Journal of Balkan Science and Technology
journal homepage: www.jbst.nku.edu.tr/

Investigation of Morphometric Changes in Erythrocytes in Neurological Diseases: Can New Optical Techniques Lead to the Development of a Biomarker?

Filiz DİLEK[1*], Merve UYANIK[2]

[1] *Elderly Care Program, Vocational School of Health Services, Tekirdağ Namık Kemal University, 59030, Tekirdağ, TÜRKİYE*
[2]*Department of Physics, Institute of Natural and Applied Sciences, Tekirdağ Namık Kemal University, 59030, Tekirdağ, TÜRKİYE*

Review Article

Keywords:
Erythrocyte,
Imaging,
Neurodegenerative diseases,
Neurology,
Biomarkers

Received: 02.01.2025
Accepted: 22.01.2025
Published:14.03.2025

DOI: 10.55848/jbst.2025.02

ABSTRACT

Early diagnosis in neurodegenerative diseases is important in terms of starting early treatment and increasing the quality of life of individuals. Therefore, effective diagnostic methods with low cost and fast results have an important place. In diagnosis, peripheral biomarkers are very few. Erythrocyte morphology as a potential biomarker is promising for future studies. Altered morphology may not only be used as a diagnostic biomarker but may also be important in the early pathogenesis of the disease. Here we reviewed importance of erythrocyte morphologies on the neurodegenerative pathologies- Alzheimer's disease (AD), Parkinson's disease (PD), Multiple Sclerosis (MS) Disease, and Epilepsy. Important studies on optical microscopy, scanning electron microscopy, atomic force microscopy, and quantitative phase imaging methods, which are widely used in the imaging of erythrocytes and detection of morphological changes in these neurodegenerative diseases, were reviewed from the literature.

1. Introduction

Erythrocytes are unique cells with an extraordinary ability to deform, which is crucial for their oxygen transport function and can be significantly altered under pathophysiological conditions. Their main function is the transport of respiratory gases and are in the range of 4-6 million in the blood. As non-nuclear cells, they are very sensitive to pathological conditions in which the biconcave discoid shape typical of healthy human cells changes to atypical morphologies such as spicular cells (echinocytes and acanthocytes), spherocytes, etc. Mature erythrocytes under physiological conditions are biconcave disc-shaped with a diameter of 6-8 µm, an average volume of 90 femtolitres (fL), and a membrane surface of 120-140 µm2. The maximum thickness of this disc at the edges is 2.5 µm, and the minimum thickness at the centre is 0.8 µm [1-3]. They do not have cytoplasmic organelles such as nuclei, mitochondria, or ribosomes. They cannot synthesize proteins, carry out oxidative reactions associated with mitochondria, or undergo mitosis. Four different cell shapes (biconcave, crenate, spiculated and spherocytic) are distinguished in the literature for healthy erythrocytes, and the morphology of erythrocytes changes during the ageing process [4]. During the aging process, healthy erythrocytes show an increase in the proportion of spicules and spherocytes morphological types and a decrease in the proportion of dissociates and crenate shaped cells [4,5].

The morphological changes of erythrocytes in chronic neuro degenerative diseases have been investigated in many studies [5]. Erythrocytes may contribute to chronic neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Multiple Sclerosis (MS) through impaired antioxidant capacity and altered hemorheology [5,6]. Erythrocytes in the blood circulation are exposed to the action of toxic pathological proteins that diffuse and accumulate in AD, PD, and Amyotrophic Lateral Sclerosis.

There have been many studies looking at the morphology of erythrocytes from samples taken from patients or at the effects of drugs in vitro [7-13]. Ficarra et al. and Suwalsky et al. investigated the effects of antiepileptic drugs on erythrocyte morphology in vitro [7,9]. A recent study investigated the morphological changes of erythrocytes of epileptic patients under effective therapeutic doses of antiepileptic drugs using quantitative phase imaging, SEM, and light microscopy [14].

Optical imaging has many advantages compared with clinical imaging modalities (e.g., high sensitivity, non-invasiveness, high imaging speed, low cost, three-dimensional imaging) and enables the detection of biological processes at the cellular or molecular level [15]. Erythrocyte surface observations have generally been made using optical microscopy, atomic force microscopy (AFM), and scanning electron microscopy (SEM) [16]. In recent years, quantitative phase imaging (QPI), a widely used optical measurement technique, has been used to calculate the projected surface area and structural parameters of erythrocytes, such as mean corpuscular volume and mean corpuscular haemoglobin, from

[*] *Tekirdağ Namık Kemal University, Faculty of Arts and Sciences, Department of Biology, Kampüs Cd. No:1, 59030, Tekirdağ, TÜRKİYE*
E-mail address: fdilek@nku.edu.tr

the three-dimensional (3D) height profile [17]. Studies of the clustering of erythrocytes according to their shapes in samples from healthy individuals have focused more on providing quantitative information at the single cell level [13, 17-29].

This study focuses on the importance of erythrocyte morphology in neurodegenerative diseases and the light microscopy, SEM, AFM, and QPI imaging methods that are often used to image erythrocyte morphology. A review of the literature on imaging techniques used to assess erythrocyte morphology, and the importance of applying these techniques in neurodegenerative diseases is presented.

2. Material And Method

2.1. Imaging methods used to evaluate erythrocyte morphology.

2.1.1. Optical Microscopy DNA extraction and Sequencing

Microscopy is the study of magnifying images of objects that are too small to be observed with the naked eye. It uses radiation that is emitted, absorbed, transmitted, or reflected by the specimen being imaged. The type of radiation used by optical microscopy, also called light microscopy, is the visible part of the electromagnetic spectrum. [30, 31]. In a standard upright microscope, the objective is above the specimen, in an inverted microscope the objective is below the specimen. In both cases, almost all microscopes are designed for direct imaging, and the digital camera is added only for image capture [32].

The resolution of conventional optical microscopes is expected to be limited to about 0.6λ/NA. Where λ is the wavelength of light, NA is called the "numerical aperture" and is usually marked on each objective lens. The resolution of optical microscopes is very limited compared to that of electron microscopes or atomic force microscopes. Images can be obtained quickly over a wide magnification range. Conventional optical microscopy's low capital and maintenance costs remain an important advantage [32,33].

Because of its wide range of contrast-enhancing techniques and its ease of use, light microscopy continues to hold its own. It is a traditional method used for cell imaging in neurodegenerative diseases. In a study, authors observed an Amyloid b-induced erythrocytic damage by optical microscope images [6]. Research published in 2022 used light microscopy to image the morphology of fresh and aged erythrocytes in neurodegenerative disease and healthy samples, characterizing them as biconcave, crenate, spiculated, and spherocytic [5]. A study focusing on quantitative phase imaging of erythrocytes was conducted by Deniz et al. in 2023 to evaluate the effects of high dose methylprednisolone on erythrocytes in vivo under physiological conditions in human blood samples. In that study, erythrocytes classified as biconcave or irregularly shaped from light microscopy images were examined [34]. In a study carried out by Ünal et al. with blood samples taken from 50 epilepsy patients, the samples were observed by light microscopy. While the cells observed in healthy individuals were mostly biconcave in shape, erythrocytes collected from patients showed an altered morphology. They stated that the number of biconcave shaped cells was lower in the samples collected from patients under antiepileptic treatment than that of the control group [14]. As can be seen from the literature, light microscopy is a widely used imaging technique for characterizing erythrocyte morphology in both neurodegenerative diseases and healthy samples. Although it has advantages such as the ability to observe many cells simultaneously, generate images at different magnifications, and being inexpensive, the images obtained by this method are two-dimensional, do not contain height information, and quantitative information cannot be obtained. In addition, the fact that it depends on the visual interpretation of the researcher performing the analyses is a disadvantage. Example erythrocyte images, taken by optical microscope, from healthy person and a patient with Alzheimer's disease samples are shown in Fig. 1.

2.1.2.AFM

In 1986, Binning, Gerber, and Quate developed the first AFM [35]. The AFM consists of a flexible lever and a pointed tip too small to be visible by eye. When the tip gets close enough to the sample surface, the forces between it and the surface cause the lever to bend. The bending is measured by a laser beam projected from the tip of the lever onto the detector. As the bending is proportional to the force, the force is also measured when the bending is measured. As the lever scans the surface, it detects the peaks and ridges on the surface. If the peak height is constant, the surface can be damaged. Therefore, while scanning is performed by moving in the X and Y directions, the height is adjusted by moving in the Z direction. When the bends are recorded in the computer, the topography of the surface is also obtained [36,37]. AFM can image biological membranes in their native state with a lateral resolution of 0.5-1 nm and a vertical resolution of 0.1-0.2 nm [35,38].

