Dog Nose to E-Nose in Disease Diagnosis

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Abstract

Dog has natural gift of better smelling power which can be exploited for several purposes and disease diagnosis is one amongst them. The work on the use of dog nose in disease diagnosis is in preliminary stage. The electronic noses/e-noses are sensor based physical devices which are used to detect and analyse the various volatile organic compounds (VOCs) specific for health disorders including cancer to metabolic and infectious diseases. The sensor based disease diagnosis is also in preliminary stage. The data generated through studies conducted on disease diagnosis using one of the best noses of the universe may improve the sensitivity and specificity of existing e-noses to add par and this refined artificial intelligence, web data bases and sophisticated hardware and software may play in future a major role in field of diagnosis, monitoring and surveillance of human and animal diseases.

Key words: Dog, e nose, special senses, disease diagnosis, cancer

Introduction

The dog can detect the odour forty-four to thousand times better than the human and up to forty feet underneath the ground. The structures imparting additional sense of smell to dog are wet snout, folded mucous membrane, scent receptors/scent glands, vomeronasal organ, alar fold, and olfactory and accessory lobes. The moist leathery *snout surface* of dog acts like Velcro. It traps and dissolves the tiniest smell particles and makes them available to the scent receptors [1]. The fifty times bigger folded *mucous membrane* located right behind the nose and in front of brain compared to postage stamp size in human, and 220 million *scent receptors* against 5 million in human, which are 40 times more in dog, increase the sense of smell in dog [2]. Further, the crescent shaped *vomeronasal organ* of dog, which was lost in human in embryonic development have role in detection of sex scent and pheromones [3]. The *alar fold* just inside the nostrils opens and allow the inhaled air to flow through the upper part of nasal cavity where scent receptors grew, improve the detection of smell. The *olfactory bulbs and accessory lobes* decode every smell they encounter. The bulbs weigh nearly 60 grams and are about four times larger than the human beings. In addition to olfactory bulbs, the dog also has a pair of *accessory olfactory lobes*. It is to be noted that dog brain is one tenth of the size of human brain, that means the dog brain has forty times as much of its brain developed to smell as human do. Little wonder then that a dog's sense of smell is reckoned to be 100,000 times better than human [4]. The breeds of dogs which have longer muzzle, more scent receptors, long ears for scooping up more scent particles and wrinkles to catch scent in atmosphere are better suited for scent work.

ROLE OF DOG IN HUMAN LIFE

The dog is having multifaceted use in human life. Traditionally, it was used for, hunting preys, alerting the presence of predators, transportation, walking companion/ companionship, emotional supporter, children's playmate and for recreational activities such as sports and shows. Besides, the dog itself is used as human food in countries like Mexico, Korea, China, Vietnam etc where dog soup is a famous dish.

The sniffer dog is used for a number of human good activities. The detection of human theft is one amongst them. Each individual has specific sweat odour which is made up of eight kinds of volatile odorous chemicals emitted from pheromones, mucous, sebum, hormones and metabolites produced by five kinds of bacteria

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present on the body surface [5]. The great diversity has been reported from human sweat. There are roughly 5500 or more types of human sweat [6]. The dog identifies the thief in odour match test. The dog can differentiate the odours in footprints, which is naturally used in tracking the path and direction of its prey by detecting the residual odour persisting in the area after source has left, but, now a days, this peculiarity is used in tracking the path of kidnapped/missing persons, schizophrenic patients, criminal escapees and militants etc which a dog can do correctly up to twenty minutes after a person has used it. In military, paramilitary and other security services it is used for detection of explosives, mines, guns, contraband narcotics and cadaver materials. It is also used for clearance of train before deport at railway station having security threats. Recently, dog's brilliance has been used in sniffing the fake currency. The special sense of smell makes them ideal to locate the leaking gas pipelines, building moulds, winery moulds etc. Other uses of dog are in detection of the endangered species, locate termites and bedbugs.

