CHANGES OF OXIDATIVE STRESS AND SERUM ISCHEMIC MODIFIED PROTEIN LEVELS IN PATIENTS WITH CARDIAC ARREST AFTER CARDIOPULMONARY RESUSCITATION

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ABSTRACT

Objective: *Clarify the change rule of oxidative stress index and ischemic modification protein content in patients with cardiac arrest after cardiopulmonary resuscitation and determine the effective prognostic indicators of patients with cardiac arrest after cardiopulmonary resuscitation.*

Methods: *By collecting blood from patients with cardiac arrest treated with CPR and normal people, the oxidative stress indicators in the blood pool of the two groups are measured, including the activity levels of malondialdehyde, superoxide dismutase and glutathione peroxidase, and the changes of serum oxidative stress index between the groups are analyzed. The normal population is used as a control to detect changes in IMA levels in patients who underwent CPR-treated cardiac arrest to achieve main circulation recovery and those who did not achieve spontaneous circulation recovery.*

Results: *In healthy people, the MDA, SOD and GSH-Px levels in serum are normally distributed. One-way ANOVA shows that the mean MDA levels in the serum of group A1 and group A2 are higher than those of the control group, and both reach statistically significant differences (P<0.05), but there is no statistical difference between group A1 and group A2 (P>0.05); the levels of SOD activity and the average activity level of GSH-Px in serum of group A1 and group A2 are significantly lower than those of normal control group (P<0.05). The IMA level in the serum of group A2 is higher than that of group A1 and normal control group, and there is statistical difference (P<0.05), while the level of IMA in serum of group A1 is higher than that of normal control group. There is also a statistical difference (P < 0.05).*

Conclusion: *The oxidative stress index in serum can objectively reflect the damage of tissues and organs in patients with cardiac arrest after CPR. The change of IMA level can effectively reflect the ischemic state of tissues and organs in patients with cardiac arrest, and the level of IMA in serum has a certain reference value for effective prognosis of patients with CPR.*

Keywords: *Cardiac arrest, cardiopulmonary resuscitation, oxidative stress, ischemic modified protein.*

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Introduction

Cardiac arrest refers to the sudden termination of cardiac ejection function due to the combined effects of various diseases in the body[1]. Common pathophysiological mechanisms of cardiac arrest include rapid ventricular arrhythmia, slow arrhythmia, ventricular pause or pulseless electrical activity[2]. The current technical aspects of the treatment of cardiac arrest are mainly cardiopulmonary resuscitation (CPR).

Cardiac arrest is a pathophysiological process of ischemia and hypoxia in the whole body. The process of CPR reaching the ROSC in patients with cardiac arrest is actually the process of systemic organ reperfusion. It is currently believed that tissue damage or irreversible damage occurs after reperfusion of ischemic organs. This is called ischemia reperfusion injury (IRI). After ischemia and reperfusion, a large number of reactive oxygen species (ROS) are produced, which cause severe oxidative stress[3]. Within a few minutes of cardiac arrest, significant oxidative stress occurs before and after normal oxygen supply and circulation. These oxidants may affect defibrillation success, tissue survival, and cranial nerve rehabilitation after CPR[4].

Therefore, by understanding the level of oxidative stress in patients with cardiac arrest, the severity of the body's IRI can be reflected.

Malonaldehyde (MDA) is an important lipid peroxidation product that reflects the degree of cellular oxidative stress damage and is elevated during oxidative stress[5]. Superoxide dismutase (SOD) and Glutathione peroxidase (GSH-Px) are antioxidant enzymes of the body, which can effectively remove free radicals generated by the body and is reduced in the event of oxidative stress damage in the body[6]. By studying the levels of MDA, SOD and GSH-Px, the level of oxidative stress in the body can be inferred. To investigate the protective effects of anisodamine on oxidative stress and myocardial ischemia/reperfusion (I/R) injury, Hackenhaar et al. observed the changes of anisodamine on plasma, myocardial oxidation and antioxidant markers in patients with oxidative stress, and the results showed that anisodamine can effectively reduce the MDA content of cardiopulmonary resuscitation in patients with cardiac arrest, and it has a certain protective effect on its cell function[7]. At present, there are few reports on the level of oxidative stress in patients with cardiac arrest after CPR. Therefore, it is meaningful to study the oxidative stress response in patients with cardiac arrest after CPR.

