CHAPTER IV

LIPIDOMICS STUDY OF BLOOD PLASMA AND ERYTHROCYTES FROM PATIENTS OF Eβ THALASSEMIA
4.1 INTRODUCTION

Phospholipids are the major building blocks of a cell membrane. They not only provide a permeability barrier to water and solutes but also act as anchors for macromolecules and an interface for cell-cell communication \(^1\). These phospholipids can be divided into specific classes depending upon the polar head groups. The heterogeneity of this mixture is further complicated by the number of individual fatty acyl substituents that are esterified to two additional hydroxyl groups of the glycerol backbone \(^2\). These asymmetric cell membranes maintain a particular composition and organization of the phospholipid population. In stressed conditions this asymmetry is perturbed leading to premature removal of erythrocytes from the circulation by phagocytosis by macrophages of the reticulo-endothelial system \(^3\).

Furthermore, due to the presence of double bonds in the lipids they are susceptible to oxidative damage by the reactive oxygen species (ROS). The lipid peroxidation process is a radical reaction that leads to the formation of oxidized intact phospholipids, known as long-chain products, such as hydroxy and/or hydroperoxide derivatives, phospholipids that contain a truncated lipid at the sn-2 position and lysophospholipids or formyl-phospholipids generated on plasmalogen oxidation \(^1\),\(^4\). It has already been shown that lipid peroxidation is increased in certain diseases like cancer, rheumatoid arthritis, drug-associated toxicity, and post ischemic reoxygenation injury, as well as in the degenerative processes associated with aging \(^5\).

In our study for the first time we are studying the changes in the lipid population that occur in the plasma and erythrocyte membrane in HbEβ thalassemia. Thalassaemias as a whole belongs to the family of autosomal recessive blood disorders which are caused due to mutations in the globin chain gene giving rise to defective globin chains \(^6\). Eβ thalassemia is the most
common form of thalassemia which is prevalent in the Indian subcontinent and south-east Asia. This form is associated with the most common variant of hemoglobin, HbE, caused by the single mutation in β-globin gene (β26 Glu→Lys). HbE does not result in clinical severity, but its combination with β-thalassemia is responsible for the pathophysiological severity of the disease. These patients suffer from varying phenotypes ranging from severe transfusion dependent thalassemia major to thalassemia intermedia that mainly include ineffective erythropoiesis, faster aging and premature hemolysis of the red blood cells (7).

Due to the defective synthesis of the β globin chain there is an accumulation of the α globin chain. The excess α globin chains form unstable homotetramers. These homotetramers precipitate as inclusion bodies and the iron is released leading to the oxidative damage of membrane lipids (6, 7).

Most studies have focused on end products of lipid peroxidation such as aldehydes and volatile hydrocarbons. However, recent focus has shifted to the oxidatively modified lipids as they may have significant biological activities (8). In our study apart from the lipid populations we have tried to monitor this population of oxidatively modified lipids in the three fractions.

4.2 MATERIALS AND METHODS

SUBJECTS:

Blood samples, collected in EDTA vials (BD Biosciences), from six normal healthy volunteers and six patients of HbEβ thalassemia, diagnosed for the first time who did not receive any blood transfusion, were used for the lipidomic studies (table I). Blood samples were
Table 4.1: sample details.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>CONDITION</th>
<th>AGE</th>
<th>GENDER</th>
<th>Hb F</th>
<th>Hb A</th>
<th>HbA2/ HbE</th>
<th>% PS</th>
<th>EXPOSURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011_N</td>
<td>normal</td>
<td>38 years</td>
<td>Male</td>
<td>0.6%</td>
<td>87.7%</td>
<td>2.7%</td>
<td>0.62</td>
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</tr>
<tr>
<td>2011_N1.</td>
<td>normal</td>
<td>28 years</td>
<td>Male</td>
<td>0.4%</td>
<td>88.1%</td>
<td>2.6%</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>2011_N2</td>
<td>normal</td>
<td>49 years</td>
<td>Female</td>
<td>0%</td>
<td>88.1%</td>
<td>2.5%</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>2012_N1</td>
<td>normal</td>
<td>26 years</td>
<td>Female</td>
<td>0.1%</td>
<td>87.5%</td>
<td>2.5%</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2012_N2</td>
<td>normal</td>
<td>26 years</td>
<td>Male</td>
<td>0.4%</td>
<td>89%</td>
<td>3.1%</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2012_N3</td>
<td>normal</td>
<td>26 years</td>
<td>Female</td>
<td>0.3%</td>
<td>88.3%</td>
<td>2.6%</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2011_Eβ</td>
<td>HbEβ thalassemia</td>
<td>27 years</td>
<td>Female</td>
<td>21.8%</td>
<td>3.2%</td>
<td>81.7%</td>
<td>3.39 %</td>
<td></td>
</tr>
<tr>
<td>2011_Eβ1</td>
<td>HbEβ thalassemia</td>
<td>3 years 6 months</td>
<td>Female</td>
<td>25.5%</td>
<td>3.3%</td>
<td>70.4%</td>
<td>3.6 %</td>
<td></td>
</tr>
<tr>
<td>2011_Eβ2</td>
<td>HbEβ thalassemia</td>
<td>11 months</td>
<td>Male</td>
<td>41.4%</td>
<td>6%</td>
<td>50.9%</td>
<td>3.9 %</td>
<td></td>
</tr>
<tr>
<td>2012_Eβ1</td>
<td>HbEβ thalassemia</td>
<td>6 years 6 months</td>
<td>Female</td>
<td>68.4%</td>
<td>0.7%</td>
<td>27.5%</td>
<td>3.11 %</td>
<td></td>
</tr>
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<td>2012_Eβ2</td>
<td>HbEβ thalassemia</td>
<td>7 years</td>
<td>Female</td>
<td>45.8%</td>
<td>3.2%</td>
<td>50.3%</td>
<td>8.45 %</td>
<td></td>
</tr>
<tr>
<td>2012_Eβ3</td>
<td>HbEβ thalassemia</td>
<td>4 years</td>
<td>Female</td>
<td>6%</td>
<td>47.2%</td>
<td>40.7%</td>
<td>4.5 %</td>
<td></td>
</tr>
</tbody>
</table>
obtained from Ramakrishna Mission Seva Pratishthan, with informed written consent following the guidelines of the Institutional Ethical Committee of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata 70026, INDIA and Institutional Animal & Bio ethics committee of Saha Institute of Nuclear Physics. The Institutional Ethical Committee of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, and Institutional Animal & Bio ethics committee of Saha Institute of Nuclear Physics have also specifically approved the current study.

ANNEXIN BINDING

Erythrocytes were labeled using FITC Annexin V Apoptosis Detection Kit (BD Biosciences). The erythrocytes were suspended in the buffer provided in the kit to a final concentration of $1 \times 10^6$ cells/ml and incubated with FITC-annexin. FITC-annexin binding was measured with respect to unlabeled samples. Flow cytometry of FITC-annexin labeled erythrocytes were performed in FACs caliber flow cytometer (Becton Dickinson). Acquisitions were taken following the protocol described in our previous work (9).

LIPID EXTRACTION

The plasma and erythrocytes were separated according to density. Erythrocytes were further lysed and the erythrocyte membranes were taken separately. Lipid extraction was done as described in (10). Briefly, to the plasma, and the erythrocyte membranes 1 mL of MTBE was added and the mixture was vortexed at 20°C for one hour. Then 250 µL of water was added and
thoroughly vortexed. After centrifuging for 1 minute at 4000 rpm 800 µL of the upper organic phase was transferred into a new vial and stored at -20°C until analysis. For mass spectrometric analysis, tenfold dilutions were made of the extract with CHCl₃/MeOH/2-propanol 1/2/4 (v/v/v) containing 7.5 Mm ammonium acetate in 96 well plate and then sealed with aluminum foil. The lipids of the two fractions were then analyzed with a LTQ Orbitrap XL mass spectrometer enabling to perform a lipidomics screen.

MASS SPECTROMETRIC ANALYSIS

Mass spectrometric analysis was performed on a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source using chips with 4.1 mm nozzle diameter. The ion source was controlled by chipsoft 6.4. software (Advion BioSciences) and operated at the ionization voltage of 0.95 kV and gas
pressure 1.25 psi. MS survey scans were acquired in positive and negative ion mode using the Orbitrap analyzer operated under the target mass resolution of 100,000 FWHM (Full Width at Half Maximum). Targeted MS\textsuperscript{n} experiments were performed using pulsed Q-dissociation (PQD) for positive ion mode and high energy collisional dissociation (HCD) for negative ion mode using the LTQ Orbitrap machine.