In Agriculture, the dogs are used for identifying the weeds hazardous for agriculture and in choosing and picking up the fruits and vegetables that may ship dangerous insects and diseases. In animal husbandry, they are used for herd protection and heat/oestrus detection in animals through smelling the urine, vaginal fluid, milk, blood, saliva. Farmers train them to do this, so they knew the best time to introduce a bull to breed [7]. The dogs are also used in search and rescue operations conducted to identify the living or dead in disasters like earthquake, tornado, hurricane & war victims. They are used to trace the hazardous chemicals like mercury, lead etc which are harmful to the health of school going children. The dog's company is one of the best therapies. It boosts the morale and reminds the owner that he is a special and unique individual. It helps in guiding blind, aiding handicapped and as a therapeutic agent in reducing the chance of coronary heart blockade many times.

DOG NOSE IN DISEASE DIAGNOSIS

The dog's nose can smell tiny odour concentration/trace of the volatile odorous biomarkers emitted in exhaled breath, sweat, skin, faeces, urine etc but the diagnostic potential of dog is still underestimated, understudied, under recorded and poorly documented [8]. The dog has an extraordinary ability of recognizing the alterations in the magnetism, electromagnetic wave frequencies and odorous biochemical signature/indicator expressed only in ailing individuals and not in healthy individuals in much earlier and better ways and with an accuracy comparable or superior to readily available sophisticated diagnostic instruments/equipment's of present time. The dog also tries to express and communicate it to his master/owner. If the help of dog in early diagnosis of ailments which left no time for therapy is considered, it may be a breakthrough in the field of medicine [9]. The branch using animals in biological diagnosis is yet to be recognise/establish, however, there are few case control and experimentally designed reports that document the diagnostic ability and potential of dogs and are presented here.

The dog has tremendous smelling power. If trained properly, it can sniff out the minute concentration of disease specific odorous volatile organic biomarkers produced in pathological processing of cancer, infectious diseases, metabolic & genetic disorders and emitted in breath, blood, milk, skin and urine samples and can avoid the unnecessary painful procedures on patients and minimize the time and expenditure on diagnosis made through the biopsy and other tests having compromised sensitivity, specificity and predictive values resulting into inadequate/notorious accuracy. It may be effective weapon in fighting the life-threatening diseases and saving human/animal lives [10]. Whether, actual dogs will play active role in future diagnosis is uncertain, but they possess a pretty powerful tool that can be used to understand the olfaction-based diagnosis and development and refinement of futuristic artificial sniffing sensors as electronic nose.

Epilepsy

The dogs are able to predict the attacks of epilepsy in their owner [11]. The way in which dogs detect the imminence of fits in man is not known but it is useful to speculate on possible explanations. It is a well-known fact that an animal is capable of detection of electrical disturbances which can be correlated with epileptic episodes in human subjects. There may also be distinctive odours generated in the aura phase of epilepsy, which may be detected by sniffers. The extraordinary sense of smell of dog may be explored in prediction of other diseases as an early warning system. Study suggests that some dogs have innate ability to respond to seizures. The success of seizure prediction by dogs mainly depends on handler's awareness and response to the dog's alerting behaviour [12].

Carcinomas

Carcinoma or cancer is a disorder which originates from epithelial cells when the DNA of a cell is damaged or altered and the cell starts growing in uncontrolled manner [13-14]. Based on cells of origin, it may be squamous cell carcinoma, adenocarcinoma, anaplastic carcinoma, adenosquamous carcinoma, large and small cell carcinoma etc. The early cancer detection may help in timely institution of remedial measures. There are several diagnostic tools available in markets which are used for cancer diagnosis but with varying sensitivity and specificity and many more are in pipeline for improvement in diagnostic criteria [15-16]. The present paragraph highlights the studies conducted in detection of cancer using dog nose. Researchers have performed several studies for detection of cancers using canine (Table 1).