Ischemia modified albumin (IMA) is produced by human serum albumin as it passes through an ischemic organ. Especially in myocardial ischemia, IMA level increases rapidly, and with the prolongation of ischemia time, it continues to increase, which is a sensitive biochemical indicator reflecting myocardial ischemia[8]. Studies have shown that IMA increases in the first few minutes after ischemia, and continues to increase in 12 hours, and then falls to normal levels after 24 hours[9]. The clinical significance of detecting IMA is mainly to diagnose acute myocardial ischemia at an early stage and to have a high negative predictive value for acute coronary syndrome (ACS). There are few reports on changes in IMA levels in serum after CPR in patients with cardiac arrest. Ruivo et al [10] found that patients with cardiac arrest after CPR had higher IMA levels in the GOS<3 group than in the GOS≥3 group and the control group. And the IMA level of the GOS≥3 group is compared with the control group by single factor analysis, and it is found that there is no significant difference between the two groups. The sensitivity and specificity of prognosis in patients with cardiac arrest after CPR are predicted by IMA in serum. The results are 65.8070 and 78.6070, respectively. Therefore, IMA is likely to be a valuable predictor of prognosis in patients with cardiac arrest. Other studies in experimental animals with cardiac arrest after CPR shows that the mean IMA level in serum in ROSC group is significantly lower than those in the unrecovered spontaneous circulation group ($P<0.001$).

In summary, oxidative stress indicators and IMA levels can effectively reflect the cellular function level of patients with cardiac arrest and the ischemic state of tissues. Clinical studies of changes in oxidative responses and changes in IMA in patients with cardiac arrest after CPR have not been reported. Therefore, by comparing the changes in IMA levels in the serum of oxidative stress indicators in patients with cardiac arrest after CPR and in the normal population, it is possible to use IMA as an indicator to provide scientific reference of effective prognosis for patients with cardiac arrest after CPR.

Materials and methods

Experimental material

The trial was conducted in The Affiliated Hospital of Hangzhou Normal University from October 2015 to February 2017. Thirty-six patients (aged ≥ 18 years old) who met the criteria for cardiac arrest were selected as the experimental group (group A) for cardiopulmonary resuscitation. Patients who were able to achieve spontaneous circulation recovery were included in the group A1, a total of 15 cases, including 11 males and 4 females, with an average age of 56.1 years; patients who failed to achieve spontaneous circulation recovery were included in the group A2, a total of 21 cases, including 14 males and 7 females, with an average age of 69.1 years. A group of healthy people with the same age and sex of the test group were selected as the healthy control group (group H), a total of 60 cases, including 39 males and 21 females, with an average age of 59.3.

After CPR in all the patients with cardiac arrest in the experimental group, 3 to 5 mL of the femoral vein blood of the patient was collected by the operation method of vacuum negative pressure blood collection tube within 5 min and placed in a blood collection tube containing a coagulant. Continuously reverse 2 to 3 times, so that the blood was in contact with the vessel wall. Then, it was allowed to stand for 30 minutes. After the blood was completely coagulated, it was centrifuged at 3000 r/min for 10 min in a low temperature centrifuge

(EPPENDORF, USA). The supernatant was serum and stored in a refrigerator at -80 °C until use. Informed consent was signed for all subjects in the study, and the study was approved by the ethics committee of The Affiliated Hospital of Hangzhou Normal University.

Experimental methods

Determination of oxidative stress indicators

Determination of malondialdehyde (MDA) content: add 0.1 ml of blood sample to the reaction system of the kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China), mix well, put it into a constant temperature water bath at 95 °C for 40 min, remove it, and stop the reaction by running water to cool down. Then, it is centrifuged at 4000 r/min for 10 min, and the supernatant is taken to measure the OD value at a wavelength of 532 nm. At the same time, the OD values of the blank tube, the standard tube and the control tube are measured, wherein the blank tube is changed to add anhydrous ethanol, the standard tube is changed to the standard product (concentration of l0 nmol/ml), the control tube is not added with the coloring agent, and then the MDA level concentration is calculated according to the following formula.

$$\text{Serum MDA levels } \left(\frac{\text{nmol}}{\text{ml}}\right) = \frac{(\text{Measurement tube OD value - control tube OD value})}{(\text{Standard tube OD value - blank tube OD value})} \times \text{Standard concentration} \times \text{Dilution factor}$$

