INTRODUCTION

Free radical-induced oxidative damage has been implicated in various clinical diseases [1], including kidney injury. Oxidative stress is described as an imbalance between the oxidation and antioxidation processes resulting in highly reactive molecules, primarily reactive oxygen species, being produced and cleared (ROS). According to studies, children with nephrotic syndrome experience oxidative stress (NS) [2]. The albumin protein mediates the majority of the antioxidant activity in plasma and serum. Human serum albumin was responsible for 70% of the free radical trapping activity (HSA) [3]. Renal perfusion decreases due to a reduction in intravascular volume. As a consequence, the glomerular filtration rate (GFR) is reduced. One of the investigations of renal failure in various clinical diseases [1], including kidney injury. Oxidative stress in NS children. Excessive production of reactive oxygen species (ROS) associated with kidney injury.

MATERIALS AND METHODS

Study Participants: This research was carried out on nephrotic syndrome patients in India. The participants were split into two groups: (A) Children suffering from Nephrotic syndrome (B) Individuals who are healthy (Age: 2-14 y). Inclusion criteria: Renal insufficiency was defined as a glomerular filtration rate (GFR) less than 80 ml/min, a urine protein excretion larger than 0.05 g/kg per 24 h, and serum albumin less than 25 g/l. NS patients diagnosed with other systemic disorders were excluded from this study. Pediatric nephrologists made the diagnosis of NS at the authorized center of Muljibhai Patel Urological Hospital. The Institutional Ethics Committee of G. J. Patel Ayurvedic College, Vallabh Vidyanagar, Gujarat, approved this study (Approval No–IEC-4/GJPM/2017-18/08). Informed consent forms were signed before performing the experiments.

Sample collection

Blood samples were collected in a vacutainer serum tube from Muljibhai Patel Urological Hospital Nadiad. After taking a blood sample, the serum was isolated from the blood and centrifuged for 10 min at 3200-3500 rpm; to obtain the red blood cells. From the fresh sample, oxidative stress markers like MDA, SOD, and GSH were estimated. Malondialdehyde (MDA) level in patients was checked using the buege method [12]. Superoxide dismutase (SOD) activity from blood serum was checked using the pyrogallol method [13]. Glutathione level in blood was measured using jollow’s method [14].
Estimation of GSH

Jollow’s methods were used to test blood glutathione. 200 µl of blood, 0.8 ml of D/W, and 0.5 ml of 10% Sulfosalicylic acid were mixed. The mixture was vortexed and centrifuged @ 3000rpm for 10 min; 0.5 ml of supernatant was added in 4.5 ml of 0.5M (pH 8.23) Tris phosphate buffer and 0.5 ml of 5,5-Dithiobis (2-nitro benzoic acid). The reaction mixture formed yellow color, which was read at 412 nm.

Estimation of MDA

1 ml serum was treated with 2 ml TCA-TBA-HCL and thoroughly mixed. The reaction solution was heated for 15 min and then centrifuged for 10 min at 1000g. The coloring complex developed in the supernatant was measured at 535 nm against a blank. Blank included all the reagents except the serum sample.

Estimation of SOD

The inhibition of pyrogallol autoxidation was used to determine SOD activity in serum samples, defined by Marklund [13]. 1 ml serum sample, 0.1 ml pyrogallol reagent, and 1.9 ml tris buffer were mixed. After a 90-second induction time, the inhibition of pyrogallol was calculated every 30 seconds for 3 min at a wavelength of 420 nm.

Statistical analysis

SPSS version 21.0 was used to conduct the statistical analysis. Comparison between control and nephrotic syndrome group was done by student t-test.

RESULTS

There is no noticeable difference in age between NS patients and controls. However, the NS group had considerably lower serum albumin and creatinine levels than the control group (p<0.05), the NS group had a higher UPCR ratio (p<0.05). On the other hand, the serum cholesterol level was normal in both groups (table 1).