After the 2000s, there have been many studies imaging erythrocytes with AFM [5,10,39,40]. AFM imaging of erythrocytes in a physiological medium was reported in 2001 [41]. Strijkova-Kenderova et al. performed ultrastructural analysis of erythrocytes for neurodegenerative pathologies, PD, AD, and Amyotrophic Lateral Sclerosis, using AFM [5]. There is a study in which the morphological changes in the erythrocyte membrane induced by antiepileptic treatment were imaged by AFM [12]. In 2015, Mukherjee published a critical review of nanoscale surface characterization of human erythrocytes using AFM [16]. Buys et al. used AFM imaging to investigate erythrocyte membrane roughness and ultrastructural changes in type 2 diabetes [10]. A study published in 2021 examined the quantification of physical differences in protein aggregates implicated in AD, using AFM to profile erythrocytes from 50 neurocognitive patients and 16 healthy individuals [42].

As suggested in the literature, AFM allows imaging of erythrocytes in air or in aqueous (buffer) solutions, simulating physiological conditions, with sample preparation steps. The major advantage of AFM is that it allows the topography of the sample to be monitored with high resolution and in three dimensions. However, due to the sample preparation and the need to change the tip of the AFM instrument for each sample, there are disadvantages in terms of time and cost when measuring topography with this instrument. Erythrocyte images obtained with AFM from healthy person and a patient with Alzheimer's disease are given as an example in Fig. 2.

2.1.3. SEM

An electron microscope essentially acts as an electron source, emitting electrons that interact with the specimen being examined, which are then processed by a detector to produce an image. The electron source is an electron gun containing a tungsten or tungsten filament wire. A high voltage is applied to the filament, which heats the wire and releases electrons. For this oscillation to occur, both the current and the voltage applied to the wire must be increased. The oscillating electrons are guided by the anode plate, pass through lenses with electromagnetic properties, and fall onto the sample. This environment is under 10^{-4} Pa vacuum. The accelerated electron beam interacts with the sample, and the electrons which change direction because of elastic collisions with the sample atoms are scattered back. Inelastic collisions of the beam electrons with the outer orbital electrons of the sample atoms also result in the formation of lower energy Auger electrons, which provide information about the sample surface. As a result of all these collisions, electrons that have left their orbits and lost energy form secondary electrons. These secondary electrons from 10nm below the sample surface provide a high-resolution topographic image of the sample [43,44,45].

Numerous studies have been published investigating erythrocyte morphology using SEM [5,10,40,44]. A study published in 2012 focuses on thalassemia screening using geometric features that characterize the morphology of erythrocytes based on SEM images [46]. The classification of erythrocytes in anaemic cases using SEM was investigated by [47]. In a recent study, SEM images were used to observe erythrocyte populations with different morphological stages of stomatocytosis and echinocytosis [48]. The morphology, structure, and function of human and animal erythrocytes have been studied using SEM [49]. Also, there is an important study in which erythrocyte membrane roughness and ultrastructural changes in type 2 diabetes were examined by SEM [10]. Ünal et al. reported on the morphological changes of erythrocytes in epileptic patients under effective therapeutic doses of antiepileptic drugs. Among other techniques, they used SEM images of erythrocytes to show the morphological changes of the cells [14].

As stated in the literature, structural, morphological, and topographical features of erythrocyte surfaces can be realized with SEM, and it is possible to observe the samples at different magnifications. SEM provides a two-dimensional surface image with scale information [18]. In addition, blood cells must be subjected to a preliminary preparation process prior to imaging [10,14]. During imaging, distortion of the sample can occur due to the pressure applied within the instrument. In these respects, SEM has disadvantages compared to surface profilometers or quantitative phase imaging techniques. SEM images are not dynamic but provide a qualitative measurement result. SEM images of erythrocytes from healthy person and a patient with Alzheimer's disease are given as an example in Fig. 3.

2.1.4. Quantitative Phase Imaging

In basic sciences such as biology and physics, quantitative studies have come to the forefront in recent years. Making optical measurement techniques used in basic laboratories capable of performing healthy measurements without damaging or contacting the human sample and using them in medical laboratory studies will support the design of more accurate and inexpensive methods in the diagnosis and treatment phase. Increasing the sensitivity of profilometric measurement systems which are used in many fields of industry and research is necessary for more accurate and reliable measurement [50,51]. Quantitative phase imaging is the general name for a few different experimental setups that use techniques to record the phase of light, such as interferometry and holography. By recording the interferogram, which carries the phase information of the light from an object, and analysing it using phase calculation techniques, a profile of the object can be created with a known height at each point [52-54]. If the object is micrometre sized, it is possible to obtain quantitative information about the morphology of the object by using the microscope and interferometer together [22,55-57]. Relying on the current success of quantitative phase imaging, researchers have recently applied these techniques to a variety of clinically relevant problems in their research covering a wide range of diseases [52,58].

Eliminating the need to label the sample means that several steps in tissue preparation can be avoided, reducing the time and cost of analysis. Perhaps most interestingly, the quantitative information on the structure of the biopsied tissue allows the diagnosis to be made objectively and independently of the observer or preparation [59]. These techniques can provide quantitatively detailed information about cell structure at the level of a single cell [18]. In medical applications, parameters related to erythrocyte count and morphology are frequently used in the diagnosis and monitoring of many diseases [56,60,61]. As the health sciences and biomedical fields clearly require more quantitative data, quantitative phase imaging will play an incrementally significant role in the generation of quantitative clinical data [17,18,20,62-64].

There are several different approaches to quantitative phase imaging, such as common path, phase shift, off-axis, and white light interferometry, and each has its own advantages and disadvantages [61]. In principle, common-path geometries are extremely stable because both arms of the interferometer overlap and are highly correlated, but subject to noise. Some common-path methods are Fourier phase microscopy (FPM) [65] and diffraction phase microscopy (DPM) [66]. Phase shift methods perform temporal phase modulation and require a large number of raw images (more than 2) to reconstruct a single-phase image. Therefore, phase shifting methods are usually not used for high*speed applications [67]. Off-axis methods modulate spatially and require only a single image to create a phase image. An off-axis approach is used in most conventional digital holography methods [18,68-70]. Such methods are well suited to high-speed measurements and single shot imaging. For imaging erythrocyte morphology, white light diffraction phase microscopy (WDPM) offers the speed and phase sensitivity of off-axis holographic imaging [14,34,59,71].

To acquire the surface profile of erythrocytes in a dynamic three-dimensional structure and to determine the parameters related to erythrocyte morphology from this structure, it is first necessary to obtain an interferogram image of the sample and the reference. The WDPM setup used for this purpose consists of a Zeiss Axio Observer A1 inverted microscope, a Mach-

Zehnder-like interferometer and a Hamamatsu Orca Flash 4.0 camera [14,34,59,71]. With this setup, blood samples from patients and healthy individuals can be imaged without any preliminary preparation. These interferogram images are analysed using phase calculation techniques such as Fourier or wavelet transforms to generate surface height profiles of erythrocytes [14,34,59,71]. From this profile, many 2D and 3D parameters such as mean cell thickness, mean corpuscular volume, projected surface area, mean corpuscular haemoglobin, and surface area to volume ratio can be calculated for morphological assessment [34,59]. Examples erythrocyte profiles derived from samples taken from a healthy person and a patient with Alzheimer's disease is shown in Fig. 4. The morphology-related parameters calculated from these two profiles are given in Table 1.

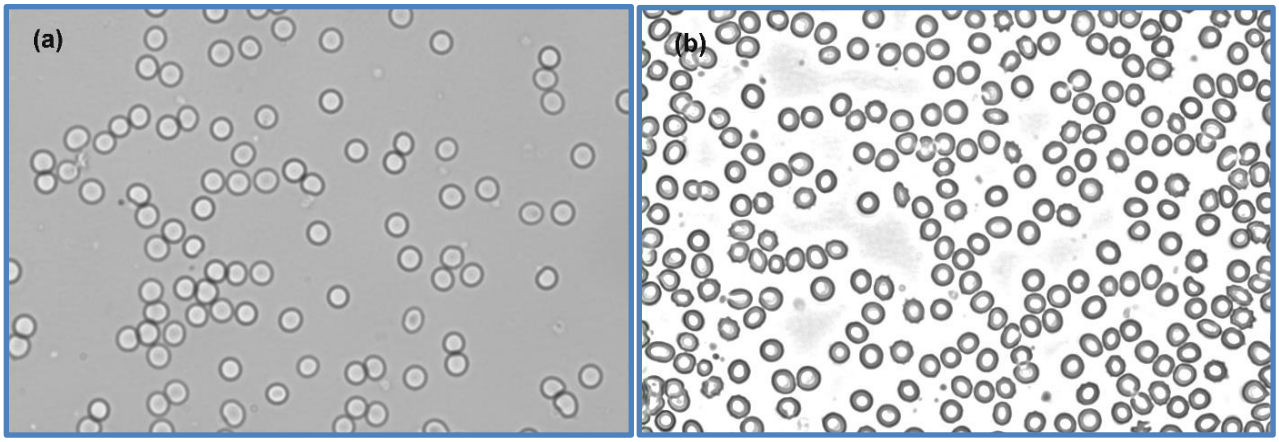

Fig. 1 Optical microscope images taken from samples **(a)** a healthy person and **(b)** a patient with Alzheimer's disease.

