognize a predilection of AD for persistence in adulthood, with a lifetime prevalence accounting for 34.1% Recent years brought significant improvement in elucidating the complex interactions between skin barrier, genetic and environmental factors. The better understanding of pathologic pathways is a stepping-stone to improved management for AD patients. The goal of this review is to summarize the topic of severe refractory atopic dermatitis from the perspective of novel therapeutic immunomodulatory methods: IgE directed therapy (Omalizumab), anti IL-4 (Dupilumab) and anti IL-4/IL-13 agents (Lebrikizumab, Tralokinumab), IL-31 directed therapy (Nemolizumab), anti IL-12/23 (Ustekinumab), IL-22 blockade(Fezakinumab), thymic stromal lymphopoietin directed therapy (Tezepelumab), phosphodiesterase inhibitors (Apremilast, Crisaborole), and JAK inhibitors (Tofacitinib).

Methodology

Mepolizumab, a humanized monoclonal anti-IL-5 antibody, Rituximab, a chimeric monoclonal antibody against CD20, a pan marker of B lymphocytes, as well as inhibitors of tumour necrosis-α factor/receptor (TNF-α), such as Infliximab, Etanercept, and Adalimumab, brought moderate and intermittent improvement of AD. High-dose intravenous immunoglobulins (IVIGs) were investigated for their immunodulatory effects in moderate to severe AD, and failed to bring significant improvements. Earlier studies on recombinant human interferon-γ (rhIFN-γ) proved its efficacy in reducing clinical severity of AD. However, rhIFN-γ is not currently approved by the FDA for AD. T-cell modulating agents, such as Efalizumab and Alefacept failed to bring spectacular results in adult patients with moderate to severe AD. In addition, Efalizumab was voluntarily withdrawn from the market because of the risk of severe neurological adverse reactions caused by reactivation of the John Cunningham human polyoma virus .

Allergen-specific immunotherapy

There is still controversy regarding the use of allergen immunotherapy (AIT) in AD patients. Data suggests that AIT improves the clinical course of AD, pleading for its potential form of treatment. Case reports and small cohort studies showed effectiveness of AIT on AD. A multi-centre randomized study that enrolled 89 adult patients with AD and sensitization to house dust mites, of whom 51 completed the study, assessing the usefulness of AIT, observed the improvement of disease and a reduction in the need for topical corticosteroids. A meta-analysis of eight randomized trials that included 225 patients brought moderate-level evidence for the efficacy of AIT in AD. As shown by a prospective placebo-controlled study that included 168 patients with AD, AIT is beneficial only in severe AD, with a SCORAD score greater than 50. A systematic review using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach reported improvement of clinical symptoms of AD. However, the study also noted the strength of recommendation for use of SIT in patients with AD as weak, pinpointing the need for high-quality evidence to support AIT in AD. Several studies on sublingual immunotherapy in AD patients bring arguments in favour of AIT as a safe and effective strategy. There are no contraindications for AIT in AD; AIT does not worsen AD.

Novel topical agents for AD:1.Tofacitinib-Initially approved for the treatment of rheumatoid arthritis, Tofacitinib, an oral small-molecule JAK inhibitor that acts by blocking several Th2 cytokine signalling (IL-4, −5 and −13), shows promise in AD. Many of the cytokines involved in AD use JAK biological pathways. The latter encompasses several tyrosine kinase proteins that interact with the common γ-chain of cytokine receptors to initiate cytokine mediated responses.
Crisaborole-A topical PDE inhibitor ointment, has been approved by the FDA in the topical treatment of AD patients of at least 2 years of age. It proved to reduce skin inflammation and pruritus, as compared to controls, with the disadvantage of being less effective than low potency topical corticosteroids (10). In contrast to topical corticosteroids, topical PDE inhibitors do not pose the risk for telangiectasia and skin atrophy.
CONCLUSION: Atopic dermatitis poses a challenge for clinicians and patients alike, particularly in severe forms of disease. Recent progresses in understanding the pathophysiology of atopic dermatitis underlie its multiple facets and allow the introduction of novel substances for the systemic and topical treatment of severe atopic dermatitis. New therapeutic strategies brought tremendous advances in the management of refractory to conventional treatment, severe atopic dermatitis. Robust evidence pleads for efficacy of Dupliumab, while other immunomodulatory agents, such as Nemolizumab, Lebrikizumab, Tralokinumab, Ustekinumab and Apremilast show promise, but further data are needed to confirm their usefulness and safety in atopic dermatitis. Allergen specific immunotherapy may be of use in selected cases of atopic dermatitis.

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DEVELOPMENT AND APPLICATION OF STEM CELL TECHNOLOGIES DURING SKIN WOUNDS HEALING

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Abstract

The main clinical focus of stem cell application in wound care is to target improved quality of wound healing. The medical practitioner would anticipate achieving acceleration in healing, prevention of wound contracture and scar formation, earlier wound closure, and ideally regeneration of the skin and its appendages using stem cells.]. Stem cells, defined based on the findings obtained by McCulloch and Till, are characterized by their capacity for self-renewal, asymmetric replication, and differentiation to other cells building different tissues and organs.
Keywords: Stem Cells, wound Healing, regeneration

Background

Stem cells replenish lost cells throughout an organism's lifespan. They have the capacity for unlimited replication providing a population of "sister" SCs. These cells are responsible for self-renewal and differentiate tissue-specific cells. This process maintains the constant number of aging somatic cells, which have become apoptotic. Their therapeutic potential is largely due to their capability to secrete pro-regenerative cytokines, causing them to be an attractive choice for the treatment of chronic wounds [1]. Among the main sources of cells that might be used for wound healing and regeneration of injured skin are embryonic stem cells (ESCs), induced pluripotent stem cells (iPS), and adult stem cells [2]. However, the remaining challenge of stem cell application for skin regeneration is still to describe the optimum source and the method of processing and administration from a clinical standpoint and to define the roles of stem cells in the real clinical situation [3-5]. Table 1 shows stem cells used for wound therapeutic. Embryonic stem cells (ESCs)-the ESCs were first established from the inner cell mass (ICM) of mouse blastocysts in 1981, and the term "embryonic stem (ES) cell" was coined. ES cells are pluripotent stem cells derived from the inner cell mass of the preimplantation blastocyst (35-day-old embryo) and obtained from mice, humans, and nonhuman primates. ES cells have the ability to differentiate cell types, including neural cells, blood cells, adipocytes, chondrocytes, muscle cells, and skin cells. In an attempt to utilize the remarkable regenerative potential of ESCs for cutaneous repair, Guenou et al. showed that human embryonic stem cells growing in induction medium containing BMP4 (bone morphogenetic protein-4) and ascorbic acid could differentiate between basal keratinocytes, which were subsequently used to reconstitute the epidermis composed of multiple layers of differentiated cells. These tissues were also successfully transplanted into nude mice to facilitate wound healing. In another report, Shroff et al. evaluated the effect of human embryonic stem cell (hESC) therapy in six patients with non-healing wounds. It showed that the wounds of all the patients healed after receiving hESC therapy. Reduction in the size of wounds and granulation was observed among all the patients [5.6]. Despite these promising findings, the use of embryonic stem cells has remained controversial. The cells could be the most suitable ones over adult stem cells for skin tissue regeneration owing to their capacity of self-renewal and the unlimited supply of differentiated keratinocytes or keratinocyte progenitors for treating cutaneous injuries [7]. In addition to the widespread clinical use of ESCs, which is currently elusive due to the potential for immunogenicity and tumorigenicity, another major limitation of using ESC-derived cells for regenerative wound healing is ethical controversy and substantial legal restrictions [8]. Induced pluripotent stem cells (iPS cells)-The iPS cells are the newest class of pluripotent stem cells, which potentially combines the advantages of MSCs and ESCs, ushering in a new era of regenerative medicine [6]. In 2006, Yamanaka et al. at Kyoto University in Japan observed that the introduction of four genes (Oct-3/4, Sox2, c- Myc, and KLF4) into cells from the mouse tail could reprogram the cell back to an embryonic state. In 2007, iPS cells were produced from human cells These induced pluripotent stem cells were shown to be remarkably similar to ESCs in morphology, proliferation potential, gene expression pattern, pluripotency, and telomerase activity. Like ESCs, iPSCs can differentiate between all types of cells from the skin to nerve and muscle [7]. This revolutionary technology allows for generation of autologous pluripotent stem cell populations, thereby circumventing the major limitations of ESC, including ethical concerns and potential for immunological rejection. Taking advantage of these characteristics, significant progress has been made in the differentiation of iPSCs into skin cells—including folliculogenic human epithelial stem cells, fibroblasts, and keratinocytes—to engineer skin substitutes [10]. Bilousova et al. induced iPS cells in vitro to differentiate skin-like cell lines and to form multi-differentiated epidermis, hair follicles, and sebaceous glands. Additionally, Itoh et al. generated in vitro 3-D skin equivalents exclusively composed of human iPSC-derived keratinocytes and fibroblasts. Two recent studies conducted by Umegaki-Arao et al. and Sebastiano et al. have further proven this concept. One of the most recent studies in this regard suggested that exosomes derived from human-

induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) facilitated cutaneous wound healing in rats by promoting collagen synthesis and angiogenesis [9]. However, despite experimental evidence supporting the therapeutic benefits of iPSCs, there are still numerous issues such as associated cancer risk development through using retroviral vectors, epigenetic memory retained from parent cells, genetic instability, inefficient cell re-programming yielding low cell numbers with high processing costs, and potential immunogenicity [10].

Methodology

Therefore, iPSC-based therapies for wound-healing applications require further extensive analyses for safety and reliability of the reprogramming technology. Adult stem cell-the most stem cells used in skin regeneration and wound healing are adult stem cells owing to containing significant proliferative capacity, long-term self-renewal potential, and having the ability to differentiate into other lineages. They are found in various tissues, including the skin, heart, liver, brain, and bone marrow. Among the different types of adult stem cell, mesenchymal stem cells (MSCs) and adipose-derived stromal cells (ASCs) have gained considerable attention as a suitable candidate to enhance tissue regeneration. Mesenchymal stem cells (MSCs)-MSCs harvested from various sites (bone marrow, adipose tissue, amniotic fluid, and dermis) are considered a source for therapeutic approaches owing to their multilineage differentiation, high frequency, facility of isolation and characterization, and the ability of MSCs to migrate to injury sites in the body. These cells are involved in all three phases during the wound-healing process. They also enhance wound healing by immune modulation, production of growth factors, which enhance neovascularization and re-epithelialization, stimulate angiogenesis, and accelerate wound closure. One case study has reported that increased wound closure occurs when MSCs are administrated and accelerated dermal fibroblast and keratinocyte migration . Furthermore, Nakagawa et al. suggested that the combination of hMSCs with bFGF in a skin defect model improved cutaneous wound healing as the hMSCs transdifferentiate into the epithelium. Smith et as showed that MSCs secreted soluble factors inducing dermal fibroblast proliferation, migration, and chemotaxis. Endogenous bone marrow-derived mesenchymal stem cells in the dermis may provide an important early signal for dermal fibroblast responses to cutaneous injury. Li et al. demonstrated that activated MSCs promoted wound healing in acute incisional wounds, as reflected in regained tensile strength. A clinical study was performed to test a new technique for the treatment of chronic non-healing wound (diabetic ulcer) using autologous graft composed of autologous skin fibroblasts on biodegradable collagen membrane (Coladerm) in combination with autologous MSC derived from the patient's bone marrow. The wound showed a steady overall decrease in wound size and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed after 29 days of combined treatment. The treatment of burn injuries, especially severe ones, has always been a challenging issue, but the use of MSCs had beneficial therapeutic effects on burns wound healing. A case report of radiation burns has indicated the efficiency of a new therapeutic approach combining surgery and local cellular therapy using autologous MSCs, which this benefit of the local cell therapy could be linked to the "drug cell" activity of MSC by modulating radiation inflammatory processes. During the normal wound-healing process, angiogenesis is one of the most important stages in which MSCs secret various pro-angiogenic factors such as VEGF to promote endothelial cell proliferation and form new vessels. There is evidence that suggests topical VEGF accelerates diabetic wound healing through increased angiogenesis as well as mobilizing and recruiting bone marrow-derived cells. likely by regulating most a network of several angiogenic molecules. Experimental studies established that MSCs could orchestrate the inflammatory response following tissue injury. Transplantation of human umbilical cord MSCs into cutaneous rat wounds significantly accelerated wound healing and remarkably decreased the quantity of infiltrated inflammatory cells and levels of IL-1, IL-6, and TNF-a and increased levels of IL-10 and TSG-6 in wounds.

Conclusion

Bone marrow-derived stem cells (BM-SCs)- BM-SCs are considered the primary source of MSCs in adults and a good candidate for the treatment of different types of wounds. Preclinical studies using autologous BM-MSC have reported the potential therapeutic effect of these cells in dermal rebuilding and scarring reduction in chronic wound Although BM-MSC is successfully implemented in clinical treatment, other limitations in therapeutic efficacy are challenges that need to be addressed through an extensive investigation of BM-MSC. The risks of BM-MSC during clinical translation are harvesting invasiveness, in vitro culture, and further cost-time resource.

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FACTORS RELATED TO DERMAL REGENERATION

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ABSTRACT
Normal wound healing is a dynamic and complex multiple phase process involving coordinated interactions between growth factors, cytokines, chemokines, and various cells. Any failure in these phases may lead wounds to become chronic and have abnormal scar formation. Chronic wounds affect patients' quality of life, since they require repetitive treatments.
Keywords: Growth factors, Interleukins, wounds

