Review Article

Need of Blood Doping Test- From Sports to Recruitment

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Abstract

Doping is not a new trick to enhance endurance in sports. But now a days doping is not restricted in sports only. Many instances had been observed where the candidates were found doping during recruitment exam of Police and Defence services during physical test. Government is trying to combat this situation. Gujarat became the first state who took initiative in this matter and conduct dope test in recruitment of Police Inspector The test was performed for six recreational drugs such as THC, amphetamine benzodiazepine, Barbiturates, cocaine and opiates.But as now the people are aware of these doping drugs and they know that these drugs can be detected in blood and urine sample .So they are now using another trick of doping i.e blood doping because it is easier way of cheating and its detection is very difficult .Blood doping is also not new in sports , its first case was observed in 1980 in Summer Olympic in Moscow .But it was not prohibited that time. Blood Doping is not only unfair mean of getting success however there are serious health hazards associated with this. Blood doping thickens the blood due to increased RBCs mass, which leads to several deadly diseases such as heart disease and stroke. So, there is need to prevent such kind of practices. Many sophisticated techniques are evolved nowadays to detect blood doping.

This article outlines the procedure of blood doping, and the brief review is made on the testing procedures. Emphasis is placed on the recent development and need of introduction of this blood doping test in recruitment process.

Key words: Blood, Doping, recruitment, sports

Introduction

In January 2023, many Agniveer applicants were found doping during Agneepath recruitment scheme and Police recruitment exam in Maharashtra. Similar incidence has been reported in in 2016 in Punjab Police recruitment where 294 aspirants were found positive for drugs. Due to increasing tendency of dope Punjab Chief Minister ordered a mandatory dope test for all Government employees. An investigation by India Today in 2016 reveal how candidates in Haryana Police recruitment drive used banned drugs to enhance performance. In Bareilly similar matter surfaced in army recruitment. So, initiative has been taken by the Government in many ways.

On recommendation of Gujarat Public Service Commission, National Forensic Science University conducted dope test in recruitment of police inspector. The tests were conducted for six recreational drugs such as Tetra Hydro Cannabinol, Benzodiazepine, Barbiturates, Cocaine Amphetamine.

But as the people are aware of these drugs and their testing and they know that theses drugs can be detected in blood or urine sample, so they are trying

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to find out some other way to enhance performance which is easy to commit but difficult to detect So Blood doping is the easier way for enhancing endurance.

Blood Doping

The World Anti-Doping Agency (WADA) defines blood doping as "the misuse of certain techniques and/or substances to increase one's red blood cell mass, which allows the body to transport more O_2 to muscles and therefore increase stamina and performance"[1].

Therefore, the more red blood cells that you have, the greater the oxygen capacity, and the greater the supply of oxygen available for muscles to generate energy without "burning out" during extreme physical activity[2].

Methods of Blood Doping

There are many ways of blood doping but few are very easy and common methods such as

1. **Blood Transfusions-** Blood transfusion is the process of transferring blood products into a person›s circulation intravenously[3].

   Blood transfusions can be traditionally classified as

   (a) Autologous blood transfusion: It is collection and re-infusion of person's own blood or blood components Blood transfusion begins by the withdrawal of 1 to 4 units of blood (1 unit = 450 mL of blood) several weeks before competition. The blood is centrifuged, the plasma components are immediately reinfused, and the corpuscular elements, principally red blood cells (RBCs), are stored refrigerated at 4 °C or frozen at −80 °C[4].

   (b) Homologous blood transfusion: In this blood transfusion blood is transfused into someone other than the donor.

2. **Blood substitute** are the substance which have same functions of biological blood

   The main categories of blood substitutes are:

   (a) HBOC"oxygen-carrying" blood substitutes being pursued are hemoglobin-based oxygen carriers (HBOC). These are chemicals that have the ability to carry oxygen[5]. Haemoglobin is the main component of red blood cells, comprising about 33% of the cell mass.

   (b) PFCs -Perfluorocarbon-based blood substitutes are completely man-made; this provides advantages over blood substitutes that rely on modified haemoglobin, such as unlimited manufacturing capabilities, ability to be heat-sterilized, and PFCs' efficient oxygen delivery and carbon dioxide removal. PFCs in solution act as an intravascular oxygen carrier to temporarily augment oxygen delivery to tissues. PFC particles in solution can carry several times more oxygen per cubic centimeter (cc) than blood, while being 40 to 50 times smaller than haemoglobin[6].

3. **EPO injections.** Erythropoietin is a hormone produced by the kidney. It is a glycoprotein with attached sugar. It stimulates the bone marrow to produce more red blood cells. The resulting rise in red cells increases the oxygen-carrying capacity of the blood[10]. It regulates the body's production of red blood cells. In medical practice, EPO injections are given to stimulate the production of red blood cells. For example, a synthetic EPO can be used to treat patients with anaemia related to chronic or end-stage kidney disease.

   As the prime regulator of red cell production, erythropoietin's major functions are to:

   1. Promote the development of red blood cells.

   2. Initiate the synthesis of haemoglobin, the molecule within red blood cells that transports oxygen.

EPO injections are abused by the people to encourage their bodies to produce higher than normal amounts of red blood cells to enhance performance because of its easily availability and it is not a banned drug[8].

Detection of Blood Doping

Earlier it was very difficult to detect blood doping, but now novel techniques are available which are used to detect blood doping.

