"Characterization of recombinant Streptokinase and its role in treatment of cardiovascular disease"

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Abstract:

Cardiovascular Disease are the main source of death on the planet, speaking to just about 32 % of all passings in ladies and 27 % in men in 2004 (WHO Report; 2016). Almost 50% of all passings in Europe are because of cardiovascular maladies, coronary illness being the main source of mortality in men more than 45 years, and in ladies more than 65 years. Stroke is the primary driver of long haul inability in the Western culture (British Heart Foundation; Report; 2013). Patients determined to have thrombosis in one blood vessel bed have a 35 % possibility of sickness in at least one other blood vessel beds. This multivessel malady is probably in patients with fringe blood vessel ailment (PAD) with one of every two having additionally coronary illness and one out of two cerebrovascular sickness.

Biochemical parameters of cardiovascular Disease adjoin advantageous controls are presented in Table no.2. As accepted the Cardiovascular Disease had acutely higherlevels of LDL-C (P=0.0006***) and TG (P=0.0192*). Cogent association with cardiovascular Disease were apparent in Systolic BP (P<0.0001***) and Diastolic BP (P<0.0001***) if this parameter were compared to that of advantageous ascendancy subject. Nominal aberration was as well empiric in Pulse pressure (P=0.0024**). Post-Prendial Glucose, Blood urea level, HbA1C, and HDL-C level was not significantly adapted amid two groups. Streptokinase is fibrinolytic protein utilized in cardiovascular illness. During myocardial dead tissue (cardiovascular sickness), creatine kinase-MB level is expanded in blood. Streptokinase by implication control the degree of creatine kinase-MB. The degree of creatine kinase-MB were diminished step by step with time after treatment of various doges of streptokinase. ANOVA measurable examination uncovers all treatment of streptokinase are not fundamentally unique (P=0.9785) to one another yet treatment of streptokinase were related with cardiovascular illness.

Keywords: Streptokinase, cardiovascular illness, creatine kinase-MB, BP, TG, LDL.

Introduction:

Thrombosis is a condition of the blockage of blood vessel by fibrin clots that cause an acute myocardial infarction and stroke, and becoming leading of death. In 2007, Ministry of Health stated that the highest rate of death has changed from infectious to degenerative diseases, and stroke has the leading causes of death. The incidence of heart diseases has related to lifestyle changes. Thrombolytic agents are the major options than surgery. It works as plasminogen (PG) activator by changing inert PG enzymatically in the fibrinolytic system becomes plasmin (PN) and dissolves the fibrin clot into soluble degradation products, and then degradation products will be phagocyte. Several fibrinolytic drugs commonly used are streptokinase (SK), urokinase (UK), and tissue plasminogen activator **[1].**

The drug selection is dependent on the price, side effect, severity, in vivo stability, specificity to fibrin, and immunological reactions. UK and tPA are immunological inert, but has a lower in-vivo stability and more expensive than SK that is naturally secreted from *Streptococcus pyogenes* M12 strain CS24. Biotechnological research at School of Pharmacy ITB has developed several mutants of streptokinase (SKA) to obtain better in vivo activity which is associated with increasing of its biological

half life and low immunological reaction. Based on the in-vitro experiments, the observation of pharmacological parameters and in vivo stability has to be explored **[2].**

Streptokinases are a well-defined group of proteins exported by many strains of hemolytic streptococci to the growth medium. They interact stoichiometrically with the enzymatically inert plasma plasminogen to yield the active enzyme plasmin. The plasmin so formed then degrades, by limited proteolysis, the fibrin network to form soluble products (1, 2). Although, unlike other plasminogen activators, streptokinases are not proteases, the recently determined amino acid sequence of one streptokinase species revealed homology to the sequences of bovine trypsin and Streptomyces griseus proteases, suggesting that it evolved from a seine protease **[3-6].**

The role of streptokinases in the pathogenicity of streptococci is unclear. Potentially, these substances may be determinants of virulence that contribute to the invasiveness of the organisms by preventing the formation of fibrin barriers around infectious lesions. Physical and immunological differences, paralleled by differences in substrate specificity, testify to the molecular heterogeneity of streptokinases from different sources. Although these proteins are closely related in function, the genetic basis of their heterogeneity is unknown. To achieve a better understanding of the genetic aspects of this important streptococcal product, we have undertaken undertaken to clone a streptokinase gene from a group C Streptococcus and report here its expression in Escherichia coli. Besides providing approaches to studying the molecular and epidemiological relationships between streptokinases, the cloning of this gene should provide alternative organisms for commercial streptokinase production. As a drug, streptokinase has a place in thrombolytic therapy **[7].**