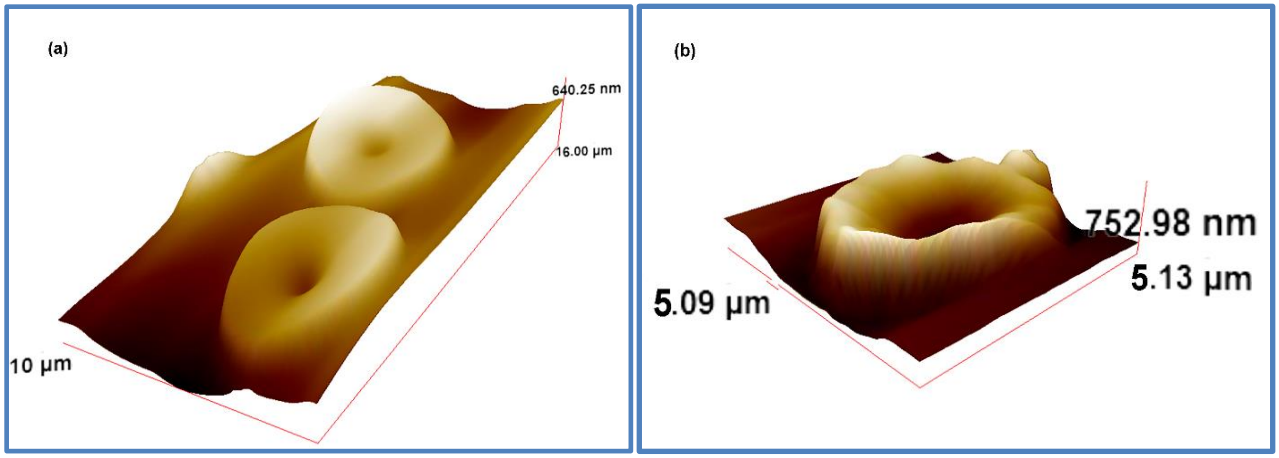

Fig. 2 Erythrocyte images obtained with AFM, for samples **(a)** a healthy person and **(b)** a patient with Alzheimer's disease.

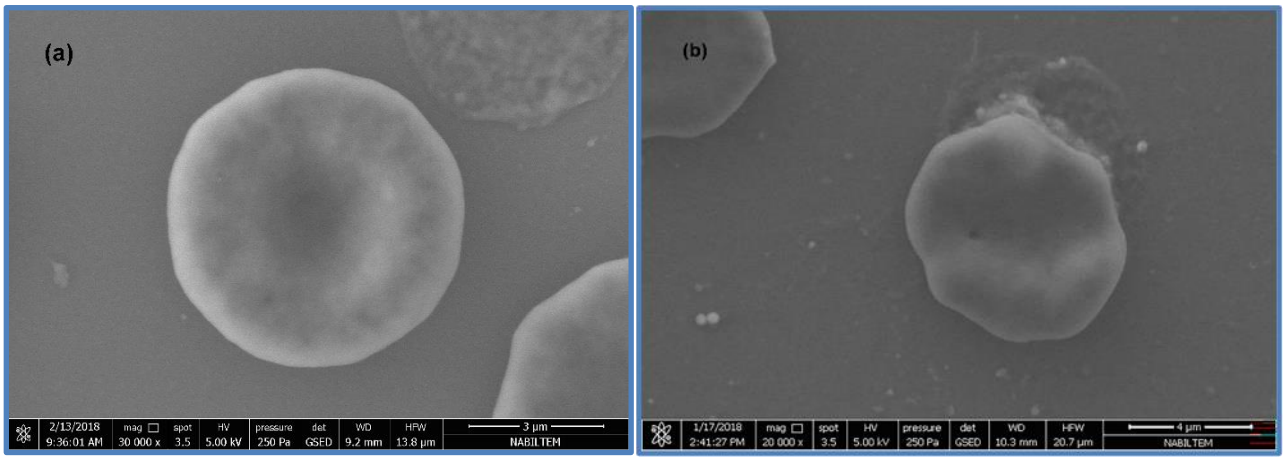

Fig. 3 SEM images of erythrocytes from samples (a) a healthy person and (b) a patient with Alzheimer's disease.

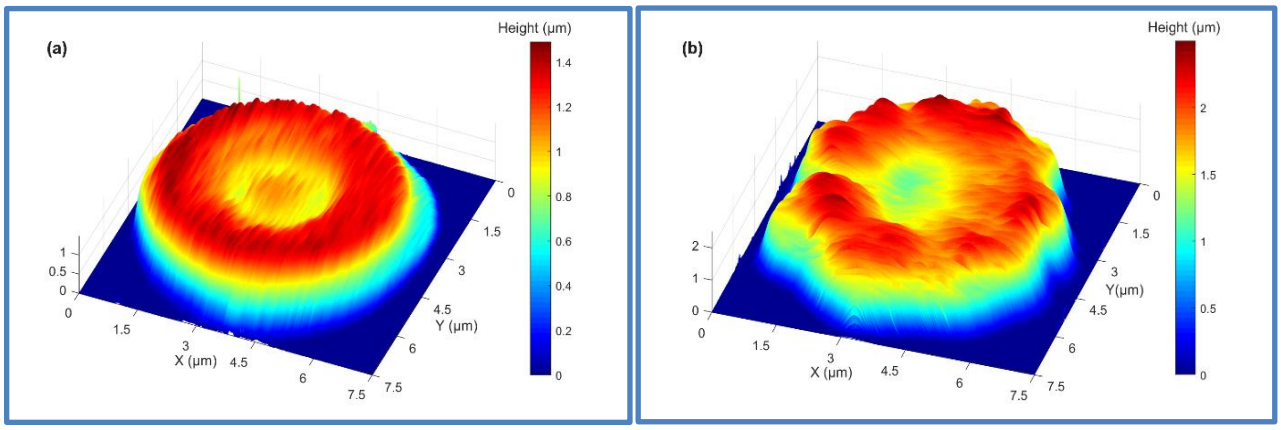

Fig. 4 Erythrocyte height profiles obtained from samples from (a) a healthy person and (b) a patient with Alzheimer's disease.

Table 1. Parameters related to erythrocyte morphology for a healthy person and a patient with Alzheimer's disease estimated by WDPM

	For a healthy person	For a patient with Alzheimer's disease
Mean corpuscular volume (µm^3)	78.5043	94.0952
Average cell thickness (µm)	1.4463	1.4096
Projected surface area (µm^2)	71.9283	68.4627
Mean corpuscular haemoglobin (picogram)	35.8518	31.8403
Diameter (µm)	6.9110	6.7424
Entire cell surface area (µm^2)	109.5537	116.7166
Surface area to volume ratio	1.3955	1.2404

2.2. Reported erythrocyte morphological changes in Neurodegenerative Diseases

2.2.1. Alzheimer's disease

AD, the most common cause of dementia and cognitive disorder among the aging population, is a progressive neurodegenerative disease that affects cognition, function, and behaviour [72,73]. AD is thought to account for 60-80% of all dementia cases. It is important to prevent, diagnose, treat, and manage AD because of its progressive nature and high resource consumption due to the need for care. AD is a disease that progresses in three stages: early, moderate, and advanced, and the cost of care increases as the stage of the disease increases [74]. Early diagnosis is important in terms of low cost and preserving the patient's level of independence; from this perspective, informative biomarkers are of great value for population-based screening. Developing new diagnostic approaches and discovering new reliable, more cost-effective, and easily accessible diagnostic biomarkers are also important for the development of new treatments [5].