LIPID ANALYSIS

The raw data files acquired were converted to \*.mzXML format using MS converter from ProteoWizard \cite{11}. Mass spectra were further processed by Lipidxplorer software \cite{12} and lipids annotated by matching the m/z of their monoisotopic peaks to the corresponding elemental composition constraints using molecular fragmentation query language (mfql) (given in supplementary) \cite{13}. Peaks recognized in blank controls with an average intensity more than 0.1 fold compared to the averaged intensities of all the acquisitions for every lipid species were discarded. Further lipid species with less than 50% occupancy were also ignored in the analysis. The mfql for around 19 major lipid classes were used: cholesterol (chol), cholesteryl ester (chol-FA), ceramides (cer in positive mode, cer-adduct in negative ion mode), glucosylceramide (glyCer), diacylglycerides (DAGs), triacylglycerides (TAGs), glycerophospholipid diethers (GPL diethers), lysophosphatidylcholine (LPC), phosphatidylcholines (PC, PC-O), lysophosphatidylethanolamines (LPE), phosphatidylethanolamines (PE, PE-O), lysophosphatidic acid (LPA), phosphatidic acid (PA), lysophosphatidylinositol (LPI), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM).
For analyzing the oxidized lipids we have limited our search to the PCs only. From the list of PCs generated after lipidXplorer run as described above we have restricted the search for oxidized PCs to only those containing 14,16,18,20 and 22 fatty acid chains with a maximum of 8 double bonds in the parent PC. The oxidized species covered are the hydroxyl, keto, hydroperoxide, epoxy and polyhydroxy derivatives of PC as long chain oxidized PCs and saturated or unsaturated aldehyde and carboxylic acid derivatives of truncated PCs. After matching the m/z of their monoisotopic peaks to the corresponding elemental composition constraints using mfql (query files in the supplementary 1) and processed as discussed before.

STATISTICAL ANALYSIS

Hierarchical clustering (single linkage) study was done on the entire data set of the two fractions separately using Cluster 3.0 (14). The similarity protein expressions data was measured by correlation (centered). Further PCA was applied to the peak lists produced from all samples analyzed in each fraction separately using XLSTAT (version 2014.3.01) software.

4.3 RESULTS

CHARACTERISTIC OF THE SAMPLE POPULATION

The percentage PS exposure is measured in terms of percentage binding of annexin V – FITC to the exposed PS on the erythrocyte membranes. It was found to be greater in case of the diseased samples as compared to normal samples. This value for the normal was found to be 0.997 (±0.33) whereas for the disease it was found to be 4.49 (±2). This data shows that the
diseased samples taken in this study have a large percentage of erythrocytes with PS externalization.

![Scatter plot representing the percentage PS exposure of the samples.](image)

**Fig 4.2:** Scatter plot representing the percentage PS exposure of the samples.

**LIPID IDENTIFICATION**

A comprehensive characterization of the major abundant lipid classes was performed leading to the identification of around 260 lipids in total distributed among 19 lipid classes in the plasma, erythrocytes and erythrocyte membrane fractions on combining the lipids identified in the positive as well as the negative ion mode. Representative spectra of the plasma, erythrocyte and erythrocyte membrane fractions are shown in figure 4.3 A, B and C respectively.

In the plasma fractions, as evident from the spectra in positive ion mode (figure 4.3 A) lipids such as TAGs, chol esters, LPCs and PCs are the most intense. Greater amount of LPCs and TAGs species were detected in the plasma fractions as compared to the other two fractions. Cholesteryl esters and DAGs were only detected in the plasma fractions (figure 4.3 D).

Whereas, in case of erythrocyte and erythrocyte membrane fractions PCs, SMs and LPEs are the most intense (Figure 4.3 B, C, E and F).
Figure 4.3: A, B and C are the representative spectra of the plasma, erythrocyte and erythrocyte membrane fractions in the positive ion mode respectively. D, E and F are pie charts showing the distribution of the lipid populations in plasma, erythrocyte and erythrocyte membrane respectively.