Breast Cancer

Breast cancer is most common cancer of women. The clinical signs include change in shape of breast, lump in the breast, fluid coming from the nipple, dimpling or red scaly patch of skin. It may undergo metastasis to distant organs and show various signs such as swollen lymph nodes, bone pain, shortness of breath, yellow skin etc [17]. In a study on breast cancer, Phillips *et al* (2006) evaluated VOCs in 51 asymptomatic women with biopsy proven breast cancer and 42 healthy women of similar age group [18]. Following random assignments to a prediction set and training set fuzzy logic model was constructed. In training set five breath volatile organic compounds were detected which can predict breast cancer in prediction set with 93.8 percent sensitivity and 84.6 percent specificity. However, the same model predicted no breast cancer in 32 per cent (16/50) women having abnormal mammograms. These women were not detected cancer on cancer biopsy [18]. The above reports confirmed the presence of VOCs in breath and blood samples of cancer patient. These VOCs can be assigned as predictor of breast and lung cancer in a two-minute breath test. The test is accurate, safe and painless for breast cancer. However, further studies and validations are required for such tests. In further study different methods viz. gaseous phase, chemiluminescence analyser for nitric oxide, gas chromatography/ mass spectrometry analysis, electronic nose and exhaled breath condensate was evaluated in exhaled breath for detection of biomarkers of various malignant and non-malignant ailments [19].

The role of dog in scent based breast cancer diagnosis was documented well in an observational report of Ms Claire specifying that how her pet Labrador dog named Daisy pawed and bruised her chest where after few days a tiniest lump of harmless cyst followed by deep seated breast cancer was diagnosed and treated through lumpectomy and some lymph nodes removed with six months radiotherapy which otherwise going to spread in her whole body before recognition, itself explains that dogs have specific sense of smell and its proper usage may help in early diagnosis required for saving human/animal life [20].

The double-blind study was conducted with five trained dogs to sniff the odorant signature of breast cancer in exhaled breath samples from 31 breast cancer patients and 83 healthy controls. The dogs correctly detect the samples with overall sensitivity of 88 per cent (95% CI, 0.75, 1.00) and specificity of 98 per cent (95% CI, 0.90, 0.99). Both sensitivity and specificity were remarkably similar across all the four stages of breast cancer [21].

Urinary Bladder Cancer

Bladder cancer is one of the common cancers in human being. Urothelial cell carcinoma or transitional cell carcinoma is most common type of bladder cancer. Rarely, bladder can also be involved by non-epithelial cancers, such as lymphoma or sarcoma, where abnormal cells multiply without control in bladder (http://www.mountsinai.org/health-library/diseases-conditions/bladder-cancer). Bladder cancer is characterised by hematuria, frequent urination and pain during urination or feeling the need to urinate without being able to do so. Weber *et al,* (2011) identified the volatile organic compound marker of transitional cell carcinoma of bladder with the help of a gas sensor array composed of 10 metal-oxide-semiconductor field-effect transistor, 12 metal-oxide sensors, an infrared-based CO_2 sensor and a capacitance-based humidity sensor and using partial least squares discriminant analysis algorithm with a detection sensitivity of 70 per cent [22]. The urinary bladder cancer was the first disease for which the diagnostic potential of dog sniffing was systematically analysed. Willis *et al* (2004) evaluated the ability of dog in identifying the transitional cell carcinoma of bladder, based on the detection of cancer related odour in the dried or liquid urine samples of 36 patients and 108 diseased/healthy and male/female controls [23]. The six dogs, trained for scent discrimination as two cohorts of two dogs on dried urine samples and four dogs on wet urine samples, correctly detected the cancer sample amongst the six healthy controls matched one each for age of ±8 years & ±12 years, sex, urological problems and blood in nine test panels, on 22 of 54 occasions with a mean success rate of forty one per cent that was more than expected by chance [23]. The dogs trained on wet urine samples performed better and had the success rate of 50 percent.