Background
For develop better substitutional medical approaches to improvement of injured skin tissues, regenerative medicine applications have been widely investigated. Regenerative therapies consist of different technological approaches such as gene targeting, stem cell treatment, soluble molecules, cell reprogramming, and tissue engineering [1]. In particular, a basic principle for these applications is using engineering techniques to facilitate a natural wound-healing cascade by providing proper physicochemical and biochemical factors [3]. A number of bioactive factors, including growth factors and cytokines, are involved in various tissue repair stages and are necessary to promote dermal regeneration. Cytokines are extracellular signaling proteins secreted by many cell types affecting the activity of other cells, including immune cells. They include interleukins, lymphokines, interferons, and tumor necrosis factor. The study of cytokines in wound healing is challenging, since examination of isolated cytokine responses in the human body usually represents an oversimplification of the phenomena. Additionally, modifying the healing process by regulating the cytokine milieu is a considerable challenge, since cytokine responses depend on time and concentration in the wound bed [2]. Growth factors are signaling proteins that release at the wound site and are required for communication between various cells such as smooth muscle cells, fibroblasts, myofibroblast, keratinocytes, endothelial cells, and immune cells. They can induce angiogenesis supplying oxygen and nutrients to cells transplanted for organ substitution to maintain their biological functions [4]. Different studies on human patients have confirmed that growth factors such as PDGF are involved in enhancing the wound-healing rate in acute wounds and even provide complete healing in chronic wounds [5]. Therefore, the development of regenerative medicine applications with the aid of exogenous growth factors could be an alternative clinical solution for skin regeneration. Transforming growth factor beta (TGFβ)-the TGF-β superfamily consists of 33 members. In mammals, mainly TGF-β1, β2 and β3 isoforms are found, but TGF-β1 predominates in cutaneous wound healing. They are produced by macrophages, keratinocytes, fibroblasts, and platelets].

Methodology
These three isoforms share 60–80% homology and are encoded by different genes. All three isoforms are believed to bind and signal through the two TGF-β receptors (TβRI and TβRII). TβRI activates the SMAD intracellular signaling pathway through Phosphorylation of Smad2 and Smad3 binding to Smad4, translocates into the nucleus, and activates transcription of target genes [65]. TGF-β can also activate a number of nonSmad signaling pathways, including ras/MEK/ERK, which requires the heparan sulfate-containing proteoglycan (HSPG) syndecan 4; p38, which requires the HSPG β-glycan; and c-Jun N-terminal kinase (JNK), which requires focal adhesion kinase and TGF-β-activated kinase (TAK) [66]. Much of the current knowledge on TGF-β action in wound healing has been obtained from animal studies using incisional and/or excisional wound models [6]. Preclinical studies indicated a significant reduction in scarring and considerably improved dermal architecture after intradermal injection of avotermin (TGF-β3) in adult rats. In adult mammals, high levels of TGFβ1 and TGFβ2 and low levels of TGFβ3 facilitate scar-forming healing, while in fetal mammals, high levels of TGFβ3 and low levels of TGFβ1 and TGFβ2 favor scar-free healing [7]. Other evidence support the involvement of TGFβ in regeneration, including using the potent small molecule inhibitor [8] and experiments with zebrafish [69]. Overall, these experimental observations support the role of TGFβ signaling in wound healing, including both non-specific scar formation and tissue-specific regeneration [7]. Vascular endothelial growth factor (VEGF)-the VEGF is the most important signaling growth factor in angiogenesis and vasculogenesis. VEGF is involved in wound healing and is secreted by platelets, macrophages, fibroblasts, and keratinocytes [59]. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E and placental growth factor. VEGF-A is one of the most potent proangiogenic molecules in the skin. It has been widely investigated as an exogenous cargo growth factor for skin tissue regeneration [60]. VEGF-A is a 45 kDa heterodimeric heparin-binding protein. Multiple isoforms of VEGF-A can be generated through alternative splicing. VEGF-A interacts with multiple receptors, including VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2). These are tyrosine kinase receptors that differ in their ligand binding properties and tyrosine kinase activity. Although VEGF-A binds VEGFR1 with a higher affinity than VEGFR-2, VEGFR-2 exhibits stronger inherent tyrosine kinase activity. VEGFR-2 is believed to be

more important than the two receptors in terms of controlling endothelial cell function and regulating angiogenesis based on its superior ability to stimulate downstream signaling cascades. On ligand binding, VEGFR-2 dimerizes, resulting in kinase activation and autophosphorylation of tyrosine residues. Phosphorylation of these residues leads to activation of protein kinase B (inhibits apoptosis), the mitogen-activated protein kinase (MAPK) pathway (induces proliferation), Src kinase, focal adhesion kinase, and p38 MAPK (leads to cell migration). It has been demonstrated that VEGF acts as an important regulator of angiogenesis (physiological and pathological) by inducing proliferation of fibroblasts and endothelial cells as well as by promoting neovascularization, re-epithelialization, and collagen deposition. Artificial three-dimensional scaffolds have been used as efficient dermal regeneration templates f to treat skin defects created by burns, trauma, and chronic diseases in regenerative medicine. Inadequate angiogenesis is often caused during application of such scaffolds. Tan et al. used collagen scaffolds with VEGF in a diabetic rat wound model and found that the treatment resulted in an enhanced healing rate, improved vascularization, and increased level of VEGF in the granulation tissue. Using plasmid DNA encoding activated VEGF-165 in collagen-chitosan dermal equivalents to treat the full-thickness burns could result in a significantly higher number of newly formed and mature blood vessels, enabling fast regeneration of the dermis. Platelet-derived growth factor (PDGF)-the PDGF is an important biochemical mediator of wound healing and promotes cellular reactions throughout all phases of the wound-healing process. PDGF is known to improve dermal regeneration, promote local protein and collagen synthesis, and cause angiogenesis. PDGF comprises a family of homodimeric or heterodimeric growth factors: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. It is mainly secreted from the α-granules of the platelet, but it is also released by different cells such as keratinocytes, macrophages, fibroblasts, and endothelial cells [6]. There are two PDGF receptors (PDGFR), PDGFR-alpha (PDGFRA) and PDGFR-beta (PDGFRB), engaging several well-characterized signaling pathways such as Ras-MAPK, PI3K, and PLC- γ, which are known to be involved in multiple cellular and developmental responses [7]. Dermal fibroblasts are one of the major target cells of PDGF in initiation and propagation of skin tissue repair. They secrete PDGF-BB and express PDGFRB receptor. PDGF-BB stimulates Wnt2 and Wnt4 mRNA expression. In terms of its relevance to wound healing and skin tissue regeneration, PDGF-BB exhibits chemotactic, mitogenic, angiogenic, and stimulatory effects, leading to modification of the extracellular matrix by stimulating collagen, collagenase, and glycosaminoglycan synthesis [8]. PDGFRB targeted deletion studies into dermal fibroblasts have demonstrated its role in transducing wound-healing signals accounting for an 85% reduction of granulation tissue mass [9]. Therefore, wound treatment using exogenous PDGF has been studied by developing a cellular collagen-chitosan temporary matrix of a wound site for in vivo dermal regeneration. This study suggested that PDGF supplementation could have altering effects on oxidative events depending on the duration of the wound-healing process [8]. In another study, a combination of AMD3100 (which mobilizes marrow-derived progenitor cell) and PDGF-BB therapy has been synergistically shown to improve progenitor mobilization and trafficking, resulting in significantly improved diabetic wound closure and neovascularization [10]. Fibroblast growth factor (FGF)-the FGFs include a family of polypeptides growth factors which have been demonstrated to have considerable capability in tissue repair and regeneration. It was originally identified to induce proliferation and differentiation in various types of the cell. The interaction of FGFs with their receptor tyrosine kinases (FGFRs) in the presence of heparin/heparan sulfate (HS) proteoglycans (HSPG) as a cofactor results in activation of FGFRs by phosphorylation of tyrosine residues. Activated FGFRs lead to triggering a number of signaling pathways such as the RAS/MAP kinase pathway, PI3 kinase/AKT pathway, and PLCγ pathway, resulting in specific cellular responses. Regeneration is controlled by a different type of growth factors among which FGFs are the key players in tissue regeneration, including the neural, liver, bone, skin, intestine, cardiac, and muscle. According to the amino acid sequence, the FGF family is divided into seven subfamilies. However, FGF2 (basic FGF) is indicated to be widely applied for scarless wound healing and skin wound regenerative therapy. It has been reported that the sustained release of basic FGF from a chitosan film as a delivery vehicle could accelerate wound healing in full-thickness skin wounds made on the backs of genetically diabetic mice and promote proliferation of fibroblasts and granulation tissue formation. In another study, incorporation of bFGF with gelatin microspheres significantly accelerated dermal tissue regeneration. Furthermore, studies have identified that FGFs are key regulators of keratinocyte migration in wounded skin, as the loss of FGFR1 and FGFR2 in keratinocytes results in a wound-healing defect [10]. Hepatocyte growth factor (HGF)-the HGF was originally discovered as a mitogen of hepatocytes to be produced by stromal cells. HGF stimulates many properties of the epithelial cell, including proliferation, motility, morphogenesis, and angiogenesis via tyrosine phosphorylation of its receptor, tyrosine-protein kinase Met (c-Met) [9]. The mature form of HGF is composed of α/β heterodimers linked by a disulfide bond. The α-chain contains an N-terminal hairdomain and first Kringle domain and exhibits a high-affinity binding site for Met, and the β-chain has a serine protease-like structure; although the α-chain is required for Met binding, it is not able to activate Met and the β-chain induces the activation of Met and biological responses [9]. The binding of HGF to its receptor, c-Met, results in structural changes in c-Met and phosphorylation of protein tyrosine kinase (PTK) domain. Subsequently, two other phosphotyrosines in the carboxy-terminal multifunctional docking domain recruit intracellular signaling molecules Grb2 (growth factor-receptor-bound protein 2), Gab1 (Grb2-associated binder 1), phosphoinositol 3-kinase (PI3K), MAPK, PLCγ (phospholipase Cγ) and Shp2 (SH2-domain-containing protein tyrosine phosphatase 2), signal transducer and activator of transcription factor (STAT) pathway. Therefore, c-Met and its related signaling pathways play a crucial role in the diverse process, including embryogenesis, wound healing, organ regeneration, and mature tissue survival. It promotes mitogenic, morphogenic, and mitogenic activity in various cell types. HGF/Met contributes to immune regulation by modulation of DC migration and activation of