Detection for homologous blood doping

In 2004, a test for detection allogeneic/homologous blood transfusion doping was implemented. Flow cytometry is the method of choice. By examining markers on the surface of blood cells, the method can determine whether blood from more than one person is present in an athlete›s circulation. The test utilizes 12 antisera directed against the blood group antigens, obtained from donor plasma. The antigens are labeled with secondary antibodies, which are conjugated with phycoerythrin to label IgG or IgM-coated RBCs and enhance the detection by flow cytometry. The flow cytometry is able to detect minor variance in blood group antigens. The assessment was able to distinguish the blood of subjects who had earlier received at least one unit of allogeneic blood[7].

Modern flow cytometers are able to analyse many thousands of particles per second, in "real time" and, if configured as cell sorters, can actively separate and isolate particles with specified optical properties at similar rates. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers high-throughput, automated quantification of specified optical parameters on a cell-by-cell basis[9].

A flow cytometer has five main components: a flow cell, a measuring system, a detector, an amplification system, and a computer for analysis of the signals. The flow cell has a liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing. The measuring system commonly uses measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals. The detector and analog-to-digital conversion (ADC) system converts analog measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye-specific fluorescence signals into digital signals that can be processed by a computer.

Detection for autologous blood doping

Currently, no test exists to directly detect autologous transfusions. Instead, indirect methods are used. One method is based upon a transfusion-induced immune-response resulting in specific changes in gene expression related to leukocytes such as T lymphocytes. Another method relies on detecting increased plasticizer metabolite levels in the urine caused by the leakage of plasticizers from the blood bags used during the blood storage. These methods need further development and validation across different types of transfusion regimes before they can be implemented[8].

Detection method for haemoglobin-based oxygen carriers:

It is done in four separate steps. Step one involves the elimination of abundance proteins in the blood samples by immunodepletion (i.e. Proteo Prep 20 plasma immunodepleting kit). This process ensures that other proteins (i.e. albumin and immunoglobulin) do not interfere with capillary electrophoresis (CE) separation by changing the ionization. Second step, CE separation is done under certain condition, in this case background electrolyte consisting of ammonium formate (75mM at pH 9.5) in order to provide sufficient resolution between HBOC and Hb. Third step, UV/Vis detection was performed at 415 nm to selectively detect HBOC and HB. Fourth step, time-of-flight or mass spectrometer allowed increased accuracy in selectivity between haemoproteins and other proteins and definite determination of HBOC uptake[12].

EPO injections

Blood and urine tests can detect the presence of synthetic EPO. ESAs is prohibited according to the World Anti-Doping Code and its prohibited list of substances and methods. Since the first publication of a direct and urine-based detection method in 2000, which uses changes in the Epo isoform profile as detected by isoelectric focusing in polyacrylamide slab gels (IEF-PAGE), the method has been constantly adapted to the appearance of new ESAs (e.g., Dynepo, Mircera). Blood had to be introduced as an additional matrix, because Mircera (a PEGylated Epo) is best confirmed in serum or plasma after immunoaffinity purification. A Mircera ELISA was developed for fast

screening of sera. With the appearance of Dynepo and copy epoetins, the additional application of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE or equivalent) became necessary[11].

Risks of Blood Doping

The simple act of increasing the number of red blood cells in blood may be associated with hyper viscosity syndrome which is characterized by increased blood viscosity and decreased cardiac output and blood flow velocity which results in the reduction of peripheral oxygen delivery. Because blood doping increases the volume of red blood cells, it effectively introduces a condition called polycythaemia, a blood disorder that has known adverse outcomes such as heart attacks or strokes[13].

Blood doping via transfusion carries additional risks. Tainted blood can spread infectious diseases such as HIV, hepatitis B etc Blood contamination during preparation or storage is another issue. Contamination was seen in 1 in every 500,000 transfusions of red blood cells in 2002.[9] Blood contamination can lead to sepsis or an infection that affects the whole body[14].

Over time, repeated blood transfusions can cause a dangerous buildup of iron in the body. Improperly stored blood and improperly administered transfusions can cause acute lung injury , bacterial infection ,allergic reactions such as fever and rashes. Other risks of EPO injections include: hyperkalaemia (potentially dangerous elevation of plasma potassium l**e**vels in the body),high blood pressure, mild flu-like symptoms[15, 16].

Erythropoietin (EPO) injections can cause a variety of side effects, some of which can be serious.

The most common side effects of EPO injections include Hypertension (high blood pressure), Headache, Nausea and vomiting, Joint pain, Fever, Dizziness, Edema (swelling of the legs, feet, or hands). Injection site reactions, such as pain, swelling, or redness[17,18].

The following are more severe adverse effects of EPO injections:

- Thrombosis (blood clots), including deep vein thrombosis (DVT), pulmonary embolism (PE), and stroke.
- Increased risk of heart attack.
- Seizures.
- Allergic reactions, including anaphylaxis.
- Pure red cell aplasia (a rare condition in which the bone marrow stops producing RBCs).
- Hypertension (high blood pressure)[19,20].

Conclusion

Blood doping is not only means of unfair method of being selected in recruitment process or enhancing performance in sports but also carries many risks such as Blood clot, heart attack, Stroke , HIV, Hepatitis B, Hepatitis .Individual can even die during performance due to heart blockage as viscosity of blood gets increased by high mass of RBCs.

The recent advancement in technology can resolve the cases based on blood doping. The upgraded technology would be helpful in fair selection in Police and Defense recruitment.

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