A review of research into plasma/serum biomarkers for the diagnosis of dementia identifies several relevant and potentially promising erythrocyte biomarkers for dementia subtypes such as erythrocyte membrane proteins like glucose transporter (GLUT-1), amyloid-β, IgG, Hsp90, calpain-1 and band 3 protein, oxidative stress, and erythrocyte morphology. The most promising biomarkers are preclinical Hsp90, amyloid-β, calpain-1, and band 3. However, the most interesting aspect of erythrocytes is their changing morphology during dementia [75]. Erythrocytes are easy to obtain and do not have any internal organelles in the red blood cells, which makes them easy to analyse.

In AD, erythrocytes are exposed to toxic pathological proteins (such as Aβ peptide, α-sin, τ-protein, and their heterocomplexes) that replicate and accumulate [5]. Since there is a characteristic decrease in cerebral perfusion and energy metabolism in AD, it is thought that the capillary disruptions often found in the brain of AD patients lead to hemodynamic changes that alter the delivery and transport of essential nutrients to neuronal and glial cells, especially glucose and oxygen [76,77]. Mohanty et al. reported a possible link between changes in the erythrocyte membrane proteome and AD pathology. They reported that 15% of erythrocytes in Alzheimer's patients were elongated and there were changes in erythrocyte membrane architecture. It has been suggested that this may be due to erythrocyte-beta-amyloid interactions and/or changes in the expression of membrane proteins [78]. Based on the information that amyloid β (Aβ) binds to erythrocytes and that this process damages them, Lan et al. conducted a study in 50 healthy individuals and 50 individuals diagnosed with AD; Aβ amyloid fibrils and/or aggregation in peripheral erythrocytes, Thioflavin T (ThT) staining to confirm the presence of Aβ amyloid fibrils and/or aggregation in erythrocytes, followed by immunofluorescence testing after optimizing fluorescent staining and imaging conditions, and analysis of the images obtained using image processing software. They found that 16.8% of erythrocytes in the peripheral blood of Alzheimer's patients were elongated, versus

6.7% in normal controls, and there was a downward correlation between the two parameters. The study found that 98% of peripheral erythrocytes from Alzheimer's patients could bind amyloid peptides, compared to 38% in healthy individuals. This result showed that the binding of Aβ to erythrocytes in the peripheral blood of Alzheimer's patients alters the morphology of the erythrocytes [39].

The abundance of the biconcave type decreases significantly by day 40 of ageing in healthy cells and by day 20 in those with AD. The proportion of the crenated type does not change significantly during ageing in healthy erythrocytes until day 30 and then decreases, whereas in Alzheimer's patients, it decreases significantly as early as day 10. The contribution of spherocyte shape to the morphology of Alzheimer's erythrocytes increases much more rapidly with age, reaching 30-40% compared to healthy cells by day 10. After 40 days of ageing, the contribution of Alzheimer's erythrocytes is about 70% and that of healthy cells is about 60%. Therefore, spherocytic is the predominant morphological type found in all cells examined in aged erythrocytes in healthy and AD patients. When the morphology of erythrocytes from healthy and AD patients was characterized, the predominant shape of erythrocytes in healthy individuals was biconcave discoid (73%), crenate (21%), and spiculated (6%). The morphological composition of Alzheimer's erythrocytes is 61% biconcave, 33% crenate, and 5-6% spiculated cell types [5].

2.2.2. Parkinson's disease

PD, a chronic disease that causes progressive motor impairment and affects more than 1% of the population over the age of 65, is the second most common age-related neurodegenerative disease after AD [79]. The Global Burden of Disease Study estimates that the number of Parkinson's disease cases will double from approximately 7 million in 2015 to approximately 13 million in 2040 [80]. The neurotransmitter dopamine is involved in regulating movement, motivation, memory, and other physiological processes. Loss of dopaminergic neurons in the individual leads to a decrease in dopamine levels, which causes motor impairment and contributes to the cognitive deficits observed in some patients [81]. Since most of the motor features of PD are caused by the loss of dopaminergic neuronal cells in the substantia nigra pars compacta region of the midbrain, most of the current pharmacological treatment approaches aim to provide dopaminergic replacement [82]. Imaging techniques (magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) and positron emission tomography (PET)), and biomarkers (alpha-synuclein (αS), amyloid-beta (Aβ) and tau protein, neurofilament light chain (NfL) etc.) are used in the diagnosis [83].

An important trend in recent PD research is the search for biomarkers that may help identify individuals at risk or monitor disease progression and response to treatments. One of these trends is the study of erythrocyte structure [5]. The literature suggests that inflammatory signalling molecules involved in the pathophysiology of PD may affect the haematological system of these patients and that this may be used as a potential prognostic or diagnostic tool [84]. Signalling molecules involved in PD (cyclooxygenases, prostaglandins, thromboxanes iron) are intimately involved in the development of the variable programmed cell death of erythrocytes known as erythroses. The possible interaction of these abnormal signalling molecules involved in Parkinson's disease affects the erythrocyte structure of Parkinson's patients. In a study of 30 healthy and 30 Parkinson's patients, erythrocytes in healthy individuals showed a typical discoid shape, whereas most erythrocytes from Parkinson's patients did not have a typical discoid shape as seen in axial ratios but instead showed a typical erythristic and/or longitudinal morphology. Elastic modulus calculations from force-distance curves showed an increase in the Young's modulus of PD erythrocytes compared with erythrocyte measurements from control subjects (this increase in Young's modulus reflects a decrease in the elasticity of the cell membrane). In the same study, light microscopy showed that the shape of erythrocytes in whole blood smears was altered and that most erythrocytes had a typical erythrocytic shape. The average axial ratio of healthy subjects' erythrocytes was 1.13 and PD patients was 1.18 [79].

2.2.3. Multiple Sclerosis Disease

MS is a chronic disease of the central nervous system commonly affecting young adults between the ages of 20-40 years, and its main feature is the presence of foci of demyelination [85,86]. MS is a global problem, and its prevalence is increasing. The prevalence is highest in North America, Western Europe, and Australasia and lowest in countries centred around the equator. Clinically, MS follows two pathways: relapsing and progressive. Most commonly, the relapsing form of MS (RMS) is a form of MS that manifests as discrete episodes of neurological dysfunction followed by initial, partial, complete, or no remission. Over time, relapses usually decrease in frequency, but a gradual worsening usually occurs, resulting in uninterrupted progression (secondary progressive MS). Less than 10 per cent of patients with MS experience progression from onset; this category is called primary progressive MS (PPMS) [87,88]. In RMS, women are affected approximately 3 times more frequently than men, and the average age of onset is 30 years, whereas in PPMS, the proportion of affected men and women is similar, and the average age of onset is 40 years [88]. Typical acute neurological symptoms include visual, sensory, and motor deficits. Symptoms include both physical and psychosocial elements that negatively affect many aspects of patients' quality of life and perception of health [89]. The diagnosis is made by demonstrating the involvement of different neurological systems at different times with history, examination, and laboratory (evoked potentials, Magnetic Resonance Imaging) findings [90]. Today, increasing treatment options in MS offer the possibility of reducing disability and prolonging survival, but complete cure is not possible. In treatment, it is important to identify individuals with MS early in their disease to prevent relapses and long-term disability [91]. There is not yet an accepted biomarker that can predict disease activity and guide the treatment strategy. MRI is the most frequently used method to assess disease activity in addition to clinical and Cerebrospinal Fluid (CSF) findings. However, the search for a more accessible biomarker continues due to its financial burden and difficulties in its widespread and reliable applicability [92]. Therefore, it is crucial to identify new pathways underlying the

pathogenesis of MS. Previous studies have suggested that Erythrocytes may participate in the pathophysiological mechanisms of MS via reduced antioxidant capacity and altered hemorheology, leading to increased peripheral oxidative stress and potential ischaemic tissue damage, respectively. Nearly half a century ago, in a sample of 73 MS patients and 38 healthy controls, erythrocyte osmotic fragility was shown to be significantly higher in patients with MS than in controls. In 2009, there was report of erythrocyte membrane fluidity abnormalities in MS [93]. Based on the fact that peripheral blood erythrocytes (PBER), which are in close contact with all tissues and enter into morpho-functional relationships with them, reflect the physiological and pathological changes occurring in the body through their qualitative and quantitative reorganization. Churpiy et al. examined 62 patients with MS according to MacDonald criteria in the neurology department in 2024. Patients were divided into three groups as RMS, SPMS, and PPMS. A control group was also included in the study. It was found that SPMS and PPMS patients showed changes not only in the form of PBER but also in its chemical composition, and this was most prominent in PPMS patients. It is stated that as MS progresses, erythrocytes in the double convoluted discs transform into spherocytes and microrelief irregularities appear on their surfaces. The ratio of spherocytes to dissociates is 1:7 in the MS compensation stage, 1:4 in the sub compensation stage, and 1:9 in the decompensation stage. In the control group, this ratio was determined as 1:60. It was stated that erythrocyte deformation may be one of the pathogenetic factors of the progression of multiple sclerosis [94].