Materials and Methods:

Study population:

The study population consisted of 240 unrelated subjects comprising of 110 cardiovascular disease patients and 130 ethnically matched controls of central Indian population were included in this study. In this region Hindu, Muslim and some Sikh peoples are mainly living but most people belong to Hindu religion in this region.

Inclusion and Exclusion criteria for Cases:

Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi. Cardiovascular disease was diagnosed in accordance with World Health Organization (WHO Expert committee 2003) criteria. Pregnant women, children under age of 18 years from the study.

Inclusion and Exclusion criteria for Controls:

Control group composed of healthy individuals that were collected during "Cardiovascular disease Awareness Camps" organized in urban regions in and around SSMC Rewa and many volunteers were also included to collect control sample. The control subjects were recruited from the regions that from homogenous cluster in Vindhyan region India in accordance with a recent report of genetic landscape of the people of India. (Indian Genome Variation Consortium 2008)

Biochemical and clinical Analysis:

Biochemical parameters related to cardiovascular disease were estimated for both cases and controls subjects. Measurement of Serum levels of Triglycerides (TG), HbA1c, High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C), Urea were measured based on spectrophotometric method using automated clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany). Systolic and diastolic blood pressures were measured twice in the right arm in sitting position after resting for at least 5 minute using a standard sphygmomanometer and the average of the two reading was used.

Blood collection and plasma/serum separation:

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C.

Association Study of Streptokinase with cardiovascular disease:

Bovine Serum Albumine (TCI), N,N,N',N'-tetramethiletilene diamine (TEMED), coomassie blue R-250 (Pharmacia), protein marker (Amersham), chloramine-T (Sigma), sodium metabisulfite (Merck), Whatman no. 1 paper, and DOWEX 1x8 (Merck). The equipment used was protein electrophoresis (Bio-Rad), paper chromatography apparatus, dose calibrator (RI Deluxe Iisotop Calib II, Victoreen) and single channel analyzer (ORTEC). Streptokinase were obtained from Overproduction of *Escherichia coli* BL21 (DE3) consist of pET-32b ska.

Animals

Male Swiss mice with age of 3 months, and body weight of 40-45 g were used for pharmacokinetic study. Animals used in the experiments received care in compliance with the "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals".

Over-production of SKA

The SKA Over-production was performed on *E.coli* BL21 (DE3) containing pET-32b. Single colony of *E.coli* inoculated in 10 ml of liquid Luria Bertani broth containing 100µg/ml ampicillin as a selective medium. The culture was incubated for about 18h in a shaking incubator at 37oC, and then a total of 10 mL of medium inserted into 200 mL selective medium. Cultures were re-incubated for 3 h at

37^0C in a shaking incubator until OD600 reached 0.3 to 0.4. Furthermore, the culture was induced with 0.5 mM IPTG and incubation was continued for an additional 3 hours, and then centrifugated at 4^0C, 4500g for 15 min. Pellet cells obtained from sediment was re-suspended in 5 mL of lysis buffer (50 mM NaH2PO4 and 300 mM NaCl at pH 8.0) and PMSF to get a final concentration of 1 mM, and lyzed by sonication at the amplitude of 15 for 5 minutes. To prevent temperature elevation, the cells were sequentially sonicated and cooled on ice for several times, each time for 30s. Soluble protein were separated by centrifugation at 12,000 g, 4°C for 15 min, and then purified using nickel affinity column according to the manufacturer's protocol (Protino® Ni-Ted), and characterized by SDS-PAGE. Protein concentration was determined using Bradford method based on coomassie blue staining.