Prostate Cancer

It is a cancer of prostate gland of male reproductive system. It grows slowly and may spread to other parts of the body including the bones and lymph nodes [24]. Initially it produces no symptoms but in later stages it may cause pain in the pelvis, blood in the urine, difficulty in urination etc. Researcher has conducted a study conducted with trained Belgian Malinois shepherd dog and reported that by smelling the urine samples, dog could detect the prostate cancer in man with the sensitivity and specificity approaching to the degree of 91 per cent [25]. The study also suggested the release of significant amount of VOCs in urine of patient. Recent study showed that highly trained dogs can discriminate urine samples of patients having prostate cancer from healthy ones, achieving a diagnostic accuracy in terms of both sensitivity and specificity of over 97 percent, which can subsequently detect the biochemical recurrence following radical prostatectomy [26-27].

Lung Cancer

Lung cancer is a malignant tumor characterized by uncontrolled cell growth in lung tissues. It can spread to other nearby parts of the body by metastasis [28]. The common symptoms of lung cancer are coughing with blood, shortness of breath, weight loss and chest pains. Solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) based study identified the hexanal and heptanal volatiles in breath and blood samples of patients suffering with lung cancer which was not detected in healthy persons [29]. It means the volatile odour signature of lung cancer remain present in body fluid, secretions and excretions of incubating patients and may be detected much earlier before the clinical manifestation. A double-blind study was conducted using five dogs trained to recognize the lung cancer in exhaled breath samples collected from 55 patients of lung cancer and 83 healthy controls [21]. The dogs correctly detected the samples with an overall sensitivity of 0.99 (95% confidence interval, 0.99, 1.00) compared to conventional biopsy confirmed assays with overall specificity of 0.99 (95% CI, 0.96, 1.00) [21]. Recently, Ehmann *et al.,* (2012) conducted similar study on trained dogs to sniff out lung cancer in breath samples of patients suffering with lung cancer, chronic obstructive pulmonary disease (COPD) and healthy controls and whether the presence of tobacco in the samples made a difference [30]. The study showed that dogs correctly identified 71 out of 100 lung cancer samples and 372 out of 400 samples that did not have lung cancer. Thus, dogs were able to detect lung cancer with an overall 71 percent sensitivity and 93 percent specificity. The sample detection was independent of COPD and tobacco smoke [30].

Colorectal Cancer

The colorectal cancer (CRC) is one of the common cancers in human worldwide. The CRC develops from the colon or rectum of large intestine. The signs and symptoms include weight loss, blood in stool, change in bowel movements and feeling tired all the time (https://www.cancer.gov/types/colorectal). The dogs have been trained to smell the diagnosis of colorectal cancer. Sonoda *et al* (2011) conducted a study with a

Labrador retriever trained for sniff out the colorectal cancer in breath sample and reported an accuracy of 95 per cent compared to colonoscopy. The accuracy was 98 per cent with stool samples [31].

Dog was especially found effective for early stage cancer detection as well as differentiation of polyps from malignancies, which a colonoscopy cannot do. The other interesting report published in the European Respiratory Journal where dog was used in identifying the colorectal cancer and lung cancer from breath samples further drew the attention of scientific workers on the idea of using dog in cancer screening process [19].

Skin Cancer

The skin cancer is a locally destructive cancerous growth of skin cells. It can be of three types, most common basal cell carcinoma, second most common squamous cell carcinoma, and less common melanoma, which originates from melanocytes/pigment-producing skin cells. Melanoma can be metastasized to other body organs [32]. The observations on the dog smelling the scent and forcing the owner for confirmatory diagnosis pressed on the start of systematic research specifying the role of dog in diagnosis of skin diseases. A report where Dalmatian dog helped in diagnosis of skin cancer in a lady of 19 years old, where, dog agitation for mole on her right leg allowed her to get it diagnose in hospital reaffirmed the above idea. D'Amico *et al* (2008) observed the good sensitivity of electronic nose sensor arrays used in detecting the modified airborne chemicals emitted from altered metabolism of melanomas cancer cells from those of benign nevi affection of melanocytes [33]. The sensor arrays were used on 40 cases, 10 of which were diagnosed for melanomas referred to surgical intervention and out of them 9 were confirmed true and one false positive through histological examination of skin tissues [33]. The results of sensor arrays were compared with gas chromatographic investigation and had good sensitivity in detecting the volatile organic biomarkers emitted by malignant skin lesions.