monocytes and macrophages. HGF is a cytokine known to play multiple roles during the various stages of wound healing and accelerates wound healing by promoting the dedifferentiation of epidermal cells through1-integrin/ILK pathway [9]. According to this study, HGF increased the expressions of the cell adhesion molecules 1-integrin and the cytoskeleton remodeling protein integrin-linked kinase (ILK) in epidermal cells in vivo and in vitro. Baek et al. [98] demonstrated that Met signaling in skin-resident DCs was essential for their emigration toward draining lymph nodes upon inflammation-induced activation. These findings were supported using a conditional Met-deficient mouse model where activated skin resident DCs failed to migrate toward the skin-draining lymph nodes despite an activated phenotype. Epidermal growth factor (EGF)-the EGF is primarily secreted by platelets, macrophages, fibroblasts, and keratinocyte and is present during dermal wound healing and facilitates skin regeneration. The binding of EGF to EGFR activates EFGR through ligand-induced dimerization, leading to a downstream signaling cascade, including Ras/MAPK, PLCγ/PKC, PI3K/Akt, and STAT [9,10].

Conclusion

These signaling pathways are classified into four different categories: migration, proliferation, cytoprotection, and EMT among which migration and proliferation pathways have been required for wound healing [10]. EGF is influenced by different components of the keratinocyte migration machinery and induces contraction of keratinocytes, which are critical to wound re-epithelialization. Despite extensive progress in the exogenous EGF in the treatment of acute wounds, its efficacy in chronic wound therapy is limited owing to their short half-life in vivo and poor transdermal permeability. To overcome these restrictions, EGF was conjugated to an efficient delivery system to extend the residence time of EGF in the wound area and significantly regenerated skin tissue.

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NOVEL AND DEVELOPING THERAPIES FOR ATOPIC DERMATITIS

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ABSTRACT

The number of promising new medications for treatment of atopic dermatitis (AD) are in developmental stage. Novel topical medicines can be especially helpful for children, providing an alternative to the need for chronic topical corticosteroid use. While many patients with mild or moderate disease can be managed with topical treatments, there are unmet needs for recalcitrant and severe cases. New and developing therapies promisiong for real advances in management of the disease.

Keywords: Dermatitis, atopic; Eczema; Phosphodiesterase inhibitors; Novel Therapeutics

Introduction

Atopic dermatitis (AD) is a very common, chronic inflammatory skin disease affecting up to 20% of children and 10% ofadults in industrialized countries.1 Clinical features of AD include erythema, edema, lichenification, excoriations, oozing, and crusting. Pruritus is a crucial and dominant feature of AD and generates comorbidities such as sleep loss and psychological distress, creating a continuing disease burden for patients, parents and siblings, pathogenesis is not clearly elucidated, though skin barrier defects and altered immune responses are accepted as key components in disease development. Genetic and environmental factors strongly affect AD expression{1,3}. Disease prevalence is increasing in developing countries, especially in urban regions. 1. MILD ATOPIC DERMATITIS usually successfully managed with a combination of TCS and general recommendations such as moisturizing, preventing heat and sweating and reducing psychological stresses{2.4}. 2. MODERATE ATOPIC DERMATITISUsually requires topical therapy with TCS, possibly supplemented with topical calcineurin inhibitors. In patients with moderate. 3.SEVERE ATOPIC DERMATITIS Current guidelines recommend the use of traditional immunosuppressant medications including cyclosporin (CYA), methotrexate (MTX), mycophenolate mofetil (MMF), and azathioprine (AZA) in patients who fail conventional topical therapy or phototherapy. {5.6}Traditional immunosuppressive therapies can show effectiveness in AD, their routine use is limited by often inadequate disease responses and by adverse effects. Biologic therapy holds promise for providing those patients who suffer from severe disease with effective, long-term options by virtue of their targeted effects on the dysregulated inflammatory responses that cause chronic and recalcitrant disease. As our specificunderstanding of the complex pathogenesis of AD improves, including immune and molecular pathways, a variety of experimental biologics are targeting these pathways with the hope of less toxicity and greater efficacy. Novel therapies: Phosphodiesterase (PDE) inhibitors (Crisaborole) Patients with AD showed significantly elevated leucocyte PDE activity compared to non-atopic normal individuals or to patients with allergic contact dermatitis {8}. NEW AND EMERGING SYSTEMIC THERAPIES Anti IL-4 and IL-13 (Dupilumab) is a fully-humanized, monoclonal antibody targeting the alpha subunit of the IL-4 receptor to block signaling of IL-4 and IL-13. Early phase I and II trials demonstrated its effectiveness in improving the symptoms of adult patients with AD in a dose dependent manner.{9,10}It is has proven that suppressed mRNA expressionin lesional skin of genes related to activation of T cells, dendriticcells, eosinophils, inflammatory pathways, and type 2 cytokines, potential to reverse multiple molecular defects in patients with AD. Although adverse effects were relatively few, conjunctivitis, injection-site reactions, nasopharyngitis, and upper respiratory tract infection are worth mentioning.{7} Topical ophthalmic anti-inflammatory medications were typically needed to control eye symptoms while other cases resolved spontaneously. Nemolizumab subcutaneous injections were well-tolerated with adverse effects mostly in AD exacerbations, to provide significant itch relief in patients with AD in a dose-dependent manner. Further studies are warranted to better clarify the effects on skin inflammatory lesions and to further understand the side effects.