2.2.4. Epilepsy Disease

Epilepsy is a neurological disorder characterized by abnormal neurophysiological activity resulting from abnormal and excessive electrical discharge in cortical neurons, leading to sudden, recurrent epileptic seizures or abnormal behaviour not triggered by an identifiable event, and accompanied by varying degrees of sensory or consciousness loss [95,96]. Epilepsy is one of the most common neurological disorders affecting approximately 1 per cent of the population and accounts for 0.75 per cent of the global burden of disease [97,98]. With limited treatment options other than surgery, approximately 30% of patients eventually progress to drug-resistant epilepsy with unpredictable recurrent seizures. Two important pathohistological features of drug-resistant epilepsy are neuronal death and glial scarring in the epileptogenic focus [98]. The diagnosis is made by neuroimaging techniques such as non-invasive electroencephalography (EEG) and invasive electrocorticography (ECoG), positron emission tomography (PET), and magnetic resonance imaging (MRI) [96].

To date, clinicians involved in the treatment of epilepsy have mostly studied the effects of antiepileptic drugs on haematological cell counts using antiepileptic drugs and peripheral smears (thrombocytopenia, neutropenia, or anaemia). Laboratory researchers have focused on and reported the in vitro effects of antiepileptic drugs on red blood cell morphology in a self-created environment. Although all antiepileptic drugs, especially older ones, rarely cause clinically significant or relevant haematological adverse events, antiepileptic drugs have been found to cause morphological changes in erythrocytes [14]. In a study by Ünal et al. focusing on quantitative phase imaging of erythrocytes and aiming to compare the morphological differences between epilepsy patients under antiepileptic treatment without any other disease that may affect erythrocyte morphology and healthy control group, it was determined that epileptic drugs cause erythrocyte morphological changes. In 50 epilepsy and 30 healthy individuals, the erythrocyte morphology was analysed by white light diffraction microscopy, SEM, and light microscopy. In epilepsy patients, the diameter of the cell was 7.104 μm, the height of the erythrocyte was 1.4 μm, and the surface structure of the erythrocyte was determined as distorted thick-edged. In healthy individuals, the diameter of the cell was 7.387 μm, the erythrocyte height was 2.7 μm and the surface structure of the erythrocyte was biconcave [14]. In a different study investigating the morphological changes induced by antiepileptic treatment in erythrocyte membranes, the sample group consisted of 6 patients under drug treatment and one healthy individual. When the surface texture parameters calculated from the AFM images of erythrocyte membranes obtained for each healthy and treated patient were analysed, the average erythrocyte height in healthy individuals was 7.1 μm, the average smoothness was 1.2 μm, the distortion coefficient was -0.02, and the surface structure of the erythrocyte was normal. In a 63-year-old female patient who has been on anticonvulsant therapy for 24 years of age and psychiatric treatment for 3 years of age, the mean erythrocyte height was 17.7 μm, the mean smoothness was 2.7 μm, the skew coefficient was 1.05, and the erythrocyte structure was platicuric [12].

3. Conclusion

The progressive degeneration and/or loss of neurons in the central nervous system is generally considered to be involved in neurodegenerative diseases. They share common underlying pathogenic mechanisms, including reactive oxygen species generation and oxidative stress [99-103], mitochondrial dysfunction [104,105], neuroinflammation [106-108] by abnormal accumulation, and misfolding of specific proteins, mainly β-amyloid peptide (Aβ), τ-protein, and α-synuclein (α-syn), in the brain and in peripheral blood cells. [102,109-111]. There is no cure for neurodegenerative diseases, and the treatments available only alleviate symptoms or slow disease progression in spite of advances in diagnostics and therapeutics. The development of new diagnostic approaches, the discovery of new reliable, inexpensive, and easily accessible diagnostic biomarkers, and the establishment of new therapies for these diseases are of extreme importance. The analyses in this review show that RBCs together with their ultrastructural properties are promising candidates to be biomarkers in neurodegenerative diseases and may help in the diagnosis and prognosis prediction of the diseases studied.

Declaration

Author Contribution: Conceive-F.D. and M.U.; Design-F.D. and M.U.; Supervision-F.D.; Computational Performance, Data Collection and/or Processing-M.U.; Analysis and/or Interpretation Literature Review-F.D. and M.U.; Writer- F.D. and M.U.; Critical Reviews– F.D. and M.U.

Conflict of interests: The authors have declared no conflicts of interest.

Acknowledgements

We would like to thank Dr Aysun ÜNAL and Dr Özlem KOCAHAN YILMAZ for their support in the preparation of the article.

Orcid-ID

Filiz Dilek https://orcid.org/0000-0002-0245-0713
Merve Uyanık https://orcid.org/0000-0003-2537-1273

References

[1] Y. Kim, K. Kim, and Y. Park, 'Measurement Techniques for Red Blood Cell Deformability: Recent Advances', in Blood Cell - An Overview of Studies in Hematology, vol. 11, no. tourism, InTech, 2012, p. 13. doi: 10.5772/50698.

[2] J. Kim, H. Lee, and S. Shin, 'Advances in the measurement of red blood cell deformability: A brief review', J Cell Biotechnol, vol. 1, no. 1, pp. 63–79, Jul. 2015, doi: 10.3233/jcb-15007.

[3] F. Köse, N. Bahtiyar, F. B. Cinemre, and B. Aydemir, 'Hastalıkların Fizyopatolojisinde Eritrosit Deformabilitesinin Önemi', İstanbul Gelişim Üniversitesi Sağlık Bilimleri Dergisi, no. 21, pp. 1262–1272, Jan. 2024, doi: 10.38079/igusabder.1313165.

[4] S. Dinarelli et al., 'Erythrocyte's aging in microgravity highlights how environmental stimuli shape metabolism and morphology', Sci Rep, vol. 8, no. 1, Dec. 2018, doi: 10.1038/s41598-018-22870-0.

[5] V. Strijkova-Kenderova et al., 'Morphometry and stiffness of red blood cells—signatures of neurodegenerative diseases and aging', Int J Mol Sci, vol. 23, no. 1, Jan. 2022, doi: 10.3390/ijms23010227.

[6] K. Nakagawa et al., 'Amyloid β-induced erythrocytic damage and its attenuation by carotenoids', FEBS Lett, vol. 585, no. 8, pp. 1249–1254, Apr. 2011, doi: 10.1016/j.febslet.2011.03.060.

[7] S. Ficarra et al., 'Antiepileptic carbamazepine drug treatment induces alteration of membrane in red blood cells: Possible positive effects on metabolism and oxidative stress', Biochimie, vol. 95, no. 4, pp. 833–841, 2013, doi: 10.1016/j.biochi.2012.11.018.

[8] P. Zambrano, M. Suwalsky, M. Jemiola-Rzeminska, and K. Strzalka, 'A1-and B-Adrenergic Antagonist Labetalol Induces Morphological Changes in Human Erythrocytes', Biochem Biophys Res Commun, vol. 503, no. 1, pp. 209–214, 2018, doi: 10.1016/j.bbrc.2018.06.004.

[9] M. Suwalsky, S. Mennickent, B. Norris, F. Villena, and C. P. Sotomayor, 'Effects of the antiepileptic drug carbamazepine on human erythrocytes', Toxicology in Vitro, vol. 20, no. 8, pp. 1363–1369, 2006, doi: 10.1016/j.tiv.2006.05.010.

[10] A. V Buys, M.-J. Van Rooy, P. Soma, D. Van Papendorp, B. Lipinski, and E. Pretorius, 'Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study', Cardiovasc Diabetol, vol. 12, no. 1, p. 25, 2013, doi: 10.1186/1475-2840-12-25.

[11] J. Kurantsin-Mills, N. Samji, M. A. Moscarello, and J. M. Boggs, 'Comparison of membrane structure, osmotic fragility, and morphology of multiple sclerosis and normal erythrocytes', Neurochem Res, vol. 7, no. 12, pp. 1523–1540, 1982, doi: 10.1007/BF00965095.

[12] B. Oprisan, I. Stoica, and M. I. Avadanei, 'Morphological changes induced in erythrocyte membrane by the antiepileptic treatment: An atomic force microscopy study', Microsc Res Tech, vol. 80, no. 4, pp. 364–373, 2017, doi: 10.1002/jemt.22804.

[13] N. Z. Piety, W. H. Reinhart, P. H. Pourreau, R. Abidi, and S. S. Shevkoplyas, 'Shape matters: The effect of red blood cell shape on perfusion of an artificial microvascular network', Transfusion (Paris), vol. 56, no. 4, pp. 844–851, 2016, doi: 10.1111/trf.13449.