MDA levels in the serum were substantially higher in NS patients than controls (P<0.05). The activity of SOD was markedly higher in control patients than in NS patients (p<0.05). GSH concentrations were substantially lower in NS patients than in the control group (p<0.05) (table 2). In patients with NS, a significant positive relationship between SOD activity and serum albumin and creatinine levels was observed (P<0.05). No significant association was seen when serum albumin and creatinine levels were compared to MDA and GSH levels (p>0.05) (fig. 1, 2, 3).
The glomerular basement membrane is degraded by superoxide-mediated oxidative damage, and denovo proteoglycans synthesis is reduced [15]. The Glomerular Filtration Barrier (GBF) is negatively charged by heparan sulfate, a proteoglycan, and thus repels the transport of negatively charged molecules. Oxidative damage to heparan sulfate may lead to increased glomerular permeability. Therefore, glomerular injury due to ROS may be responsible for the pathogenesis of glomerular diseases [16]. Clinical data of the participants suggest that creatinine clearance is helpful in clinical practice to measure glomerular filtration rate, and decline in serum albumin level indicates reduced antioxidant level. Urine protein/Creatinine ratio and serum cholesterol level are significantly different in both groups. Many clinical disorders are caused due to oxidative damage by free radicals. Lipid peroxidation enhances protein permeability in the glomerulus and impairs the structural integrity of tubular epithelial cells due to the formation of reactive oxygen species (ROS). It is also reported that ROS is responsible for the pathogenesis of childhood nephrotic syndrome [17]. One of the possible factors which are responsible for damage in DNA is ROS. The rapid injury and damage to DNA caused by ROS result in proteinuria and subsequent glomerulosclerosis [18]. Many illnesses, including cancer, heart disease, diabetes, aging, liver, kidney, and lung disorders, are influenced by oxidative stress [19].

### Table 1: Clinical characteristic of selected participants

| Parameters                     | Control               | Nephrotic syndrome | p-Value |
|-------------------------------|-----------------------|--------------------|---------|
| Age                           | 8.72±4.81             | 7.12±4.37          | <0.05   |
| S/P Creatinine mg/dl          | 1.099±0.66            | 0.467±0.048        | <0.05   |
| S/P Serum Albumin g/dl        | 4.459±0.09            | 2.916±0.133        | <0.05   |
| Urine protein/Creatinine ratio | 0.2518±0.028          | 3.160±0.657        | <0.05   |
| S/P Cholesterol mg/dl         | 116.725±6.954         | 91.733±1.726       | <0.05   |

S. Serum; P. Protein; p-Value=0.05 considered as significant; Data were in mean±SD. Significant value for student t-test was considered<0.005 (p-value).

The assessment of lipid peroxidation status is an essential criterion in determining the degree of oxidative stress. Studies have indicated that free radical lipid peroxidation can harm the cell membrane and create enzymatic and receptor binding membrane activity abnormalities, resulting in damage to the organ [20]. The majority of phospholipids on the membrane are readily oxidized, resulting in lipid peroxide, which decomposes to aldehyde like MDA [21]. Our investigation discovered that the NS group had a significantly higher MDA level than the control group. Similarly, another study revealed a rise in the MDA level in NS compared to the control group [6-7, 9]. Turi et al. reported that eight children with post-streptococcal glomerulonephritits in the acute stage had significantly increased plasma MDA and decreased erythrocyte SOD activity compared to healthy controls [22]. Additionally, Templar et al. [23] demonstrated that patients with chronic renal failure due to chronic glomerulopathies had significantly higher plasma MDA levels than those with non-glomerular diseases, implicating a role for oxidative stress in the pathogenesis of glomerular injury [23]. Another study discovered a fourfold increase in the amount of MDA in the blood [24]. The superoxide radical produced by active neutrophils is required for the neutrophil-mediated acute inflammatory response, which results in severe tissue damage [25]. Superoxide dismutase has been found as a molecule that acts as an anti-inflammatory and anti-superoxide scavenger [26]. Bulbul et al. discovered that the antioxidant enzyme SOD had a similar activity at disease start, during remission, and in control participants [27]. SOD is also expected to increase in response to excessive oxidative damage, compensating for and protecting tissue damage [28]. Thus, the absence of an increase in acute stage SOD activity could result from oxidative stress-induced depletion, as demonstrated by increasing MDA levels.

Glutathione is the most potent antioxidant that is found in every cell [29]. It is a thiol-containing tripeptide found in high concentrations in live cells in its reduced form (GSH). When it comes into contact with reactive oxygen species (ROS), it is oxidized to glutathione radical, regenerated to its reduced form via glutathione reductase activity. We noticed a significant decrease in GSH concentrations in the patients’ group, ascribed to increased reactive oxygen species generation, converting the reduced form to the oxidized form (GSSH). Glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase all use GSH as a substrate (GRx). The decrease in concentrations may be due to the increased turnover of GSH required to prevent oxidative damage under these
conditions [30]. Previously, similar discoveries of lower GSH concentrations in nephrotic syndrome were documented [31], implying that nephrotic syndrome is related to increased oxidative stress. In contrast, nephrotic syndrome has been associated with unchanged erythrocyte GSH concentrations [6].