Conclusions

Therapies for AD have long been relatively stagnant with few dramatic breakthroughs. While new topical therapies are emerging, none has yet matched the efficacy of mid-strength TCS. That approach has been hampered by “steroid phobias” and misleading claims of “steroid addiction”, in part a consequence of over-prescribing by physicians, but also from emotion-based internet-generated fears. Clearly there remains a need to find more potent topical agents with fewer side effects. The most gratifying advances in AD therapy have come from better understanding of immune and inflammatory mechanisms. The shining example is the development of dupilumab which has shown remarkable reduction in clinical severity with relatively few adverse effects. The many other new compounds in the pipeline should continue to provide real advances in management of this severe, common and complex diseases.

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SYSTEMIC IMMUNOTHERAPY IN ATOPIC DERMATITIS (AD)

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ABSTRACT

The atopic dermatitis, which affects nearly all aged population worldwide, which affects mark able decline of patients' quality of life. Etiological factors are currently a topic of study and interpretation, the main features of atopic eczema are skin barrier disturbance and immune dysregulation. Pharmaceutical progress in understanding immunopathology of atopic dermatitis have allowed identification of therapeutic molecular targets in the field of biological therapy and Systemic immunotherapy.
Keywords: Atopic Dematitis, Immunotherapy, Interleukins

Introduction

Atopic dermatitis is a chronic, itchy skin condition that is very common in children but may occur at any age. It is also known as eczema and atopic eczema and was formerly known as Besnier prurigo. It is the most common form of dermatitis. Atopic dermatitis usually occurs in people who have an 'atopic tendency'. This means they may develop any or all of three closely linked conditions; atopic dermatitis, asthma and hay fever (allergic rhinitis). Often these conditions run within families with a parent, child or sibling also affected. A family history of asthma, eczema or hay fever is particularly useful in diagnosing atopic dermatitis in infants. Atopic dermatitis arises because of a complex interaction of genetic and environmental factors. These include defects in skin barrier function making the skin more susceptible to irritation by soap and other contact irritants, the weather, temperature and non-specific triggers: see Causes of atopic dermatitis.

Methodology

Immunotherapeutical agents:T-helper 1 (Th1)/Th2 imbalance and their associated cytokines are one facet of the pathological processes of AD. Several experimental agents have been investigated for their potential beneficial effects in the treatment of AD, by modulating Th1/Th2 homeostasis.

Omalizumab-Blocking the consequences of mast cells and basophil activation during the allergic inflammatory cascade has been a major therapeutic goal, and the use of Omalizumab has inaugurated a new era in the treatment of atopic disease. Initially, Omalizumab was approved by the FDA for the treatment of moderate to severe persistent asthma that is uncontrolled with a combination of a medium to high-dose inhaled corticosteroid and a long-acting β2-agonist, in adults and patients of 6 years and older who are sensitized to perennial aeroallergens. In 2014, FDA also approved Omalizumab for the treatment of chronic spontaneous urticaria in adults and children over 12 years of age who exhibit severe symptoms, inadequately controlled by high doses of H1 antihistamines. In addition, Omalizumab was proven to bring favourable results in patients with different subtypes of chronic inducible urticaria, allergic rhinitis, eosinophilic esophagitis, food allergy and anaphylaxis, as well as premedication in allergen specific immunotherapy, Churg-Strauss disease, eosinophilic otitis media, allergic bronchopulmonary aspergillosis, chronic rhinosinusitis, bullous pemphigoid, contact dermatitis and atopic dermatitis (1–5).

Mechanism of action; Omalizumab is a recombinant humanized monoclonal IgG1 antibody composed of 5% murine and 95% human sequence. It prevents the interaction of IgE with its receptors by recognizing and binding to the Fc portion (the CH3 domain) of free serum or membrane-bound on B cells immunoglobulin E molecule, but not IgE bound to its high (FcεRI) or low (CD23) affinity receptors. The CH3 domain serves as the site by which IgE binds to its receptors. Binding of Omalizumab to free, soluble IgE blocks the binding of IgE to its receptors, and subsequently blunts allergen-induced mediator release. Once Omalizumab is administered, it results in the formation of soluble immune complexes with free IgE, typically trimers, which are cleared by the reticuloendothelial system(2). Administration of Omalizumab dramatically reduces the serum levels of free IgE (by 99% in the first two hours after administration), which subsequently downregulates the expression of IgE high-affinity receptors on immune cells. Expression of FcεRI facilitates antigen presentation by DCs. The latter cells appear to be crucial in the phenotypic development of Th1/Th2 cells and have a documented overexpression of FcεRI on the surface of DCs in allergic individuals. Moreover, IgE neutralization therapy decreases the serum expression of several cytokines (such as IL-5, −8, −13) and negatively regulates the recruitment of immune cells (T-cells, eosinophils, and macrophages) to inflammatory sites. Thus, Omalizumab decreases both the immediate and the late phase allergic inflammation. Another mechanism of action involves mast cells and eosinophils' apoptosis in allergic patients treated with Omalizumab compared to controls. Omalizumab - Anti-IgE therapy in AD brought conflicting results. Although most data from small randomized trials, case series and case reports documented clinical benefit and resolution of eczema, a small number of studies showed no improvement of disease with Omalizumab. The