Amongst the PCs the species PC (34:2) was the most abundant in the three fractions. Cholesteryl ester (18:2) was the most abundant in the plasma. LPCs 16:0 and 18:0 were the major LPCs in all three fractions. In case of LPE, species 20:4 is the most abundant in the erythrocyte and erythrocyte membrane. In case of PI species 38:4 is the most abundant in all three fractions with PI (36:2) being the second abundant in case of plasma. PS (38:4) is the most abundant in the erythrocytes and erythrocyte membrane fractions. SM (34:1) and SM (42:2) being the most abundant in all three fractions.
Clustering studies clearly show that the lipid populations of normal and Eβ thalassemic samples show changes in the lipid levels in the three fractions (Fig 4.4 A, B and C). However the changes observed in the erythrocyte fraction does not clearly indicate the difference between the normal and diseased samples, but the plasma and erythrocyte membrane fraction clearly show the separate clustering of the normal and diseased samples in the figure.

**Figure 4.4: Hierarchical clustering (single linkage) using Cluster 3.0 shows the changes in the level of lipid species in diseased condition with respect to normal (A, B and C) in the three fractions.**
PLASMA

The primary function of plasma is transportation of the blood cells as well as nutrients, hormones, proteins and lipids. Hence, lipids which act as secondary messengers such as ceramides (Fig 4.6 A) and lyophospholipids (LPE and LPC) (Fig 4.6 C) show changes in their levels in case of diseased samples. Ceramides and LPEs show an increase in case of Eβ thalassemia whereas LPCs show a decrease (Fig 4.5 A and C). Phosphatidylcholines (Fig 4.6 D) and sphingomyelins (Fig 4.6 B) show a decrease; however PC-Os (Fig 4.6 E) show an increase in case of the diseased plasma samples. The PCA plots of all the lipid classes are shown in figure 4.5 (A – H) in the next page.

Figure 4.6: Bar plot showing the differential expression of representative lipids of cer (A), SMs (B), LPCs (C), PCs (D) and PC-Os (E) of the plasma fraction.
Figure 4.5: A – H PCA analysis of plasma samples of lipid classes Cer and SMs (A), TAGs and DAGs (B), and LPCs and LPEs (C), PCs and PC-Os (D), PEs and PE-Os (E), PI (F), PS (G), oxidized lipids (H).
ERYTHROCYTES

PCA analysis of the erythrocyte fractions did not reveal any change in pattern between the normal and diseased samples (Fig. 4.7). This might be due to some sort of contamination which might have occurred during the separation of the whole erythrocyte fraction from the blood. However the on comparing few representative lipids (Fig 4.8) of each classes shows that PCs and the PC-Os follows the same pattern as described in the plasma fraction and erythrocyte membrane fraction.

Figure 4.8: Bar plot showing the differential expression of representative lipids of cer (A), SMs (B), LPCs (C), PCs (D) and PC-Os (E) of the erythrocyte fraction.
Figure 4.7: A – H PCA analysis of erythrocyte samples of lipid classes Cer and SMs (A), PAs (B), and LPCs and LPEs (C), PCs and PC-Os (D), PEs and PE-Os (E), PI (F), PS (G), oxidized lipids (H).
ERYTHROCYTES MEMBRANE

From our annexin V binding study we have already seen that a huge percentage of erythrocytes are proapoptotic. This can be initiated by the huge oxidative stress that the erythrocyte population has to undergo. Therefore we see an increase in ceramides (Fig 4.10 A) and ethers of PC (PC-O) (Fig 4.10 E), whereas a decrease in case of the sphingomyelins (Fig 4.10 B), lysophospholipids (LPE and LPC) (Fig 4.9 C and 4.10 C) and PCs (Fig 4.10 D). The PCA plot for each lipid class is shown in figure 4.9 in the next page.

Figure 4.10: Bar plot showing the differential expression of representative lipids of cer (A), SMs (B), LPCs (C), PCs (D) and PC-Os (E) of the erythrocyte membrane fraction.
Figure 4.9: A-H PCA analysis of erythrocyte membrane samples of lipid classes Cer and SMs (A), PAs (B), and LPCs and LPEs (C), PCs and PC-Os (D), PEs and PE-Os (E), PI (F), PS (G), oxidized lipids (H).
On looking into the oxidized lipid populations the saturated and unsaturated aldehydes and carboxylic acid derivatives of truncated PCs show an increase in the plasma and erythrocyte membrane fractions but the number of these species detected in the plasma fraction “(Fig 4.5 H) is much less compared to that of the other fraction (Fig 4.9 H). The hydroxyl, keto, hydroperoxide, epoxy and polyhydroxy derivatives of long chain oxidized PCs were also detected in huge amounts in both the fractions, but a consistent increase in the Eβ thalassemic samples were observed only in case of erythrocyte membrane fraction.