Results:

Biochemical and clinical findings:

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t test and p value obtained suggests the level of significant changes here. The descriptive data and comparison of biochemical parameters of cardiovascular disease versus healthy controls are presented in Table no.2. As expected the Cardiovascular disease had markedly higher levels of LDL-C (P=0.0006***) and TG (P=0.0192*). Significant association with cardiovascular disease were seen in Systolic BP (P<0.0001***) and Diastolic BP (P<0.0001***) when this parameter were compared to that of healthy control subject. Nominal difference was also observed in Pulse pressure (P=0.0024**). Post-Prandial Glucose, blood urea level, HbA1C, and HDL-C level was not significantly different between two groups and all the clinical test results are tabulated in table no. 1

TABLE No-1

Comparison of Biochemical and clinical findings of diabetic patients and controls

Characteristics	**Case (CVD patient) N=110(76/34)**	**Control (Healthy population) N=130 (88/43)**	***P-value***
Post-Prandial Glucose (mg/Dl)	126.7±12.4	125.5±10.1	0.4095 ns
HbA1C(%)	6.9±0.8	6.7±0.9	0.0725 ns
HDL-C(mmol/L)	112.2±14.8	109.8±11.6	0.1606 ns
LDL-C (mg/dL)	43.1±4.3	41.3±3.7	0.0006***
TG(mg/dL)	131.1±13.2	126.9±14.2	0.0192*
Systolic BP (mmHg)	132.20±8.1	128.8±4.7	P<0.0001 ***
Diastolic BP (mmHg)	87.1±5.8	82.5±3.0	P<0.0001 ***
Blood Urea(mg/dL)	9.1±1.6	8.8±1.8	0.1773 ns
Pulse pressure	66.7 ± 18.5	61.1 ± 8.7	0.0024**

(N – Number of individuals in study group.)

(*-Denotes level of significant change between malarial cases and healthy controls.)

Association study of Streptokinase with cardiovascular Disease:

Streptokinase (SK) is a thrombolytic medication and enzyme. As a medication it is used to break down clots in some cases of myocardial infarction (heart attack), pulmonary embolism, and arterial thromboembolism. Streptokinase protein is obtained from *Beta hemolytic streptococci but* commercially produced from cloning of *E. coli*. The role of streptokinase in the treatment of cardiovascular disease (myocardial infarction) were measured by concentration of creatine kinase-MB in mouse model system.

Cloning and Isolation of recombinant Streptokinase:

E.coli BL21 (DE3) containing pET-32b were cloned and produced recombinant streptokinase. Streptokinase detection was performed using SDS-PAGE (Depicted in figure no. 1). A thick band of 68.3 kDa of streptokinase Where M is purified marker, lane 2 is Crude protein extract, lane 3 is washing buffer, lane 4-5 are first elute, lane 6-7 are second elute and lane 8-9 are third elute.

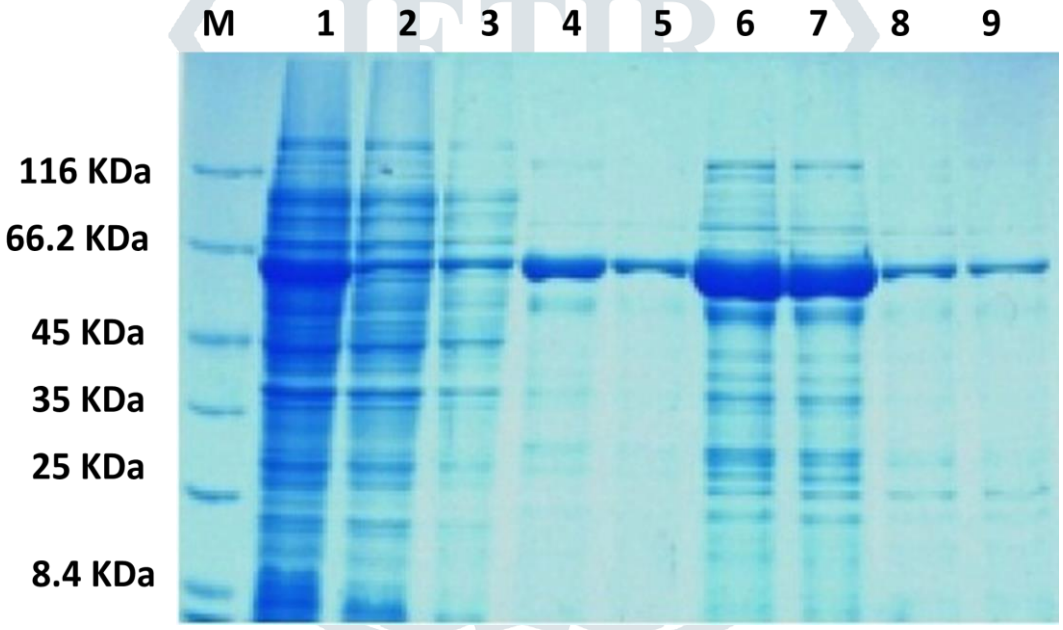


Figure No. 1: Electroforegram of SKA purified Protein marker (M); Crude protein extract (2); Washing (3); First Eluate (4-5); 2n Eluate (6-7), 3rd Eluate (8-9),