Ovarian Cancer

It is a cancer of ovary which has ability to invade or spread to other parts of the body. The symptoms include loss of appetite, pelvic pain, bloating, abdominal swelling etc. It may spread to the lining of the bowel, abdomen, bladder, lymph nodes, lungs and liver [24, 34]. The study describing the sniffing talent of Springer Spaniel dog in finding out the human ovarian cancer in blood sample of the patient emphasized upon the role of the dog's nose in the war against cancer [24, 34]. Further, it was a curiosity what the dog was exactly detecting in the sample, a change in a single odour or those from a mixture of chemicals and it was impressed upon for inventing an electronic diagnostic device that could mimic the dog's nose which will give physicians the power to find ovarian cancer long before its victims have any inkling that they are sick.

Mastitis

Mastitis is inflammation of mammary glands parenchyma which is characterized by chemical, physical and bacteriological changes in the milk. Many infectious agents have been implicated as cause of mastitis but *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Escherichia coli* are predominant infection. Fischer-Tenhagen, 2016 reported the role of dog in early and accurate diagnosis for timely initiation of antibiotic therapy in mastitis of ruminants which was discussed and appreciated in World Buiatrics Congress held in Dublin, Ireland [35]. In the study, primarily the isolates of *Staphylococcus aureus* and later of *Escherichia coli*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Enterococcus* spp., *Pseudomonas aeruginosa* and *Candida albicans* were used [35]. The isolates were cultured separately on culture plate as well as in two milliliter bulk tank milk. The cotton swab was placed on lid of culture plate to absorb smell and 2 ml of milk having bacterial concentration @ 10^{12} cfu/ml were offered to eight dogs to smell in eight training periods of one week each. When dogs smelled the swab from the culture plate in bucket first and then presented with 10 buckets with only one positive for *Staphylococcus aureus* and others nine negative detected the positive sample with very high accuracy. However, the performance of dogs offered smelling the 2 ml of milk in the buckets was not consistence. In other experiment of same study the 6 dogs trained for smelling the swabs one each of

Staphylococcus aureus, Streptococcus uberis, Enterococcus spp. and others once tested for smelling swabs in 10 buckets, one each with *Staphylococcus aureus, Escherichia coli*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Pseudomonas aeruginosa*, *Enterococcus* spp. and/or *Candida albicans* and a completely culture-negative pasteurized milk sample correctly detect the sample of the *Staphylococcus aureus* swabs with 91per cent sensitivity and 97 per cent specificity. The four dogs were 100 per cent correct [35].

Metabolic diseases

The metabolic instability having roots in genetic defects, enzyme deficiencies, transport defects, production and reproduction stress may cause abrupt, damaging change in the internal environment of body. The production and accumulation of particular metabolite (s) due to change in normal biochemical pathways of body fluids results in disease and death unless diagnosed early and treated accordingly. Some of these metabolites are associated with a characteristic odor and sometimes it is distinctive enough to be diagnosed by clinicians. For example, diabetes mellitus, this is caused by deficiency in the production, secretion and/or action of insulin. There are two major clinical classes of diabetes, type I diabetes or insulin dependent diabetes mellitus and type II diabetes or insulin resistant diabetes. The affected individuals are unable to take up glucose efficiently. It results in excessive but incomplete fatty acids oxidation, accumulation of acetyl-CoA which leads to overproduction of ketone bodies such as acetoacetate, β-hydroxybutyrate as compared to the utilization in body. The increased ketone bodies concentration can be detected in blood and urine. Acetone is volatile and is exhaled imparting characteristic fruity odor and can be detected in exhaled breath. The ketotic state of dairy cows can be detected by analysis of exhaled air/ breath, which is a potential non-invasive method for determination of metabolic state of dairy cows [36]. Phenylketonuria is another example in which accumulation of phenylalanine or its metabolites in early life impairs normal development of brain and causes mental retardation. It is a genetic defect in phenylalanine hydroxylase, the first enzyme in the catabolic pathway of phenylalanine which converts phenylalanine into tyrosine. The deficiency of enzyme results in transformation of phenylalanine into phenylpyruvate and accumulation of phenylalanine and phenylpyruvate in blood and excretion in urine which imparts a characteristic musty odour to the urine of infants that the ward boys traditionally used to detect phenylketonuria in infants. One more disease is maple syrup urine disease which arises due to defective branched chain α-keto acid dehydrogenase complex and cause abnormal development of brain, mental retardation and death in early infancy. It is a rare genetic disease in which three branched chain amino acids, valine, isoleucine and leucine accumulate in the blood and spill over into the urine imparting characteristic smell like maple syrup. There are several other metabolic diseases that are accompanied by a distinct smell and may be targeted for diagnosis by utilizing the specific smelling ability of dog.