4.4 DISCUSSION

The lipids identified so far are the major lipids in the eukaryotic system. As we know that the lipid population is tissue specific, hence the population and their abundance are unique to our sample type. The most abundant lipid species reported here also correlates with earlier studies. We also see a difference between the populations of lipids in the plasma and erythrocyte fractions. This difference is due to the fact that the plasma fractions derive lipids from the diet and the lipid population is very sensitive to dietary changes.

It has already been reported in many studies that the most common fatty acid (FA) in animals, plants and microorganisms is palmitic acid (16:0). Stearic acid (18:0) is a major FA in animals. Amongst the unsaturated FA Oleic acid (18:1; ω – 9) is the common monoenoic acid in plants and animals. Linolenic acid (18:2; ω – 6) is a major FA derived from dietary plant oils in animals. Arachidonic acid (20:4; ω – 6) is a major component of the membrane phospholipids throughout the animal kingdom, but very little is found in the diet. Therefore we see an abundance of phospholipids composed of such fatty acid chains, such as PC (34:2) was the most
abundant in the three fractions. Cholesteryl ester (18:2) was the most abundant in the plasma. LPCs 16:0 and 18:0 and LPE 20:4, PI (38:4) were the most abundant. PS (38:4) is the most abundant in the erythrocytes and erythrocyte membrane fractions. SM (34:1) and SM (42:2) being the most abundant in all three fractions.

It has been reported that in diseased conditions mature erythrocytes undergo rapid self destruction process leading to increased intracellular calcium content, reduced deformability, metabolic disruption, membrane protein modification and PS externalization (17). The increase in ceramide with a decrease in sphingomyelin and lysophospholipids in erythrocytes indicate a regulative role of lysophospholipids and ceramides for cell-to-cell communication during premature eryptosis.

CERAMIDES AND SPHINGOMYELINS

Ceramides have been implicated in suicidal death of erythrocytes and is greatly studied in cancer therapy (18-20). The increase in ceramides and a decrease in sphingomyelins clearly indicate the activation of the sphingomyelinase which is responsible for the hydrolysis of sphingomyelin to ceramides (21). This sphingomyelinase may be expressed in the plasma membrane of erythrocytes or released into the plasma (19).

Another potential source of ceramides is the reversal of synthesis. Sphingomyelin synthase converts diacylglycerols and sphingomyelin to ceramide and phosphatidylcholine. Furthermore, the ratios, PC/SM and DAG/Cer are said to be intrinsically related and may have a role in cross-talk between glycerolipids and sphingolipid signaling (19, 22).
In our case the plausible explanation is that a phospholipase mediated release of platelet-activating factor (PAF) stimulates sphingomyelinase which in turn converts sphingolipids to ceramides and choline \(^{(20)}\). Simultaneously the oxidative stress activates the Ca\(^{2+}\) cation channels, thus increasing the Ca\(^{2+}\) levels. The concerted effect of elevation of ceramides and Ca\(^{2+}\) levels play a role in PS externalization.

It has also been reported that when ceramides increase in large quantities they can also affect membrane physical properties such as deformability. Ceramides displace cholesterol from lipid umbrellas and drive its esterification \(^{(23)}\).

**LYSOPHOSPHOLIPIDS**

Signaling induced hydrolysis of glycerophospholipids lead to the formation of lysophospholipids. Lysophospholipids are also generated due to the oxidation of phospholipid ethers \(^{(1)}\). These lysophospholipids are known to carry only one aliphatic chain and hence readily leaves the plasma membrane. This explains why we have seen an increase of LPEs in the plasma fraction but a decrease in the erythrocyte membrane fractions (Fig 3). The lysophospholipids act as messenger lipids and act through membrane receptors in contrast to ceramides which remain in the membrane and employs cytosolic proteins for signaling \(^{(23)}\).