[14] A. Ünal, Ö. Kocahan, B. Altunan, A. Aksoy Gündoğdu, M. Uyanık, and S. Özder, 'Quantitative phase imaging of erythrocyte in epilepsy patients', Microsc Res Tech, no. December, pp. 1–9, 2020, doi: 10.1002/jemt.23676.

[15] Z. Luo, H. Xu, L. Liu, T. Y. Ohulchanskyy, and J. Qu, 'Optical imaging of beta-amyloid plaques in alzheimer's disease', Aug. 01, 2021, MDPI. doi: 10.3390/bios11080255.

[16] R. Mukherjee, M. Saha, A. Routray, and C. Chakraborty, 'Nanoscale Surface Characterization of Human Erythrocytes by Atomic Force Microscopy: A Critical Review', IEEE Trans Nanobioscience, vol. 14, no. 6, pp. 625–633, 2015, doi: 10.1109/TNB.2015.2424674.

[17] K. Jaferzadeh and I. Moon, 'Human red blood cell recognition enhancement with three-dimensional morphological features obtained by digital holographic imaging', J Biomed Opt, vol. 21, no. 12, p. 126015, 2016, doi: 10.1117/1.jbo.21.12.126015.

[18] E. Ahmadzadeh, K. Jaferzadeh, J. Lee, and I. Moon, 'Automated three-dimensional morphology-based clustering of human erythrocytes with regular shapes: stomatocytes, discocytes, and echinocytes', J Biomed Opt, vol. 22, no. 7, p. 076015, 2017, doi: 10.1117/1.jbo.22.7.076015.

[19] K. Jaferzadeh and I. Moon, 'Quantitative investigation of red blood cell three-dimensional geometric and chemical changes in the storage lesion using digital holographic microscopy', J Biomed Opt, vol. 20, no. 11, p. 111218, Oct. 2015, doi: 10.1117/1.JBO.20.11.111218.

[20] K. Jaferzadeh, M. W. Sim, N. G. Kim, and I. K. Moon, 'Quantitative analysis of three-dimensional morphology and membrane dynamics of red blood cells during temperature elevation', Sci Rep, vol. 9, no. 1, pp. 1–9, 2019, doi: 10.1038/s41598-019-50640-z.

[21] G. Popescu, 'Quantitative phase imaging of cells and tissues', p. 362, 2012.

[22] G. Popescu, Chapter 5 Quantitative Phase Imaging of Nanoscale Cell Structure and Dynamics, First Edit., vol. 90, no. C. Elesvier Inc., 2009. doi: 10.1016/S0091-679X(08)00805-4.

[23] H. V. Pham, B. Bhaduri, K. Tangella, C. Best-Popescu, and G. Popescu, 'Real Time Blood Testing Using

Quantitative Phase Imaging', PLoS One, vol. 8, no. 2, Feb. 2013, doi: 10.1371/journal.pone.0055676.

[24] Y. K. Park, C. Depeursinge, and G. Popescu, 'Quantitative phase imaging in biomedicine', Nat Photonics, vol. 12, no. 10, pp. 578–589, 2018, doi: 10.1038/s41566-018-0253-x.

[25] J. Nadeau, Y. K. Park, and G. Popescu, 'Methods in quantitative phase imaging in life science', Methods, vol. 136, pp. 1–3, 2018, doi: 10.1016/j.ymeth.2018.03.004.

[26] B. Bhaduri, H. Pham, D. Wickland, and G. Popescu, 'Real-time quantitative phase imaging in biomedicine', SPIE Newsroom, pp. 1–5, 2013, doi: 10.1117/2.1201304.004776.

[27] T. Kim, R. Zhou, L. L. Goddard, and G. Popescu, 'Breakthroughs in Photonics 2013: Quantitative Phase Imaging: Metrology Meets Biology', Apr. 01, 2014, Institute of Electrical and Electronics Engineers Inc. doi: 10.1109/JPHOT.2014.2309647.

[28] J. M. Higgins, 'Red Blood Cell Population Dynamics', Clin Lab Med, vol. 35, no. 1, pp. 43–57, 2015, doi: 10.1016/j.cll.2014.10.002.

[29] G. Barshtein, I. Pajic-Lijakovic, and A. Gural, 'Deformability of Stored Red Blood Cells', Front Physiol, vol. 12, no. September, pp. 1–13, 2021, doi: 10.3389/fphys.2021.722896.

[30] A. Diaspro and C. Usai, 'Optical Microscopy', in Wiley Encyclopedia of Biomedical Engineering, Wiley, 2006. doi: 10.1002/9780471740360.ebs0869.

[31] C. J. R. Sheppard, 'The optics of microscopy', Journal of Optics A: Pure and Applied Optics, vol. 9, no. 6, Jun. 2007, doi: 10.1088/1464-4258/9/6/S01.

[32] D. T. Grubb, '2.17 - Optical Microscopy', in Polymer Science: a Comprehensive Reference: Volume 1-10, vol. 1–10, Elsevier, 2012, pp. 465–478. doi: 10.1016/B978-0-444-53349-4.00035-2.

[33] JEROME. MERTZ, INTRODUCTION TO OPTICAL MICROSCOPY. CAMBRIDGE UNIV PRESS, 2019.

[34] C. Deniz, O. Kocahan, B. Altunan, and A. Unal, 'Novel Approach to Investigate the Effect of High-Dose Methylprednisolone on Erythrocyte Morphology: White Light Diffraction Microscopy', Measurement Science Review, vol. 23, no. 5, pp. 202–209, Oct. 2023, doi: 10.2478/msr-2023-0026.

[35] E. Canetta and A. K. Adya, 'Nano-imaging and its applications to biomedicine', in Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics), 2011, pp. 423–432. doi: 10.1007/978-3-642-24085-0_44.

[36] R. Hiesgen and K. A. Friedrich, 'Atomic force microscopy', PEM Fuel Cell Diagnostic Tools, no. March, pp. 395–421, 2011, doi: 10.31803/tg-20230829155921.

[37] S. Kantorovitz, 'Basic theory', Progress in Mathematics, vol. 281, pp. 3–48, 2010, doi: 10.1007/978-0-8176-4932-6_1.

[38] V. Sergunova et al., 'Investigation of Red Blood Cells by Atomic Force Microscopy', Sensors, vol. 22, no. 5, Mar. 2022, doi: 10.3390/s22052055.

[39] J. Lan et al., 'The peripheral blood of Aβ binding RBC as a biomarker for diagnosis of Alzheimer's disease', Age Ageing, vol. 44, no. 3, pp. 458–464, May 2015, doi: 10.1093/ageing/afv009.

[40] E. Pretorius, J. Bester, and D. B. Kell, 'A Bacterial Component to Alzheimer's-Type Dementia Seen via a Systems Biology Approach that Links Iron Dysregulation and Inflammagen Shedding to Disease', 2016, IOS Press. doi: 10.3233/JAD-160318.

[41] R. Nowakowski, P. Luckham, and P. Winlove, 'Imaging erythrocytes under physiological conditions by atomic force microscopy', Biochim Biophys Acta Biomembr, vol. 1514, no. 2, pp. 170–176, 2001, doi: 10.1016/S0005-2736(01)00365-0.

[42] P. Niraj Nirmalraj, T. Schneider, and A. Felbecker, 'Spatial organization of protein aggregates on red blood cells as physical biomarkers of Alzheimer's disease pathology', 2021.

[43] H. N. Southworth, 'Scanning Electron Microscopy and Microanalysis', in Physicochemical Methods of Mineral Analysis, 1975, pp. 421–450. doi: 10.1007/978-1-4684-2046-3_11.

[44] P. Hortolà, 'Secondary-electron SEM bioimaging of human erythrocytes in bloodstains on high-carbon steel substrate without specimen preparation', Micron, vol. 39, no. 1, pp. 53–55, 2008, doi: 10.1016/j.micron.2006.12.004.

[45] J. Goldstein, D. E. Newbury, J. R. Michael, N. W. M. Ritchie, J. H. J. Scott, and D. C. Joy, Scanning electron microscopy and x-ray microanalysis, Third Edit. New York: Kluwer Academic / Plenum Publishers, 2003.

[46] S. Bhowmick, D. K. Das, A. K. Maiti, and C. Chakraborty, 'Computer-aided diagnosis of thalassemia using scanning electron microscopic images of peripheral blood: A morphological approach', J Med Imaging Health Inform, vol. 2, no. 3, pp. 215–221, 2012, doi: 10.1166/jmihi.2012.1092.