Infectious diseases

The microbial infection changes the environment at the predilection site of growth and alters the host metabolism. The volatile organic compounds produced as a result of microbial metabolism itself as well as altered host metabolism in combine are released in breath, sweat, urine, faeces, skin etc and imparts an odour different from healthy control and specific for the infectious disease which is having a diagnostic value. Clinicians have long been aware of disease specific odour, which can be used as diagnostic biomarkers of infectious diseases. Ancient Greeks and Chinese had developed an interesting diagnostic method of *Mycobacterium tuberculosis.* Clinician set fire the patient's sputum and tuberculosis was diagnosed by fume's specific smell. The infection of *Clostridium chauvoei* causes black leg in animals that can be recognised by thin sanguineous fluid containing bubbles of gas and characteristic rancid odour at the site of affection. The other clostridia causing malignant oedema or gas gangrene of soft tissue and produces smell of diagnostic value. Similarly, the infection of *Dichlobacter nodosus* results in foot rot in sheep which has specific foul odour and can be explored for diagnosis. The infection of *Pseudomonas aeruginosa* produces the characteristic fruity odour that is diagnosed by clinician. The bacterial vaginosis has its distinctive 'fishy' smell. *Vibrio cholerae* infection causes vomiting, profuse watery diarrhoea and rapid dehydration. The faeces of patients with cholera are referred to as 'rice-water stools' and have a characteristic sweetish odour. VOCs analysis of faecal samples of cholera patients revealed the dimethyl disulphide and p-menth-1-en-8-ol as candidate biomarkers for the disease [37]. Recently, a lot of studies have been done to exploit the superior smelling characteristic of animals. Such newly developed scent detection assays can help in earlier detection of infectious diseases. The *Clostridium difficile* infection causes hospital diarrhoea in human. Humans can recognise the specific smell of Clostridium *difficile* diarrhoea. Recently, a dog was found capable of detecting *Clostridium difficile* infection both in faecal samples and at the patients' bedside on hospital wards with a sensitivity and specificity of 100 per cent and 94-100 per cent with stool samples and 83-93 per cent and 97-98 per cent with patients on the hospital ward [38, 39]. When tested by E-nose, faeces of *Clostridium difficile* diarrhoea patients had a significantly different VOCs pattern from faeces of asymptomatic volunteers, patients with *Campylobacter jejuni* infection, and patients with ulcerative colitis. Furthermore, the e-nose discriminated between different aerobic bacteria such as *Helicobacter pylori*, *Escherichia coli,* and *Enterococcus* species on the basis of differences in the volatile compounds [40, 41].

ELECTRONIC NOSE OR E-NOSE IN DISEASE DIAGNOSIS

In living individuals, the scent receptors of nose constitute an array that responds to a wide range of volatile odorant chemicals and impulse generated in this interaction is transmitted, decoded and discriminated in different odour types in the olfactory bulbs of brain. Based on this model, an array of large number of electronic sensors linked to a computer-based pattern recognition system that may respond and recognize a range of specific odorous compounds and aid in disease diagnosis is to be developed and refined. The volatile organic indicators of early pathogenesis may be targeted for diagnosis of disease under incubation period. Initially in VOCs based diagnostic development; the volatile and thermally stable analytes were separated and identified using gas chromatography technique with results almost comparable to potential of human nose. Later on, the gas chromatography technique was coupled with mass spectrometry which enables the researcher to improve the sensitivity, specificity and reliability up to the level of potential of trained person. In this series, sensor-based e-nose is a latest concept and may enable us to analyze and characterize sample-derived complex VOCs with or without separation of the mixture into individual components and the data generated through dog's nose experimentation may refine it to the ultimate level.