Thrombolytic Activity of isolated streptokinase:

Clot-lysis of blood samples of normal subjects (positive and negative control). Tube no. 1 is a control clot (negative control) to which water was added. No clot lysis was observed in tube no.1; a black arrow indicates the intact clot. Tube no. 2–5 (positive control) was lysed by four different concentrations of streptokinase with decreasing order. After dissolution of the clots, tubes were inverted and fluid (blue arrow) along with the remnants of clots (red arrow) could be clearly seen**.**

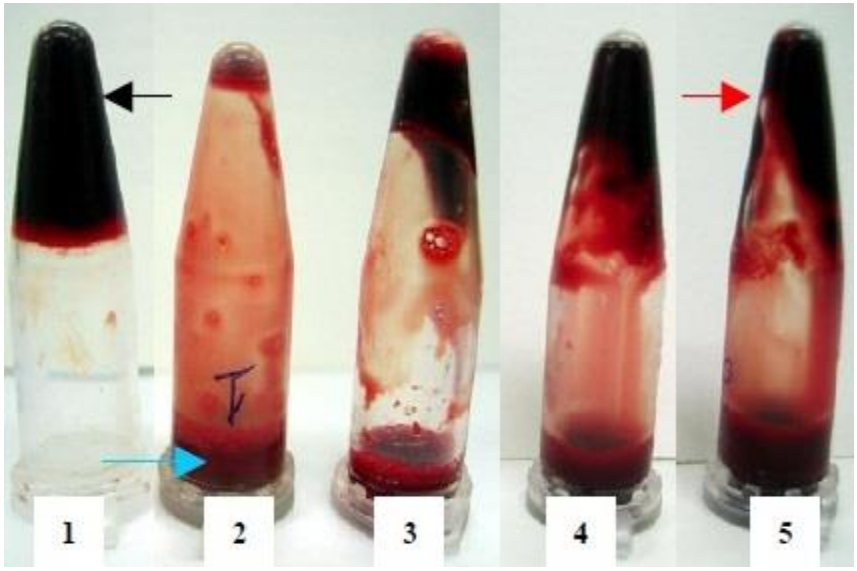


Figure No. 2: Thrombolytic activity of isolated streptokinase; 1=control, 2-5= SK treatment with different concentration.

Streptokinase treatment to Mouse model:

Streptokinase is fibrinolytic enzyme used in cardiovascular disease. During myocardial infarction (cardiovascular disease), creatine kinase-MB level is increased in blood. Streptokinase indirectly regulate the level of creatine kinase-MB. The level of creatine kinase-MB were reduced gradually with time after treatment of different doges of streptokinase. ANOVA statistical analysis reveals all treatment of streptokinase are not significantly different (P=**0.9785**) to each other but treatment of streptokinase were associated with cardiovascular disease.

TABLE No-2

Effect of different doge of Streptokinase on level of blood creatine kinase-MB with time.