Our antioxidant and oxidant status results were well supported by a study done by the reddy's group in 2016. They found a significantly increased level of MDA and decreased the level of SOD activity and GSH in NS patients [8]. In contrast, another study found unaltered GSH concentrations and increased SOD concentration in children with NS [6, 17]. We observed a statistically significant positive association between serum creatinine and SOD activity and serum albumin and SOD activity (fig. 1). Similarly, Mistry et al. also reported a positive correlation of SOD activity in Diabetic nephropathy [32]. The earlier study found that SOD is a responsible and significant mediator of the podocyte damage, causing glomerular injury influenced by purmocin and involved in many other diseases [33]. As a result, SOD activity is one of the most potent stress markers in diagnosing renal disease, including NS. No significant correlations were found in MDA and GSH levels compared with serum albumin and serum creatinine (fig. 2,3).

Table 2: Oxidative stress markers in the control and patients

| Parameters | Control | Nephrotic syndrome | % change | p-Value |
|------------|---------|--------------------|----------|---------|
| MDA (µM/ml) | 3.11±0.03 | 4.28±0.03 | +137.38 | <0.05 |
| GSH (µg/ml) | 239.86±0.01 | 175.12±0.02 | -73 | <0.05 |
| SOD (U/ml) | 1.64±0.17 | 1.09±0.20 | -66.58 | <0.05 |

Data were presented in mean±SD

In this investigation, the lack of association of MDA and GSH with serum albumin and creatinine levels indicated that these oxidative stress markers were not predominantly generated by renal insufficiency in NS. The antioxidative defense mechanism of superoxide dismutase regulates reactive oxygen species (ROS) (O2-). It is one of the most effective antioxidant defenses in all cells exposed to oxygen and plays a critical role in the body’s antioxidant defenses [34]. Thus, elevated levels of oxidative stress and decreased antioxidant defense may be related to the development of NS. Numerous studies have established that oxidative stress plays a role in developing various diseases, including NS. As a result, excessive ROS production may contribute to the progression of renal disease [31]. Thus, determining the amount of oxidative stress produced is critical for NS prediction. In vitro investigations have revealed a crucial role in oxidative stress in the development of NS [35]. The oxidation-reduction reaction of ROS can generate protein carboxylation, DNA damage and cytoskeleton disruption. The cell damage influence by ROS hampers the glomerular permeability to proteins and disorganizes the tubular epithelial cells’ integrity, eventually results in NS [17]. The limitation of this study is the sample size, so additional research with larger sample size is required to properly analyze the findings reported here. This study requires a larger sample size to determine the etiology of NS in children.

CONCLUSION

The current study's findings state that the NS group has significantly greater MDA levels and lower SOD and GSH levels, and as a result, they have higher oxidative stress. Furthermore, a positive correlation was identified in SOD activity compared with serum albumin and serum creatinine level, implying that oxidative stress defined by diminished antioxidant potentials due to hypoalbuminemia exists in children with NS. Thus, oxidative stress has a role in the development of NS in children. However, the antioxidant defense mechanisms of the body, such as SOD, GSH, and serum albumin, may attempt to alleviate this damage. Additional research involving a greater number of patients is necessary to explore the findings presented here.

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AUTHORS CONTRIBUTIONS

Conceptualization: Kinnari N. Mistry, Sishir Gang

Methodology: Glory S. Parmar, Kinnari N. Mistry, Sishir Gang

Formal analysis and investigation: Glory S. Parmar, Kinnari N. Mistry, Sishir Gang

Writing-original draft preparation: Glory S. Parmar

Writing-Review and editing: Kinnari N. Mistry, Sishir Gang

Resources: Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (affiliated to Sardar Patel University) Vallabh Vidyanagar, 388121, Anand, Gujarat, India; Department of Nephrology, Muljibhai Patel Urological Hospital, Dr. V. V. Desai Road, Nadiad,387001, Gujarat, India; Anand Agricultural University, Anand 388110, Gujarat, India.

Supervision: Kinnari N. Mistry.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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