E-noses uses three type of instrument for sample analysis viz., sensor, preprocessor, and microchip containing result analysis software. When VOCs passes through VOC Sensors, it specifically interacts with specific parts of the sensor in a unique pattern specific to that molecule. Because of specific chemical signature of a particular VOC, it can be easily identified [40]. The preprocessor collects the binding pattern information of all the VOCs to determine the unique chemical signature of the VOCs [42]. The data analysis software compared the chemical signature of VOCs to databases stored in a microchip to identify the compound [42]. The e nose technology has promised a new horizon of no invasive method of disease identification using specific chemical disease biomarkers. The improvements in design, sensor, and algorithms for discriminant analysis have increased the efficacy of e-nose in clinical trials [43-46].

The use of present e-noses is limited in discriminating the odors of different varieties of wine, beer, tea, coffee, tobacco and determining the freshness and quality of fish, fruits and foods. However, futuristic electronic noses will have ample scope in forthcoming routine life including the replacement of the human panels in deciding the odor quality of food in stared hotels to the disease diagnosis for which the physician are trained. Presently the use of e-nose sensors in disease diagnosis is in preliminary stage and is limited in identification of bacterial pathogens [47, 48], lung cancer patients [49,50], COPD [51] and asthma [51,52].

The e-nose technology is expected to expand at a very rapid speed and will offer the futuristic diagnosis with ease of use, low operating costs, excellent precision, quick sensor recovery time, rapid results and response time, smaller with greater portability and large flexibility in sensor array specificity for selective and specialized applications [53-55]. However, the removal of existing disadvantages such as sensitivity of sensor arrays to water vapour, inability to identify individual compounds in a mixture of compounds, relatively shorter life of sensor, difficulty in accurate measurement of analyte concentrations and relatively lower sensitivity than analytical chemistry instruments need to be improved [41, 53]. The few reports of using e-nose in disease diagnosis are discussed here.

The Nakhleh *et al.,* (2016) used artificial intelligent nanogold based array for diagnosis and classification of several diseases such as ulcerative colitis, irritable bowel syndrome, ovarian cancer, bladder cancer, prostate cancer, atypical Parkinsonism, colorectal cancer, gastric cancer, head and neck cancer, pulmonary arterial hypertension, kidney cancer, lung cancer, Crohn's disease, idiopathic Parkinson's, pre-eclampsia, multiple sclerosis and chronic kidney disease [56]. The diagnostic assay was based on detection of a number of combinations made up of thirteen volatile organic compounds such as acetone, ethyl acetate, ethanol, 2-ethylhexanol, 3-methylhexane, isononane, isoprene, nonanal, 5-ethyl-3-methyloctane, ethylbenzene, styrene, toluene and undecane as biomarkers in the exhaled breath of 591 healthy controls and 813 patients diagnosed for one of the above diseases in five different countries viz. USA, Israel, Latvia, France and China. The two breath samples were taken from each individual. One of the samples was analyzed using nanogold array for disease diagnosis and classification while other was analyzed using GC-MS for exploring its chemical composition. The blind experiments showed an accuracy of 86 per cent in artificial intelligent nanogold array and allowed both detection as well as discrimination among different disease conditions under investigation. Artificially intelligent nanoarray also showed that each disease has its own unique breath print. However, the sample positive for one disease would not screen out other disease. The classification and diagnosis power of nanoarray technique was also successfully validated by other analytical technique such as mass spectrometry linked gas chromatography. The Machado *et al* (2005) compared the VOCs of exhaled air from 14 patients suffering with lung cancer to 54 control patients [49]. The e-nose technique was found 71per cent sensitive and 91 per cent specific for lung cancer detection in patent. Humphreys *et al.* (2011) demonstrated the use of e-nose in diagnosis of invasive mechanical ventilation associated pneumonia and e-nose correctly detected pneumonia in 83 per cent of patients [57]. The Dragonieri *et al.* (2007) showed the significant differences in VOCs obtained from patients suffering from lung cancer, COPD and healthy controls [52]. Similarly, Fens *et al.* (2009) reported significant differences in VOCs of the exhaled gas from asthma and COPD patients along with non-smoker and smoker controls [51].