response variation to treatment helped to pinpoint patients that are most likely to respond to anti-IgE therapy. Lack of filaggrin mutations and lower elevations of total serum IgE are factors associated with a likely favourable response to Omalizumab. All of the studies noted the safety profile in both adult and paediatric population treated with Omalizumab. However, the wide variability of response to treatment remains largely obscure, while lack of standardized protocols regarding dosing is currently an unanswered task. Another notable conclusion of placebo-controlled studies showed that the improvement in clinical outcome of patients treated with Omalizumab was similar to improvements in control groups. In the authors' experience, in a case series of three patients with severe refractory atopic dermatitis with atopic diathesis (sensitization to house dust mites, and moderate serum levels of total IgE in all three cases, co-existing asthma and rhinitis in one patient), Omalizumab, 300 mg monthly, brought a significant disease improvement, which occurred within the first three months of treatment. Dupilumab-the FDA approved Dupilumab for the treatment of adult patients with moderate to severe atopic refractory dermatitis, clinical studies proving concomitant efficacy in other atopic disorders, such as asthma and nasal polyposis In addition, Dupilumab efficacy is under investigation for eosinophilic esophagitis and atopic dermatitis in paediatric patients .Mechanism of action- Dupilumab is a fully human monoclonal antibody directed against interleukin-4 (IL-4) receptor-α (IL-4Rα) that blocks the synergistic effects of IL-4 and IL-13 on allergic inflammation. Atopy is the inappropriate secretion of immunoglobulin of E isotype in response to allergen exposure. IL-4 and IL-13 are key drivers of the Th2 allergic inflammation and of consecutive production of IgE. Both IL-4 and IL-13 signal through a common receptor, IL-4Rα, to activate the signalling proteins [signal transducer and activator of transcription 6 (STAT6) and Janus kinase-1 (JAK1)] (8). IL-4 is a crucial positive regulator of allergic inflammation. It induces the immunoglobulin isotype class switch to IgE, promotes the Th2 phenotype, prevents T-cell apoptosis, renders the refractory status of T-cells to corticosteroids, and induces the expression of VCAM-1 on endothelial cells, subsequently promoting the recruitment of cells characteristic to the allergic inflammation (T-cells, eosinophils, basophils and monocytes) (9). Gene polymorphisms in IL-4, −13 and IL-4Rα have been associated with AD in certain populations (10). Additionally, IL-4 and −13 regulate expression of genes encoding proteins involved in skin susceptibility to skin pathogens . IL-4 receptors that function to stimulate the IgE receptors expression and cysteinyl leukotriene synthesis are also expressed on mast cells.
Dupilumab-Targeting Th2 polarization with Dupilumab brought unprecedented advances in the treatment of moderate to severe refractory AD. Dupilumab mono-therapy or combined therapy is associated with effective control of disease, improvement in skin lesions, significant reduction in pruritus and a substantial contribution to the reduced quality of life of affected patients (6). Dupilumab proved to reduce the expression of Th2 biomarker levels and of genes associated with the activation of T-cells, and to favour a genetic profile involved in skin barrier function. Data regarding the molecular signature showed that after 4 weeks of treatment with Dupilumab, the transcriptome of skin lesions of AD resembled that of non-lesional skin (7). A collection of clinical trials that included large number of patients with moderate to severe AD vs. control groups investigated the efficacy and safety of Dupilumab in AD (6–9). With no exception, evidence proved a rapid and marked improvement of disease activity, to the placebo group, and a safe profile of administration.IL-22 promotes epidermal hyperplasia and skin barrier dysfunction in AD.

Conclusion

Atopic dermatitis poses a challenge for clinicians and patients alike, particularly in severe forms of disease. Recent progresses in understanding the pathophysiology of atopic dermatitis underlie its multiple facets and allow the introduction of novel substances for the systemic and topical treatment of severe atopic dermatitis. New therapeutic strategies brought tremendous advances in the management of refractory to conventional treatment, severe atopic dermatitis. Robust evidence pleads for efficacy of Dupliumab, while other immunomodulatory agents, such as Nemolizumab, Lebrikizumab, Tralokinumab, Ustekinumab and Apremilast show promise, but further data are needed to confirm their usefulness and safety in atopic dermatitis. Allergen specific immunotherapy may be of use in selected cases of atopic dermatitis.

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CLINICAL APPLICATIONS OF PRENATAL SCREENING

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ABSTRACT

In a society in which substantial proportions of people believe in life after death a widespread belief in life before birth, and an aversion to its abrupt termination because of untreatable disease, is hardly to be unexpected. Even those who do not believe in life after death can hardly regard the destruction of life at any time with complete indifference, although there is a wide range in the aversion with which the gametocide of contraception, the feticide of abortion, and infanticide are regarded as tolerable, or in the toleration of any changes in legislation. Those who look for religious guidance will find little consistency, excepting the religion viewpoint which, since gametocide is regarded as unequivocally sinful, avoids the problem of defining when an embryo lives. Unfortunately this is a field of legislative and theological activity in which discussions tend to take place without reference to the realities involved, and even medically qualified advocates of various actions do not always take the trouble to obsetve the consequences of the actions they recommend. Nor do they appreciate that, in courtship, marriage, conception and birth, specialists and sell-selected members of committees and societies are unlikely to have values representative of the silent majority.

Screening procedures, such as those used for the detection of both Rhesus native women and the antibodies they are prone to produce, are so simply, reliable, and effective that a failure to ensure such tests are carried out would be regarded as negligent. The actions consequent on the results are also evidently beneficial to all parties, including the fetus, so that opposition is restricted to very small religious minorities, whose views can be overruled.

All other screening procedures for genetic disease by proxy, that is, by parental study, cannot be applied to the benefit of all parties, and will not be acceptable to substantial minorities. This imposes serious difficulties in providing an efficient and economical service for those who wish such tests to be done on them.

Although malformations of unknown aetiology can hardly be termed genetic unless the word is used to cover all disease. A test case is provided by spina bifida, since the births of many cases could be interrupted following automated screening of maternal blood for alphafetoprotein at 16 weeks, but this is administratively difficult unless done routinely on all pregnancies.

In the genetic disorders the impact on the frequency of disease is likely to be slight in most Caucasian communities, although the elimination of fetuses with some specific recessive disorders is technically possible. In some countries plans have been advanced aiming at the elimination of Tay-Sachs disease. Similar plans will eventually be feasible for the elimination of most haemoglobinopathiesin societies in which they are common, legitimacy usual, abortion acceptable, and medical standards high. Cystic fibrosis might be similarly amenable to elimination within a decade. The prospects for other genic conditions, most of which have an incidence of less than one per pediatrician lifetime, seem remote. In particular, the eye, the ear, and the brain are unlikely to have many of their metabolic derangements demonstrable by proxy in cells which are both present in the amniotic fluid and sufficiently undifferentiated to grow in culture.

Keywords: screening procedures, malformations, genetic disease, prenatal diaqnostics

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RESEARCH OF THE MORTALITY IN 2018, 2019, 2020 AND EFFECTS OF COVID-19 ON MORTALITY

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ABSTRACT

Background
In the literature, there are different studies about the mortality and epidemiology of COVID-19 and such researchs are expected to increase even more. We purposed in our study to compare data about the mortality and epidemiology of patients who died from COVID-19 and another disease in January, February, March, April, May and June of 2020 with data of patients who died because of other diseases in the same months of 2018, 2019 and 2020 .

Materials and Methods
We evaluated the files of the patients who died in the months of January, February, March, April, May and June of 2018, 2019 and 2020 retrospectively. Patients died from COVID-19 were exact cases whose reverse transcription polymerase chain reaction was positive. We noted age, gender, nationality, marital status, social security, place of residence of all patients. Mortality in our study describes number of deaths / inpatients.

Results
The number of inpatients in 2018 was 49831, in 2019 was 46429, in 2020 was 37843 and totally was 134103. 1421 of them were inpatients from COVID-19. 39 of 1421 inpatients died because of COVID-19. Average number of mortality in 2018 was 0.92%, in 2019 1.14% and in 2020 1.47% and there was significant relationship between groups ($p < 0.0001$). The mortality of COVID-19 was determined 2.74 % in our hospital.