Determination of superoxide dismutase (SOD) activity: take 50μl of blood for measurement, add it to the reaction system of the kit, mix well, the blood is subjected to a water bath in a constant temperature water bath at 37 °C for 40 min, take it out, and then mix with a color developer (provided by the kit), and allow it to stand at room temperature (21-25 °C) for 10 min, and the OD value is measured at a wavelength of 550 nm. The OD value of the control tube is also measured, and the control tube is changed to 50 μl of distilled water. The SOD activity of the serum to be tested is then calculated according to the following formula. When the inhibition rate of SOD per ml of the reaction solution reaches 50%, the corresponding amount of SOD is one SOD activity unit (Y).

$$\text{Serum SOD activity (U/ml)} = \frac{(\text{Measurement tube OD value} - \text{control tube OD value})}{(\text{Control tube OD value})} \div 50\% \times \text{Reaction system dilution factor}$$

Determination of glutathione peroxidase (GSH-Px) activity: take the diluted sample 0.l ml, add it to the kit enzymatic reaction system, mix well, water bath in a constant temperature water bath at 37 °C for 10 min, take out and mix thoroughly, centrifuge at 4000 r/min for 10 min, and 1 ml of the supernatant is taken for color reaction, and the OD value is measured at a wavelength of 412 nm. At the same time, the OD value of the non-enzymatic tube, the blank tube and the control tube is determined, wherein the non-enzymatic tube is added with an equal amount of diluted sample after the end of the enzymatic reaction to make up the reaction dose; in the color reaction, the blank tube is changed to 1 ml of GSH standard solvent application solution, and the standard tube is changed to 1 ml of GSH standard product. The activity of the blood sample GSH-Px is then calculated according to the following formula. The GSH concentration in the reaction system is decreased by 1 umol/L per 0.1 ml of serum as one unit of enzyme activity (U).

$$\text{Serum GSH} - \text{Px activity } \left(\frac{\text{U}}{\text{ml}}\right) = \frac{(\text{Non} - \text{enzymatic tube OD value} - \text{Enzyme tube OD value})}{(\text{Standard tube OD value} - \text{Blank tube OD value})} \times \text{Standard product concentration} \times \text{Reaction dilution factor}$$

Detection of ischemic modified albumin (IMA)

Take 45 μl of serum to be tested, add to the reaction system of the kit, mix well, let stand for 5 min at room temperature, then add the color developer (provided by the kit), mix and let stand at room temperature for 10 min. The resulting reaction solution is placed in an OET-N10 specific protein analyzer (Beijing Share-Sun OET. CO., LTD., Beijing, China) for colorimetry (light path is lcm, wavelength is 650 nm), and serum IMA values are directly read.

The serum samples are replaced by IMA standard products (Shanghai weiyin biotechnology co. LTD, Shanghai, China) of 50 U/ml, 80 U/ml, l05 U/ml, 130 U/ml, and 145 U/ml, respectively, and the OD values are measured to prepare a standard curve. Perform a linear analysis of the IMA kit.

The concentration of IMA in the serum of the sample is determined by the above IMA test kit (Shanghai weiyin biotechnology co. LTD, Shanghai, China), and the Cut off is analyzed by Receiver operating characteristic curve (ROC curve), and the result is determined by the positive value of Cut off.

Statistical methods

SPSS 18.0 software is used for statistical analysis of the data. The indicators of oxidative stress detection are expressed by the average value. The data of each block are tested by the single factor analysis method. The test level α is 0.05. The reference range of serum IMA in the population is analyzed, and the 95% reference interval on the left side is calculated. P<0.05 is used as the statistical difference. Serum IMA measurements after CPR in patients with cardiac arrest are analyzed by ROC curve and the area under the ROC curve is calculated.

Results

Effects of different genders on oxidative stress index in blood of healthy people

It can be concluded from Figure 1 that the normality test of MDA, SOD and GSH-Px in serum shows that the MDA, SOD and GSH-Px levels in the serum are normally distributed in healthy people. Oxidative stress indexes in blood are measured in 60 healthy people, including 39 men and 21 women. The results show that the levels of MDA and the activity level of GSH-Px in healthy men are generally higher than those in women, while the level of SOD activity in healthy women is slightly higher than that in men. However, the gender differences in healthy people don't show statistical differences (P>0.05) for the three oxidative stress indicators.