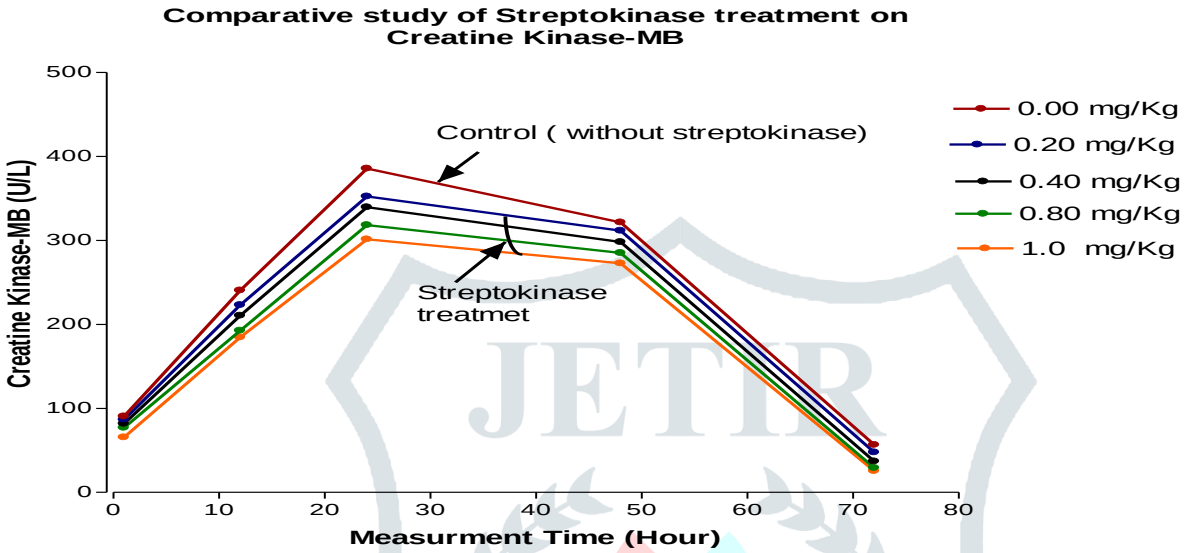
Ceatine Kinase-MB at hour	STK 0.00 mg/Kg	STK 0.20 mg/Kg	STK 0.40 mg/Kg	STK 0.80 mg/Kg	STK 1.0 mg/Kg	P value
1	90.65	86.55	81.74	76.95	65.48	0.9785 , ns
12	240.55	223.15	210.53	192.75	184.55	
24	385.75	352.35	339.74	318.23	301.41	
48	321.65	311.61	298.29	285.15	272.75	
72	56.75	47.66	36.91	29.29	25.53	

(N – Number of individuals in study group.)

(*-Denotes level of significant change between malarial cases and healthy controls.)

Graphical Representation:

Graph no. 3 showing different treatment group of streptokinase is correlated with creatine kinase-MB. The level of creatine kinase-MB gradually increased with time after that its level is reduced after the treatment of streptokinase.



Graph No. 1: Effect of streptokinase treatment on creatine kinase- MB.

Discussion:

Cardiovascular disease (CVD) is a major public health problem in India. The epidemiological transition plays out differently in different regions of India because of varied economic development. Disparate relationships between CVD risk factors are evident in regions that are at different stages of epidemiological transition **[22].** In this study, we analysed association of anthropometric and biochemical parameters with cardiovascular disease. Three major protein Urokinase, Streptokinase and fibrin are associated with cardiovascular disease**[8].**

Our statistical data for Anthropometric parameters suggest BMI are significantly associated with cardiovascular disease but WHR are not showing any association. The descriptive data and comparison of anthropometric and biochemical parameters of Cardiovascular disease versus controls were significantly different. The age, sex, BMI, WHR parameters were selected for study of cardiovascular disease had markedly higher levels of weight of women (P=0.0013**), Men (P=0.0016**). BMI of Women (P=0.0172*) and men (P=0.0240*) were significantly associated with cardiovascular disease. Waist circumference in women (P=0.5037) and men (P=0.3260) likewise WHR in Women (P=0.5263) and Men (P=0.1169) were not associated.

The study of Dudina A. *et. al.* were support to our work. His study was a strong, graded but J-shaped univariable relationship between BMI and CVD mortality in both genders. Each 5-unit increase in BMI was associated with an increase in CVD mortality of 34% in men and 29% in women. The hazard

ratios remained significant when adjusted for age, self-reported smoking status, total cholesterol, and systolic blood pressure (SBP). In all age groups except those over 60 there were significant relationships between increased BMI and CVD mortality **[9].** In the over-60 age group the only significant relationships with mortality were in underweight and severely overweight women and mildly obese men. After adjustment for age, each 1-unit increase in BMI was associated with a 1.14 mmHg increase in SBP, 0.055 mmol/l increase in total cholesterol, and a 0.024 mmol/l decrease in HDL in men. Figures were slightly lower in women **[29,10].**