Conclusion
In conclusion, in our study, average number of deaths and mortality in 2020 were more than in 2018 and 2019. In addition, COVID-19 may have begun in February 2020, not in March, in Turkey according to the number of deaths in February and March in 2020 of our hospital. Mortality in June 2020 was less than June 2019 and June 2018. We think that our study can contribute to the last epidemiologic data in the world.
Keywords: COVID-19, 2019-nCoV, SARS-CoV-2, mortality, epidemiology

Introduction
Early December 2019, in Wuhan, capital of the state of Hubai, China, a large number of cases of pneumonia of unknown origin began to appear (1,2). This disease quickly spreaded to other parts of China and even six continents. On January 3rd 2020, a new type of coronavirus family was detected from a bronchoalveolar lavage sample of a patient in Wuhan. On January 7th 2020, World Health Organization (WHO) named this virus as new type of coronavirus 2019. On February 11th 2020, WHO defined this disease as coronavirus disease 2019 (COVID-19) (3-5). On March 11th 2020, the first case was determined in Turkey and the epidemia has also started in our country too. In the literature, there are different studies about the mortality and epidemiology of COVID-19 and such researchs are expected to increase even more. We purposed in our study to compare data about the mortality and epidemiology of patients who died from COVID-19 and another disease in January, February, March, April, May and June of 2020 with data of patients who died because of other diseases in the same months of 2018, 2019 and 2020 .

Materials and methods
This study was approved by the ethics committee of İzmir Tepecik Training and Research Hospital with the decision number 2020/ 7-20 and also by the ethics committee of Republic of Turkey, Ministry of Health. We evaluated the files of the patients who died in the months of January, February, March, April, May and June of 2018, 2019 and 2020 retrospectively. We included patients died because of COVID-19 and other diseases in aforementioned months. Patients died from COVID-19 were exact cases whose reverse transcription polymerase chain reaction (RT-PCR) was positive. We noted age, gender, nationality, marital status, social security, place of residence (center of city, environmental district, neighboring province and another city) of all patients. We also recorded the results of coronavirus tests of patients who

were hospitalized because of COVID-19. We noted the number of all inpatients who were hospitalized in the months and years previously mentioned. Mortality in our study describes number of deaths / inpatients.

Statistical analysis

Statistical power analysis was made with G-Power 3.1.9.4 programme and we determined that our study had 85.4% power. Statistical analysis was performed with SPSS Version 17.0 statistic software package. Different variables were investigated with analytical methods if they were suitable for normal distribution (Kolmogorov-Smirnov/Shapiro-Wilk tests). In descriptive analysis, parameters were written as frecuency, percent, mean, standard deviation. Pearson's chi-squared, Spearman tests and one-way ANOVA were used in comparing categorical data between groups. We used independent samples t test for metric and normally distrubuted variables when we compared independent samples. P values of less than 0.05 were *regarded as* statistically significant.

Results

The number of inpatients in 2018 was 49831, in 2019 was 46429, in 2020 was 37843 and totally was 134103. 1421 of them were inpatients from COVID-19 (208 in March, 572 in April, 348 in May, 293 in June. We determined that 464 people died in 2018 (251 male, 213 female) (163 not married, 301 married), 530 died in 2019 (299 male, 231 female) (124 not married, 406 married) and 528 died in 2020 (310 male, 218 female) (223 not married, 215 married). 39 of 528 inpatients died because of COVID-19 (25 male, 14 female) (20 not married, 19 married). Average number of deaths in 2018 was 77.3, in 2019 was 88.3 and in 2020 was 88 and there was significant correlation between groups ($p < 0.0001$). Average number of mortality in 2018 was 0.92%, in 2019 1.14% and in 2020 1.47% and there was significant relationship between groups ($p < 0.0001$). 47 government official, 275 workers, 60 employer and 82 other professionals died in 2018. In 2019 42 of deaths were government officials, 284 of deaths were workers, 108 of deaths were employers and 96 of deaths were other professionals and in 2020 the numbers were 47, 313, 56, 112 in order. 4 of died patients from COVID-19 were government official, 6 deaths were workers, 22 deaths were employers and 7 deaths were other professionals. The mean age of the patients who died in 2018 was 66.02 ± 11.51 (61.64 for male, 62.56 for female), in 2019 was 61.64 ± 12.24 (60.17 for male, 63.48 for female), in 2020 was 61.53 ± 11.58 (61.51 for male, 63.2 for female). The mean age of the inpatients who died because of COVID-19 was 70.31 ± 9.14 (66.82 for male, 69.02 for female) (min 11, max 97). There is significant relationship between the mean age of inpatients who didn't die because of COVID-19 and the mean age of inpatients who died because of COVID-19 ($p = 0.001$). All other data was presented in tables (table 1-4).

Discussion

Several clinical studies and case reports were published about mortality of COVID-19 and it was noticed that data about this topic is varying in time. Therefore researchs on mortality of COVID-19 remain fresh. This is a descriptive study on the epidemiology of 2019 novel coronavirus (2019-nCoV), including data on 1421 patients with 39 cases who died because of COVID-19. In April 2020, one of 4 cases, in May one of five cases and in June one of seven patients died from COVID-19. If attention is paid, we can determine that the number of sick people with COVID-19 decreases from the month of May. We think about this result that our doctors could take control outbreak in our hospital from May. According to our research, mortality from COVID-19 is more in male patients and marital status isn't effective on mortality. In all years, we can observe that workers died mostly because of both COVID-19 and another diseases. In Turkey, government officials and employers usually prefer university hospitals and workers usually prefer the training and research hospitals for health care. This data explains why most of the people who died in our hospital were workers. Interestingly, 22 of 39 died from COVID-19 were employers in our hospital. We explain this so, because our hospital was made one of the pandemic hospitals in İzmir, employers applied to our hospital by necessity. Otherwise, we determined the mean age of cases died from COVID-19 was high both for male patients and female patients. We found that average number of deaths in 2018 was less than in 2019 and in 2020. The average number of deaths in 2019 and 2020 was close to each other, to be noted here is that our hospital is a pandemic hospital and in first six months of 2020 patients with COVID-19 were most common hospitalized therefore it can be said mortality of COVID-19 may be more than of other diseases. In this topic more studies should be made. In our study, we found mortality in 2020 because of COVID-19 was more than in 2019 and 2020 because of other diseases. Except those if you take heed of the number of deaths in January and February in 2020, the number increased significantly in February. Based on this result, we think that COVID-19 may have begun in February 2020, not in March.

Several published reports of early clinical descriptions of COVID-19 have emerged from Hubei province in China, and many more will come from different countries. Zhou et al. (6) provide further insight into the clinical course and mortality risk for adults with COVID-19 severe enough to require hospitalisation. They report findings from 191 patients with COVID-19 from Wuhan during the first month of the outbreak, and follow them through to discharge (n=137) or death (n=54). The median age of dead cases was 56 and 62% of all patients was male. The current message of this research is that mortality is high among the minority of people with COVID-19 who get severe disease. We recorded in our study 213 discharged patients and 2 dead cases in first month (March) of the outbreak in Turkey. 56% of all dead cases was male and 44% of all cases was female. The reduced susceptibility of females to

viral infections could be attributed to the protection from X chromosome and sex hormones, which play an important role in innate and adaptive immunity (7). Additionally if we compare the death rate, mortality from COVID-19 is 2.45 times the mortality because of other diseases in our work. In Chen et al.'s study with 99 patients (8), mortality of the 99 included

patients infected by 2019-nCoV was 11%. This result is resembling Huang et al.'s study (9). In other studies, the mortality of SARS coronavirus (SARS-CoV) has been reported as more than 10% (10) In our study we determined the mortality as 2.75%. Mortality in our research is less than Chen et al and Huang et al's studies but it shouldn't be forgotten that these two works were published in January 2020 so there weren't appropriate medical agents for COVID-19 in January 2020. It was later understood which treatment methods were effective for COVID-19.