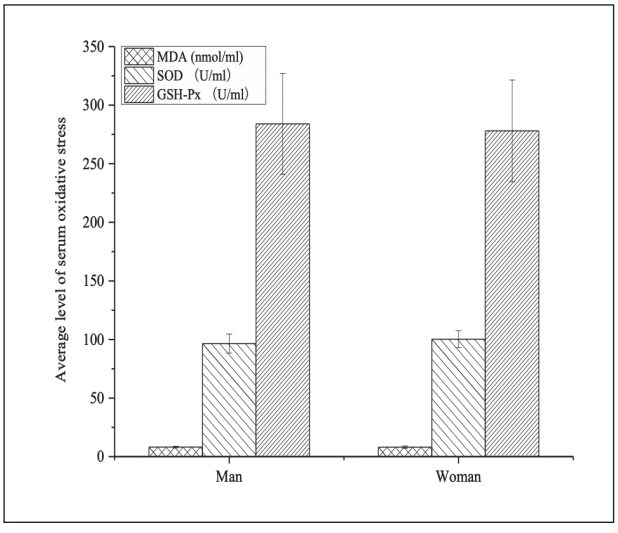

Fig 1: Average level of serum oxidative stress in healthy people of different genders.

Comparison and analysis of blood oxidative stress indicators between experimental group and control group

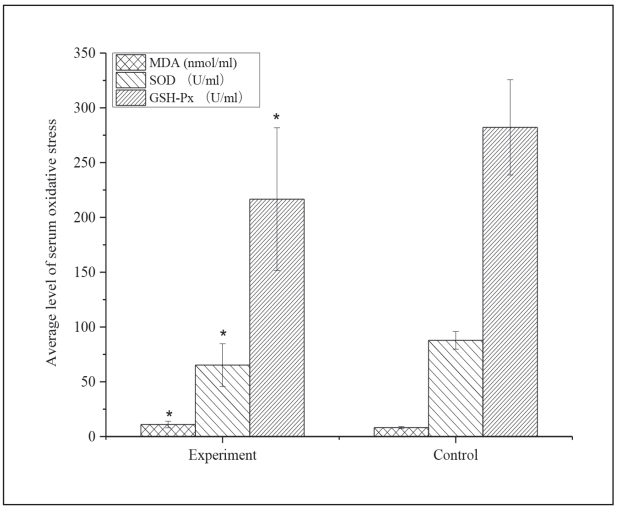

Fig 2: Average level of serum oxidative stress in experiment and control groups.

*Note: *indicates that the difference is significant at the 0.05 level compared to the control group.*

The results of oxidative stress determination in blood of the patients with cardiac arrest after cardiopulmonary resuscitation and in the normal population are shown in Figure 2. The results show that the MDA content in the blood of the experimental group is significantly higher than that of the control (P<0.05), but the activity level of SOD and the activity level of GSH-Px are significantly lower than those of the normal control group (P<0.05).

Comparison and analysis of blood oxidative stress indicators between group A1, group A2 and control group

One-way analysis of variance is used to compare the group A1 and the group A2 with the normal control group. The results are shown in Figure 3. The average level of serum MDA in group A1 and group A2 is higher than that in the control group, and both reach statistically significant difference (P<0.05), but there is no statistical difference between group A1 and group A2 (P>0.05); the activity levels of SOD and the average activity level of GSH-Px in the group A1 and the group A2 are significantly lower than those in the normal control group, indicating that there is a statistical difference between the group A1 and the group A2 and the control group (P<0.05). Similarly, there is no statistical difference between the group A1 and the group A2.