Our Biochemical and clinical findings suggest LDL-C, TG, Pulse pressure, Systolic BP and Diastolic BP were associated with cardiovascular disease. Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t test and p value obtained suggests the level of significant changes here. The descriptive data and comparison of biochemical parameters of cardiovascular disease versus healthy controls are presented in Table no.2. As expected the Cardiovascular disease had markedly higher levels of LDL-C (P=0.0006***) and TG (P=0.0192*). Significant association with cardiovascular disease were seen in Systolic BP (P<0.0001***) and Diastolic BP (P<0.0001***) when this parameter were compared to that of healthy control subject. Nominal difference was also observed in Pulse pressure (P=0.0024**). Post-Prandial Glucose, blood urea level, HbA1C, and HDL-C level was not significantly different between two groups.

The study of Robert HN., supported our work on LDL-C. Elevated levels of blood lipids are well documented risk factors for cardiovascular disease. Current classification schemes and treatment levels for hyperlipidemia are based on the National Cholesterol Education Panel's (NCEP) and Adult Treatment Program-3 (ATP-III) guidelines. He suggest hyperlipidemia may be risk factor for cardiovascular disease **[11, 30].** Another study of Philip Barter, *et. al.* on predictive value of HDL cholesterol levels in 9770 patients. The primary outcome measure was the time to a first major cardiovascular event, defined as death from coronary heart disease, nonfatal non–procedure-related myocardial infarction, resuscitation after cardiac arrest, or fatal or nonfatal stroke. The predictive relationship between HDL cholesterol levels at the third month of treatment with statins and the time to the first major cardiovascular event was assessed in univariate and multivariate analyses and was also assessed for specific LDL cholesterol strata, including subjects with LDL cholesterol levels below 70 mg per deciliter (1.8 mmol per liter). The HDL cholesterol level in patients receiving statins was predictive of major cardiovascular events across the TNT study cohort, both when HDL cholesterol was considered as a continuous variable and when subjects were stratified according to quintiles of HDL cholesterol level **[15].** When the analysis was stratified according to LDL cholesterol level in patients receiving statins, the relationship between HDL cholesterol level and major cardiovascular events was of borderline significance (P=0.05). Even among study subjects with LDL cholesterol levels below 70 mg per deciliter, those in the highest quintile of HDL cholesterol level were at less risk for major cardiovascular events than those in the lowest quintile (P=0.03). In this post hoc analysis, HDL cholesterol levels were predictive of major cardiovascular events in patients treated with

statins. This relationship was also observed among patients with LDL cholesterol levels below 70 mg per decilitre **[12-17].**

Our work on Blood Pressure reveals association with cardiovascular disease. He suggest, hypertension is common among older adults, the optimal blood pressure (BP) for survival in older adults remains unclear. he attempt to use a large cohort to assess the relationship between BP and mortality and to gain insight into what level of BP is required for optimal survival in older adults. The cardiovascular and expanded-cardiovascular mortality risks were lowest when systolic blood pressures were 120 to 129 mm Hg, and increased significantly when systolic blood pressures (SBPs) were160 mmHg or diastolic BPs were 90 mm Hg. A J-curve phenomenon for SBP on CVD and expanded-CVD mortality was observed. The impacts of stage 2–3 hypertension on mortality risks were significantly increased among women. The mortality risks of hypertension were not attenuated with older age. This is closely similar to our work systolic and diastolic blood pressure. BP make hypertension that is risk factor for cardiovascular disease **[18].**

Hypertension (HT) is a major risk factor for coronary heart disease (CHD). Among the numerous risk factors associated with CHD, HT plays a major role given its high frequency and its physiopathogenesis. Thus, roughly 15% of the general adult population manifest HT with a net male predominance, and 25% of patients with CHD have HT. CHD is the first cause of morbidity and mortality in hypertensive patients. Numerous other risk factors for CHD, such as dyslipidaemia, insulin resistance, diabetes, obesity, lack of physical exercise and certain genetic mutations are frequently associated with HT **[19-20].**