[47] S. Bhowmick, D. K. Das, A. K. Maiti, and C. Chakraborty, 'Structural and textural classification of erythrocytes in anaemic cases: A scanning electron microscopic study', Micron, vol. 44, no. 1, pp. 384–394, 2013, doi: 10.1016/j.micron.2012.09.003.

[48] N. M. Geekiyanage et al., 'A coarse-grained red blood cell membrane model to study stomatocyte-discocyteechinocyte morphologies', PLoS One, vol. 14, no. 4, pp. 1–25, 2019, doi: 10.1371/journal.pone.0215447.

[49] G. Benga and G. Cox, 'Light and Scanning Electron Microscopy of Red Blood Cells From Humans and Animal Species Providing Insights into Molecular Cell Biology', Front Physiol, vol. 13, no. July, pp. 1–13, 2022, doi: 10.3389/fphys.2022.838071.

[50] Ö. Kocahan, E. Coşkun, and S. Özder, 'Generalized Morse wavelet for the determination of the birefringence

of a liquid crystal cell', Meas Sci Technol, vol. 26, no. 8, 2015, doi: 10.1088/0957-0233/26/8/085204.

[51] O. Kocahan, E. Tiryaki, E. Coskun, and S. Ozder, 'Determination of phase from the ridge of CWT using generalized Morse wavelet', Meas Sci Technol, vol. 29, no. 3, 2018, doi: 10.1088/1361-6501/aa9d56.

[52] H. Majeed et al., 'Quantitative phase imaging for medical diagnosis', J Biophotonics, vol. 10, no. 2, pp. 177–205, 2017, doi: 10.1002/jbio.201600113.

[53] M. Takeda, H. Ina, and S. Kobayashi, 'FOURIER-TRANSFORM METHOD OF FRINGE-PATTERN ANALYSIS FOR COMPUTER-BASED TOPOGRAPHY AND INTEFEROMETRY.', J Opt Soc Am, vol. 72, no. 1, pp. 156–160, Jan. 1982, doi: 10.1364/JOSA.72.000156.

[54] Ö. Kocahan, E. Coşkun, and S. Özder, 'Generalized Morse wavelets for the phase evaluation of projected fringe pattern', Meas Sci Technol, vol. 25, no. 10, p. 105701, Oct. 2014, doi: 10.1088/0957-0233/25/10/105701.

[55] C. Edwards, B. Bhaduri, B. G. Griffin, L. L. Goddard, and G. Popescu, 'Epi-illumination diffraction phase microscopy with white light', Opt Lett, vol. 39, no. 21, p. 6162, 2014, doi: 10.1364/OL.39.006162.

[56] Y. Park et al., 'Measurement of red blood cell mechanics during morphological changes.', Proc Natl Acad Sci U S A, vol. 107, no. 15, pp. 6731–6, Apr. 2010, doi: 10.1073/pnas.0909533107.

[57] M. Jiang et al., 'Automatic Classification of Red Blood Cell Morphology Based on Quantitative Phase Imaging', Int J Opt, vol. 2022, 2022, doi: 10.1155/2022/1240020.

[58] C. Edwards et al., 'Diffraction phase microscopy: monitoring nanoscale dynamics in materials science [Invited]', Appl Opt, vol. 53, no. 27, p. G33, Sep. 2014, doi: 10.1364/AO.53.000G33.

[59] Ö. Kocahan, N. Çelebioğlu, and M. Uyanık, 'White light diffraction phase microscopy for imaging of red blood cells for different storage times', Phys Scr, vol. 99, no. 5, 2024, doi: 10.1088/1402-4896/ad3b79.

[60] C. L. Curl et al., 'Refractive index measurement in viable cells using quantitative phase-amplitude microscopy and confocal microscopy', Cytometry Part A, vol. 65, no. 1, pp. 88–92, 2005, doi: 10.1002/cyto.a.20I34.

[61] G. Popescu, Quantitative Phase Imaging of Cells and Tissues. The McGraw-Hill Companies, Inc., 2011.

[62] T. Cacace, V. Bianco, and P. Ferraro, 'Quantitative phase imaging trends in biomedical applications', Opt Lasers Eng, vol. 135, no. February, p. 106188, 2020, doi: 10.1016/j.optlaseng.2020.106188.

[63] H. S. Park, S. Ceballos, W. J. Eldridge, and A. Wax, 'Invited Article: Digital refocusing in quantitative phase imaging for flowing red blood cells', APL Photonics, vol. 3, no. 11, Nov. 2018, doi: 10.1063/1.5043536.

[64] M. Trusiak et al., 'Variational Hilbert Quantitative Phase Imaging', Sci Rep, vol. 10, no. 1, pp. 1–16, 2020, doi: 10.1038/s41598-020-69717-1.

[65] G. Popescu et al., 'Fourier phase microscopy for investigation of biological structures and dynamics', Opt Lett, vol. 29, no. 21, p. 2503, 2004, doi: 10.1364/ol.29.002503.

[66] G. Popescu, T. Ikeda, R. R. Dasari, and M. S. Feld, 'Diffraction phase microscopy for quantifying cell structure and dynamics', Opt Lett, vol. 31, no. 6, p. 775, 2006, doi: 10.1364/OL.31.000775.

[67] S. Van der Jeught and J. J. J. Dirckx, 'Real-time structured light profilometry: A review', Opt Lasers Eng, vol. 87, pp. 18–31, 2015, doi: 10.1016/j.optlaseng.2016.01.011.

[68] M. Mihailescu et al., 'Automated imaging, identification, and counting of similar cells from digital hologram reconstructions', Appl Opt, vol. 50, no. 20, pp. 3589–3597, 2011, doi: 10.1364/AO.50.003589.

[69] C. Ping, W. Xiao, L. Ping, J. Liu, feng pan, and P. Ferraro, 'Automatic removal of phase aberration inholographic microscopy allows detection ofdrug sensitivity of ovarian cancer cells', OSA Contin, vol. 3, no. 7, pp. 1856–1869, 2020, doi: 10.1364/osac.391773.

[70] M. Kumar, O. Matoba, X. Quan, S. K. Rajput, M. Morita, and Y. Awatsuji, 'Quantitative dynamic evolution of physiological parameters of RBC by highly stable digital holographic microscopy', Opt Lasers Eng, vol. 151, Apr. 2022, doi: 10.1016/j.optlaseng.2021.106887.

[71] Ö. Kocahan Yilmaz, 'Quantitative Determination of Surface Morphology of Red Blood Cell', Journal of Advanced Research in Natural and Applied Sciences, vol. 9, no. 2, pp. 385–395, Jun. 2023, doi: 10.28979/jarnas.1206923.

[72] C. Mallozzi et al., 'Activation of Tyrosine Phosphorylation Signaling in Erythrocytes of Patients with Alzheimer's Disease', Neuroscience, vol. 433, pp. 36–41, May 2020, doi: 10.1016/j.neuroscience.2020.02.050.

[73] A. P. Porsteinsson, R. S. Isaacson, S. Knox, M. N. Sabbagh, and I. Rubino, 'Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021', Jul. 01, 2021, Serdi-Editions. doi: 10.14283/jpad.2021.23.

[74] S. Eroymak and V. Yiğit, 'Alzheimer Hastaliğinin Maliyet Analizi', Journal of Süleyman Demirel University Institute of Social Sciences, vol. 4, no. 29, pp. 167–196, 2017.

[75] A. Stevenson, D. Lopez, P. Khoo, R. N. Kalaria, and E. B. Mukaetova-Ladinska, 'Exploring Erythrocytes as Blood Biomarkers for Alzheimer's Disease', 2017, IOS Press. doi: 10.3233/JAD-170363.

[76] E. A. Kosenko, L. A. Tikhonova, C. Montoliu, G. E. Barreto, G. Aliev, and Y. G. Kaminsky, 'Metabolic abnormalities of erythrocytes as a risk factor for Alzheimer's disease', Jan. 05, 2018, Frontiers Media S.A. doi: 10.3389/fnins.2017.00728.

[77] J. Bester, A. V. Buys, B. Lipinski, D. B. Kell, and E. Pretorius, 'High ferritin levels have major effects on the morphology of erythrocytes in Alzheimer's disease', Front Aging Neurosci, vol. 5, no. DEC, 2013, doi: 10.3389/fnagi.2013.00088.

[78] J. G. Mohanty, H. D. Shukla, J. D. Williamson, L. J. Launer, S. Saxena, and J. M. Rifkind, 'Alterations in the red blood cell membrane proteome in alzheimer's subjects reflect disease-related changes and provide insight into altered cell morphology', Proteome Sci, vol. 8, Mar. 2010, doi: 10.1186/1477-5956-8-11.