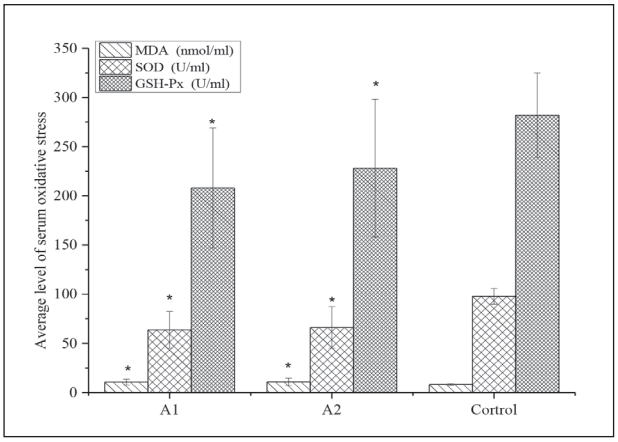

Fig 3: Average level of serum oxidative stress in groups

Linear analysis of IMA kits

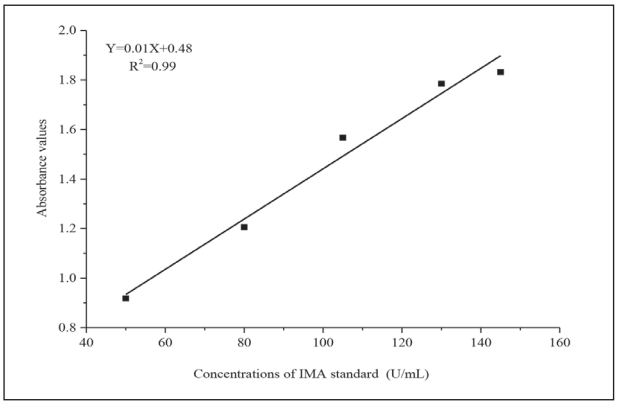

Fig 4: Linear analysis of IMA kit.

The linear analysis of the IMA kit is shown in Figure 4. The IMA standard products at concentrations of 50 U/ml, 80 U/ml, 105 U/ml, 130 U/ml, and 145 U/ml are used for the determination, and the corresponding OD values are 0.98, 1.20, 1.58, 1.76, and 1.83, respectively. According to the linear correlation regression analysis, the linear regression equation of the IMA test kit is Y=0.01 X+0.45, and the regression coefficient is 0.01. The correlation coefficient is 0.99 and the determination coefficient is 0.99. The linear relation is good.

Distribution characteristics of IMA in serum of healthy people

As shown in Figure 5, by measuring the serum IMA content in the healthy control group, it is found that the average value of IMA in the normal population is 102.2 ± 9.8 U/ml, and the results show a kurtosis of 0.6 and a skewness of 0.3, which is consistent with an approximately normal distribution. Therefore, the reference range of serum IMA in healthy people is: healthy people serum IMA<118U / ml (one side P<95%).

Comparison and analysis of blood IMA levels between group A1, group A2 and control group

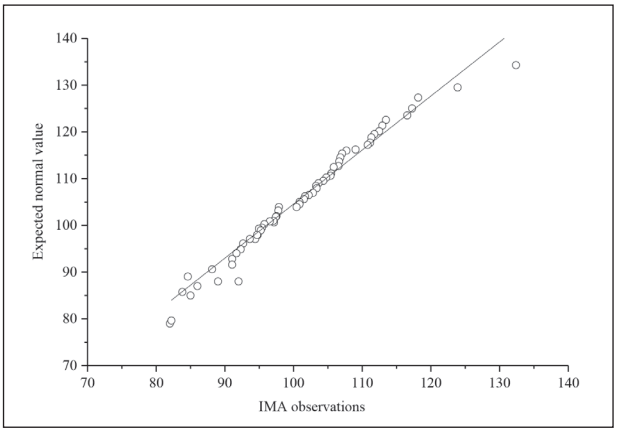

Fig 5: Normal distribution of IMA levels in healthy populations.

As shown in Figure 6, the comparison of the three groups by one-way variance shows that the serum IMA level of the group A2 is higher than that of the group A1 and the normal control group, and there is a statistical difference, while the serum IMA level of the group A1 is higher than the normal control group, there is also a statistical difference between the two groups.

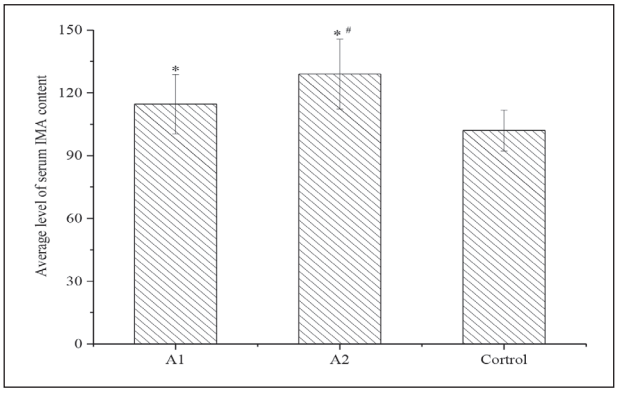

Fig 6: Average level of serum IMA content in groups A1, groups A2 and control.