The Valera *et al.* (2012) studied the e-nose in identifying the respiratory bacterial microbes either *in vitro* or *in vivo* in exhaled breath of patients suffering with asthma, COPD and tuberculosis and found it a good option for detection of respiratory diseases [58]. Bruins *et al* 2012 used a commercially available e-nose device (diagnose, C-it BV) to detect tuberculosis in exhaled air of healthy controls and TB patients which differentiated the TB patients from healthy controls with a high sensitivity (76.5%) and specificity (87.2%) [59]. Earlier, Fend *et al.* (2005) has described the possible use of e-nose in diagnosis of *Mycobacterium bovis* infection [60]. The e-nose was able to discriminate infected animals (cattle or badgers) from controls as early as 3 weeks post infection.

Recently, the exhaled breath of 18 psoriatic arthritis/ PsA and 21 rheumatoid arthritis/ RA patients with active disease was compared to 21 healthy persons using an e-nose technique. The VOCs were identified by gas chromatography and mass spectrometry. The study showed that breath prints of RA and PsA patients could be distinguished from controls patients with an accuracy of 71 per cent and 69 per cent, respectively. The GC-MS identified seven distinct key VOCs which significantly differed between the study groups [61].

Capelli *et al* (2016) exhaustively reviewed the scientific work conducted for using the e-nose in detection of bacterial, urinary tract and kidney diseases where different sensorial technique was used for analysing the gaseous composition of human urine with accuracy having promising sensitivity and specificity [62]. The researchers may find number of reviews on use of e-nose in disease diagnosis.

Table 1: Detection of human cancer using canine

Cancer	Sample Type	Cancer Samples (no.)	Sensitivity	Specificity	References
Breast	Breath	116	88%	99%	[21]
Ovarian	Tissue	40	100%	95%	[63]
Ovarian	Blood	42	97%	99%	[64]
Lung	Breath	25	71%	93%	[30]
Lung	Urine	59	73.6%	29.2%	[65]
Prostate	Urine	16	-	-	[66]
Prostate	Urine	362	99.3%	98.2%	[26]
Prostate	Urine	33	91%	91%	[25]
Prostate	Stool	37	100%	100%	[31]
Prostate	Urine	33	-	-	[67]

CONCLUSIONS AND FUTURE PERSPECTIVE

Analysis of VOCs exhaled in the breath, sweat, urine, faeces etc can provide an insight into current metabolic health status of an individual and existence of any disease state. The breath sample analysis in e-nose is in current use and offers non-invasive methods of rapid disease diagnosis with high precision, sensitivity, accuracy and reproducibility. Moreover, the sample collection is painless and disease cans be detected in early stages. This allows the early treatment and rapid recovery of patients. However, to facilitate the e-nose as main stream diagnostic technology, a world-wide volatile organic compound-based biomarker database for disease diagnosis, standard control comparability up to dog nose level and improvement in sample collection and storage technology is to be built. In future, the portable e-nose instruments can also be coupled with satellite based wireless internet communication devices in hospitals for remote care utilities. The future e-nose devices will be in miniaturized form with fewer but smart hardware and improved algorithms to recognize unique breath prints of disease-specific biomarkers. This will enhance the reproducibility and accuracy of results in early stage without using any painful invasive biopsies techniques to patients.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflicts of Interest

All the authors declare that there is no any conflict of interest.

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