However we see a decrease in case of LPCs in both the fractions. This can be correlated to the fact that the PCs are undergoing extensive oxidation. Therefore there is a tremendous pressure to maintain the PC levels in both the fractions and hence the LPCs are being continuously used up showing a decrease in their levels.
PHOSPHATIDYLCHOLINE AND ITS ETHER

Phosphatidylcholines are the most abundant glycerophospholipids present in the eukaryotic system accounting for more than 50% of the total membrane lipids \(^{(23)}\). There have been a lot of reports on the identification of oxidized products of PCs in a number of diseases \(^{(1, 8, 24, 25)}\). Being the most abundant it is greatly exposed to reactive oxygen species (ROS) and is easily oxidized. Thus there is a decrease in the levels of intact PCs in all three cases, however in case of the erythrocyte and erythrocyte membrane fractions the changes were not so pronounced compared to the plasma fraction. This might be due to the increased inward movement of PCs in the thalassemic erythrocytes \(^{(26)}\). Intact PCs have other than making up the major bulk of the membrane also acts as the biosynthetic precursor for lipids such as SMs \(^{(27, 28)}\), LPCs \(^{(29)}\), PAs \(^{(30)}\) and as well as PSs \(^{(28)}\) (Fig 4.11). Hence with an increase in Cer, LPCs and oxidized PCs (as discussed later) and a decrease in sphingomyline followed by PS externalization it is inevitable that the level of PCs decreases.

Contrary to earlier findings in case of neurological diseases or metabolic and inflammatory disorder, the levels of ethers of PCs in our case show an increase. However, long chain ethers species containing arachidonic acid (20:4) or docosahexanoic acid (22:6) shows a decrease. The phospholipid ethers have very short life span, about 30mins in case of PC ethers. Hence the populations of PC ethers are greatly adapted to changing environmental conditions. Phospholipid ethers are reported to have antioxidant function as well as they act as reservoirs of long chain fatty acids especially arachidonic acids \(^{(31)}\). Arachidonic acid in turns acts as precursor in the biosynthetic pathways of all lipids. Hence alteration in these ethers not only indicates the change in the oxidative state but also alters other lipid metabolism. Ethers also give rise to a
Figure 4.11: Schematic description of the role that phosphatidylcholine plays in the biosynthesis of other lipid classes.

number of second messengers in the signaling cascades \(^{(1, 31)}\). Keeping all this in mind we can assume that the erythrocytes which show an elevation of ether population are the ones battling against the oxidative stress that is induced in the diseased condition and the ones who could not synthesize the ethers as per demand are the ones who have lost the battle and perished. Hence we can only see those erythrocytes who have survived the test. In other words the elevation of the ethers levels might denote the defense mechanism of the body.

OXIDISED PHOSPHATIDYLCHOLINES

We have also observed an increase in the levels of oxidized PCs. There was a huge amount of long-chain products, such as hydroxy and/or hydroperoxide derivatives in both the
fractions in normal as well as diseased condition. As reported in other studies \(^{(32)}\), we also detected polyhydroxy derivatives which have been generated in case of polyunsaturated acyl chains where the addition of oxygen molecules occurred several times. The increase that we observe in case of the thalassemic samples reinforces our claim of a condition of extreme oxidative stress. We have also shown an increase in the population of aldehyde and carboxylic derivatives of the truncated PCs. However, compared to the erythrocyte membrane fractions the plasma fractions did not show very consistent changes. This might be due to the fact that the susceptibility of lipid peroxidation alters as the lipid environment changes as well as the oxidants present \(^{(32, 33)}\). The increase in the oxidized species population further explains the decrease in the level of PCs.

### 4.5 CONCLUSION

The premature eryptosis leading to acute anemia is one of the key pathological features of Eβ thalassemia. The pathophysiological symptoms observed in the diseased condition can be linked to the changes observed in the lipid population that have been reported in this study, which in turn can be due to the increase in reactive oxygen species (ROS) as shown in Fig 4.12. The changes in the lipidome combined with our already vast knowledge of the changes in the proteome in plasma as well as the erythrocytes may help us discover new insights so as to prolong the survival of the diseased erythrocytes. A detailed quantitative study of these changes might enable a “fingerprinting” approach towards a better understanding of the disease. This area has a huge potential in the therapeutic level as well as diagnostic level of this disease.
Figure 4.12: Schematic representation of the effect of ROS on the levels of lipid classes and the physiological changes that occur in turn due to the changes in their levels.
4.6 REFERENCE