[79] E. Pretorius, A. C. Swanepoel, A. V Buys, N. Vermeulen, W. Duim, and D. B. Kell, 'Eryptosis as a marker of Parkinson's disease', Aging, vol. 6, no. 10, pp. 788–819, Oct. 2014, doi: 10.18632/aging.100695.

[80] J. Jankovic and E. K. Tan, 'Parkinson's disease: Etiopathogenesis and treatment', J Neurol Neurosurg Psychiatry, vol. 91, no. 8, pp. 795–808, Aug. 2020, doi: 10.1136/jnnp-2019-322338.

[81] S. Ramesh and A. S. P. M. Arachchige, 'Depletion of dopamine in Parkinson's disease and relevant therapeutic options: A review of the literature', 2023, AIMS Press. doi: 10.3934/NEUROSCIENCE.2023017.

[82] F. C. Church, 'Review treatment options for motor and non-motor symptoms of parkinson's disease', Apr. 01, 2021, MDPI. doi: 10.3390/biom11040612.

[83] N. Öksüz, Ş. Öztürk, and O. Doğu, 'Future Prospects in Parkinson's Disease Diagnosis and Treatment', Dec. 01, 2022, Turkish Neuropsychiatric Society. doi: 10.29399/npa.28169.

[84] J. D. Meléndez-Flores, J. M. Millán-Alanís, H. de León-Gutiérrez, S. S. Rojo-Garza, N. A. Álvarez-Villalobos, and I. Estrada-Bellmann, 'Association of anemia with Parkinsons disease: a systematic review with meta-analysis of epidemiological studies', Revista Mexicana de Neurociencia, vol. 24, no. 1, Jul. 2023, doi: 10.24875/rmn.22000059.

[85] K. E. Attfield, L. T. Jensen, M. Kaufmann, M. A. Friese, and L. Fugger, 'The immunology of multiple sclerosis', Dec. 01, 2022, Nature Research. doi: 10.1038/s41577-022-00718-z.

[86] B. Ptaszek et al., 'Effect of whole-body cryotherapy on morphological, rheological and biochemical indices of blood in people with multiple sclerosis', J Clin Med, vol. 10, no. 13, Jul. 2021, doi: 10.3390/jcm10132833.

[87] S. R. Murúa, M. F. Farez, and F. J. Quintana, 'The Immune Response in Multiple Sclerosis', Annual Review of Pathology: Mechanisms of Disease Annu. Rev. Pathol. Mech. Dis. 2022, vol. 17, p. 39, 2024, doi: 10.1146/annurev-pathol-052920.

[88] S. L. Hauser and B. A. C. Cree, 'Treatment of Multiple Sclerosis: A Review', Dec. 01, 2020, Elsevier Inc. doi: 10.1016/j.amjmed.2020.05.049.

[89] L. Lakin, B. E. Davis, C. C. Binns, K. M. Currie, and M. R. Rensel, 'Comprehensive Approach to Management of Multiple Sclerosis: Addressing Invisible Symptoms—A Narrative Review', Jun. 01, 2021, Adis. doi: 10.1007/s40120-021-00239-2.

[90] M. P. McGinley, C. H. Goldschmidt, and A. D. Rae-Grant, 'Diagnosis and Treatment of Multiple Sclerosis', JAMA, vol. 325, no. 8, p. 765, Feb. 2021, doi: 10.1001/jama.2020.26858.

[91] N. Makhani and H. Tremlett, 'The multiple sclerosis prodrome', Aug. 01, 2021, Nature Research. doi: 10.1038/s41582-021-00519-3.

[92] J. Yang et al., 'Current and Future Biomarkers in Multiple Sclerosis', Int J Mol Sci, vol. 23, no. 11, Jun. 2022, doi: 10.3390/ijms23115877.

[93] G. M. Hon et al., 'Red blood cell membrane fluidity in the etiology of multiple sclerosis', Journal of Membrane Biology, vol. 232, no. 1–3, pp. 25–34, Jan. 2009, doi: 10.1007/s00232-009-9213-1.

[94] I. K. Churpiy et al., 'Morphologıcal And Functıonal Changes Of Erythrocytes And Enzymes Of The Antıoxıdant System In Multıple Sclerosıs', World of Medicine and Biology, vol. 20, no. 89, p. 177, Apr. 2024, doi: 10.26724/2079-8334-2024-3-89-177-183.

[95] G. Akdağ, D. İ. Algın, and O. O. Erdinç, 'EPİLEPSİ / EPILEPSY', OSMANGAZİ JOURNAL OF MEDICINE, vol. 38, no. 0, 2016, doi: 10.20515/otd.88853.

[96] J. Yuan et al., 'Machine learning applications on neuroimaging for diagnosis and prognosis of epilepsy: A review', Feb. 15, 2022, Elsevier B.V. doi: 10.1016/j.jneumeth.2021.109441.

[97] E. Trinka, P. Kwan, B. I. Lee, and A. Dash, 'Epilepsy in Asia: Disease burden, management barriers, and challenges', Mar. 01, 2019, Blackwell Publishing Inc. doi: 10.1111/epi.14458.

[98] Z. P. Chen et al., 'Lipid-accumulated reactive astrocytes promote disease progression in epilepsy', Nat Neurosci, vol. 26, no. 4, pp. 542–554, Apr. 2023, doi: 10.1038/s41593-023-01288-6.

[99] S. Younes-Mhenni, M. Frih-Ayed, A. Kerkeni, M. Bost, and G. Chazot, 'Peripheral blood markers of oxidative stress in Parkinson's disease', Eur Neurol, vol. 58, no. 2, pp. 78–83, Aug. 2007, doi: 10.1159/000103641.

[100] P. Jenner and C. W. Olanow, 'Oxidative stress and the pathogenesis of Parkinson's disease', 1996, Lippincott Williams and Wilkins. doi: 10.1212/wnl.47.6_suppl_3.161s.

[101] L. Ciccoli et al., 'Morphological changes and oxidative damage in Rett Syndrome erythrocytes', Biochim Biophys Acta Gen Subj, vol. 1820, no. 4, pp. 511–520, Apr. 2012, doi: 10.1016/j.bbagen.2011.12.002.

[102] J. Wojsiat, C. Prandelli, K. Laskowska-Kaszub, A. Martín-Requero, and U. Wojda, 'Oxidative stress and aberrant cell cycle in alzheimer's disease lymphocytes: Diagnostic prospects', 2015, IOS Press. doi: 10.3233/JAD-141977.

[103] A. Singh, R. Kukreti, L. Saso, and S. Kukreti, 'Oxidative stress: A key modulator in neurodegenerative diseases', Apr. 22, 2019, MDPI AG. doi: 10.3390/molecules24081583.

[104] S. Ciccone, E. Maiani, G. Bellusci, M. Diederich, and S. Gonfloni, 'Parkinson's disease: A complex interplay of mitochondrial DNA alterations and oxidative stress', Feb. 2013. doi: 10.3390/ijms14022388.

[105] Y. Wu, M. Chen, and J. Jiang, 'Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signaling', Nov. 01, 2019, Elsevier B.V. doi: 10.1016/j.mito.2019.07.003.

[106] P. L. McGeer and E. G. McGeer, 'Inflammation and the degenerative diseases of aging', in Annals of the New York Academy of Sciences, New York Academy of Sciences, 2004, pp. 104–116. doi: 10.1196/annals.1332.007.

[107] L. Minghetti, 'Role of inflammation in neurodegenerative diseases', Curr Opin Neurol, vol. 18, no. 3, pp. 315–321, Jun. 2005, doi: 10.1097/01.wco.0000169752.54191.97.

[108] V. H. Perry, 'The influence of systemic inflammation on inflammation in the brain: Implications for chronic neurodegenerative disease', Sep. 2004. doi: 10.1016/j.bbi.2004.01.004.

[109] T. B. Thompson, P. Chaggar, E. Kuhl, and A. Goriely, 'Protein-protein interactions in neurodegenerative diseases: A conspiracy theory', PLoS Comput Biol, vol. 16, no. 10, Oct. 2020, doi: 10.1371/journal.pcbi.1008267.

[110] C. Giacomelli, S. Daniele, and C. Martini, 'Potential biomarkers and novel pharmacological targets in protein aggregation-related neurodegenerative diseases', May 01, 2017, Elsevier Inc. doi: 10.1016/j.bcp.2017.01.017.

[111] J. Meldolesi, 'News about the role of fluid and imaging biomarkers in neurodegenerative diseases', 2021, MDPI AG. doi: 10.3390/biomedicines9030252.