In this case series of death patients admitted to service and intensive care unites (ICU), with laboratory-confirmed COVID-19 from March 11 to June 30, 2020, the majority were older men and ICU mortality was 26%. Graselli et al. (11) found in their study older patients (n = 786; age ≥64 years) had higher mortality than younger patients and ICU mortality was 26%. These results were compatible with our research.

In our study, since June 2020, mortality decreased significantly and we observed that mortality in June 2020 was less than June 2019 and June 2018. According to these results we think that struggle against COVID19 in İzmir became succesful. In getting these results, we believe that residents in İzmir have taken due care to the use of masks and paid attention to social isolation. In addition, doctors and all health care workers in our hospital worked most efficent for the treatment of COVID19.

This study has two limitations. First, this was a retrospective study. Second, only 39 patients with confirmed COVID-19 were included; suspected but undiagnosed cases were ruled out in the analyses. It would be better to include as many patients as possible.

In conclusion, these results can still be considerably useful for epidemiological description of the disease in terms of person-level risk. COVID-19 can be more mortal than several diseases. In our study, average number of deaths and mortality in 2020 were more than in 2018 and 2019. In addition, COVID-19 may have begun in February 2020, not in March, in Turkey according to the number of deaths in February and March in 2020 of our hospital. Mortality in June 2020 was less than June 2019 and June 2018, In getting these results, we believe that residents in İzmir have taken due care to the use of masks and paid attention to social isolation. We think that our study can contribute to the last epidemiologic data in the world.

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Table 1. Epidemiological characteristics of inpatients died in 2018, 2019, 2020

Parameters	2018 (n)						2019 (n)						2020 (n)					
	January	February	March	April	May	June	January	February	March	April	May	June	January	February	March	April	May	June
Total (death)	81	77	86	62	73	85	86	104	83	82	88	87	72	111	104	91	89	61
Male	38	38	47	34	43	51	46	55	53	47	49	49	34	70	57	57	52	40
Female	43	39	39	28	30	34	40	49	30	35	39	38	38	41	47	34	37	21
Service	19	29	37	13	18	26	20	28	24	23	36	23	29	59	37	34	33	21
Intensive case unit	62	48	49	49	55	59	66	76	59	59	52	64	43	52	67	57	56	40
Nationality																		
Turkish citizen	78	70	81	61	69	83	81	100	77	77	86	85	71	105	99	86	88	59
Not Turkish citizen	3	7	5	1	4	2	5	4	6	5	2	2	1	6	5	5	1	2
Marital status																		
Not married	55	33	24	21	12	18	20	25	19	19	26	15	19	58	44	39	48	15
Married	26	44	62	41	61	67	66	79	64	63	62	72	53	53	60	52	41	46
Social security																		
Government official	9	3	10	6	7	12	9	12	5	4	4	8	9	11	10	9	4	4
Worker	48	49	45	41	42	50	11	60	46	56	53	58	47	59	58	59	58	32
Employer	9	12	13	5	14	7	51	14	13	7	14	9	6	11	18	7	7	7
Other	15	13	18	10	10	16	15	18	19	15	17	12	10	30	18	16	20	18
Residence																		
Center	65	63	71	55	64	72	78	87	62	71	64	67	60	96	78	76	68	48
Environmental district	14	8	13	5	8	9	3	10	10	4	13	11	7	10	11	8	11	6
Surrounding province	2	6	1	1	0	3	4	5	8	5	6	9	2	5	13	4	8	5
Other city	0	0	1	1	1	1	1	2	3	2	5		3		2	3	2	2
Inpatients (dead+alive)	8547	7742	8159	7886	9137	8360	8355	7464	7763	7898	8350	6599	8471	7528	6413	4189	4744	6498
Mortality (%)	0.94	0.99	1.05	0.78	0.79	1.01	1.02	1.39	1.06	1.03	1.05	1.31	0.84	1.47	1.62	2.1	1.87	0.93

Table 2. Epidemiological characteristics of inpatients died in 2020 because of COVID-19
**p ≤ 0.05*

Parameters	March	April	May	June	Total (all months) (%)
Total	2	19	14	4	39
Male	2	13	6	4	25 (56%)
Female	0	6	8	0	14 (44%)
P					0.03*
Service	0	2	2	0	4
Intensive care unit	2	17	12	4	35
Nationality					
Turkish citizen	2	17	13	3	35
Not Turkish citizen	0	2	1	1	4
Marital status					
Not married	0	9	9	2	20
Married	2	10	5	2	19
P					0.88
Social security					
Government official	0	2	1	1	4
Worker	2	1	1	2	6
Employer	0	13	9	0	22
Other	0	3	3	1	7
Residence					
Center	1	10	9	2	22
Environmental district	1	3	2	1	7
Surrounding province	0	3	1	0	4
Other city	0	3	2	1	6
Inpatient (dead+alive)	208	572	348	293	1421
Mortality (%)	0.96	3.32	4.02	1.3	2.74

Table 3. The numbers of inpatients died from COVID-19 and another one cause

Parameters	2020 (n)						
	January	February	March	April	May	June	Total
Not COVID-19	72	111	102	72	75	57	489
COVID-19 (exact case)	0	0	2	19	14	4	39
Total	72	111	104	91	89	61	528

Table 4. Relationships about mortality rate between death from COVID-19 and death from another cause

Parameters	Death from another cause	Death from COVID-19	Inpatients	Mortality (%)	P
2018	306	0	33542	0.91	0.021*
2020 (from COVID-19)	0	39	1421	2.74	
2019	340	0	30610	1.11	< 0.0001*
2020 (from COVID-19)	0	39	1421	2.74	
2020 (from another cause)	306	0	21844	1.4	0.021*
2020 (from COVID-19)	0	39	1421	2.74	
Total (from another cause)	952	0	85996	1.1	< 0.0001*
2020 (from COVID-19)	0	39	1421	2.74	

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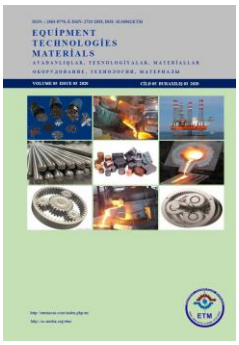

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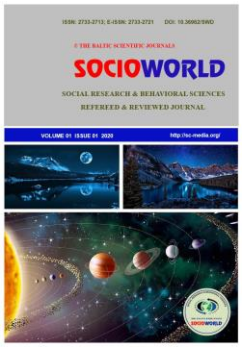

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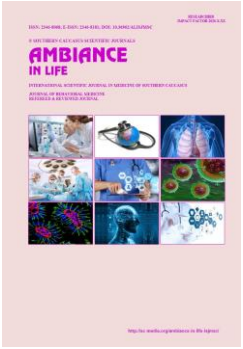

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