Discussion

At present, CPR is a common method for the treatment of patients with cardiac arrest. The main advantages of CPR treatment are its high efficiency and high quality[11]. In recent years, with the advancement of technology, although the CPR technology has been improved to some extent, the treatment of patients with cardiac arrest is still at a lower success rate.

The increase in reactive oxygen species caused by cardiac arrest is one of the main causes

of CPR rescue failure[12]. Sudden cardiac arrest can cause sudden interruption of organ perfusion, ischemia and hypoxia, free radical bursts, large consumption of antioxidant enzymes, and decreased antioxidant system function. At the same time, the membrane lipid peroxidation reaction causes the structure of the cell to be destroyed, the cell function is further lost, and the body is damaged, and MDA is the final product of membrane lipid peroxidation. In the normal body, the antioxidant enzyme system can effectively identify harmful substances such as reactive oxygen species, and the body's antioxidant enzymes mainly include SOD, GSH-Px, catalase, etc.[13]. SOD is the world's most active enzyme for removing harmful substances in reactive oxygen species. It combines free radicals and converts them into hydrogen peroxide, which in turn decomposes into harmless water and oxygen by the catalytic action of catalase, thereby exerting an antioxidant effect. GSH-Px can catalyze the oxidized glutathione and water formed by the combination of GSH and peroxide, and further exert its antioxidant effect. Studies have shown that the body's process of oxidative stress can increase serum MDA content and reduce serum SOD and GSH-Px levels[14].

In this study, by analyzing changes in serum oxidative stress index after CPR in patients with cardiac arrest, it is found that the mean level of serum MDA in the patients in this trial group is significantly higher than that in the normal control group. This indicates that CPR can guarantee the partial perfusion requirement of vital organs in patients with cardiac arrest, but it also triggers a relatively serious lipid peroxidation, which causes the patient's cell structure and function to be damaged to some extent. Secondly, the average levels of serum SOD and GSH-Px in the experimental group are significantly lower than those in the normal control group. This indicates that after CPR in patients with cardiac arrest, harmful substances such as reactive oxygen species are formed in a large amount in the patient, resulting in a certain consumption of the patient's antioxidant enzymes. However, as the consumption and supplementation of the body's antioxidant capacity don't reach a balanced state, the body's antioxidant capacity is severely damaged, showing a sharp decrease in SOD activity and GSH-Px.

IMA can well reflect the state of tissue and organ ischemia caused by cardiac arrest in patients. The study finds that when CPR is used to treat patients with cardiac arrest, the level of IMA in the blood of patients is detected and found to be higher than that of the normal population. Therefore, IMA detection can provide an effective prognosis for patients with cardiac arrest after CPR. Theodoro Xanthos et al[15] conducted an animal study and found that serum IMA levels were significantly elevated after CPR of cardiac arrest. At the same time, the level of IMA in the serum of animals that have not reached the ROSC state is also higher than the level of IMA that reaches the state of ROSC. The sensitivity and specificity of IMA for predicting cardiac arrest in CPR experimental animals with ROSC were as high as 100% and 93.8070, respectively. In this study, by analyzing changes in serum IMA levels after CPR in patients with cardiac arrest, the results show that serum IMA levels in patients with cardiac arrest after CPR are higher than those in the normal population, and there are statistical differences. Further analysis of patients who achieve spontaneous circulation recovery and patients who don't achieve autonomic circulation find that patients who don't reach the ROSC status also have higher levels of IMA in the serum, which is also statistically different. This indicates that serum IMA levels rise immediately after cardiac arrest, while patients who don't reach ROSC status have higher IMA levels in serum than those that reach ROSC status. This further indicates that after CPR in patients with cardiac arrest, different prognosis affects serum IMA levels, which may be related to coronary perfusion levels during CPR, suggesting that serum IMA levels are associated with ischemic severity.

In summary, in the normal population, the serum oxidative stress index and IMA content are normally distributed. Sudden cardiac arrest causes a large number of harmful free radicals in the patient's body, causing a certain degree of damage to the function of the cells, which in turn causes the internal organs to fail. In this case, by detecting changes in IMA levels, it can effectively reflect the ischemic state of tissues and organs of patients with cardiac arrest, and it has a certain reference value for effective prognosis of patients with CPR.

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