Our statistical data from Puls rate were also supported by Singh B.N., he suggested that heart rate is a major determinant of oxygen consumption in patients with ischaemic heart disease. Its pharmacological modulation is increasingly the focus of therapeutic approaches to alleviate symptoms and prolong survival. It is the simplest cardiovascular variable to measure accurately and reproducibly. Conversely, low heart rate reduces risk for coronary artery disease and sudden death. Primary and secondary prevention studies in myocardial infarction indicated that elevated heart rate in susceptible patients predicts risk for developing myocardial infarction and death. Prophylactic beta-blockers attenuate risks for reinfarction, sudden death and total mortality; these effects correlate with reduced heart rate, thus providing a compelling basis for developing agents that reduce heart rate exclusively as antianginal agents for the therapy of myocardial ischaemia with broader therapeutic implications **[21,31].**

Triglycerides can be measured in the non-fasting or fasting states, with concentrations of 2–10 mmol/L conferring increased risk of cardiovascular disease, and concentrations greater than 10 mmol/L conferring increased risk of acute pancreatitis and possibly cardiovascular disease. Although randomised trials showing cardiovascular benefit of triglyceride reduction are scarce, new triglyceride-lowering drugs are being developed, and large-scale trials have been initiated that will hopefully provide conclusive evidence as to whether lowering triglycerides reduces the risk of cardiovascular disease. **[23-26].**Increase in plasma triglyceride (TG) levels was associated with an increase in CHD risk, even after adjustment for high density lipoprotein cholesterol (HDL-C) levels. Very recently, two studies were published that further

extent the early observation and showed the importance of nonfasting plasma triglyceride (TG) levels in the prediction of risk on coronary heart disease (CHD). In the current review we have summarized all available evidence obtained in clinical studies showing that treatment guidelines should reconsider to include nonfasting TG in their risk assessments as nonfasting TG levels may better predict CVD risk **[28].**

The study of John CC, and Katherine AH were favour to our work of fibrinolytic properties of urokinase. Fibrin plays an essential role in hemostasis as both the primary product of the coagulation cascade and the ultimate substrate for fibrinolysis. Fibrinolysis efficiency is greatly influenced by clot structure, fibrinogen isoforms and polymorphisms, the rate of thrombin generation, the reactivity of thrombus-associated cells such as platelets, and the overall biochemical environment. Regulation of the fibrinolytic system, like that of the coagulation cascade, is accomplished by a wide array of cofactors, receptors, and inhibitors. Fibrinolytic activity can be generated either on the surface of a fibrin-containing thrombus, or on cells that express profibrinolytic receptors. In a widening spectrum of clinical disorders, acquired and congenital defects in fibrinolysis contribute to disease morbidity, and new assays of global fibrinolysis now have potential predictive value in multiple clinical settings. Here, we summarize the basic elements of the fibrinolytic system, points of interaction with the coagulation pathway, and some recent clinical advances **[27-30].**

Most acute cardiovascular events are attributable to arterial thrombosis. Plaque rupture or erosion stimulates platelet activation, aggregation, and thrombosis, whilst simultaneously activating enzymatic processes that mediate endogenous fibrinolysis to physiologically maintain vessel patency. Interplay between these pathways determines clinical outcome. If proaggregatory factors predominate, the thrombus may propagate, leading to vessel occlusion. However, if balanced by a healthy fibrinolytic system, thrombosis may not occur or cause lasting occlusion. Despite abundant evidence for the fibrinolytic system regulating thrombosis, it has been overlooked compared with platelet reactivity, partly due to a lack of techniques to measure it. **[28].**

Our study reveals *E.coli* BL21 (DE3) containing pET-32b were cloned and produced recombinant streptokinase. Streptokinase detection was performed using SDS-PAGE (Depicted in figure no. 1). A thick band of 683 kDa of streptokinase Where M is purified marker, lane 2 is Crude protein extract, lane 3 is washing buffer, lane 4-5 are first elute, lane 6-7 are second elute and lane 8-9 are third elute. The study of Horst M and Joseph JF were very similar to our study. They suggest genomic DNA from Streptococcus equisimilis strain H46A was cloned in Escherichia coli by using the bacteriophage X replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for recombinants carrying the streptokinase gene (skc). The gene was present with a frequency of 1 in 836 recombinants, and 10 independent clones containing skc were isolated and physically characterized. One recombinant clone was used to subclone skc in *E. coli* plasmid vectors **[15, 24].** Plasmid pMF2 [10.4 kilobases (kb)] consisting of pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the *Pst I* site of pBR322 were among the recombinant plasmids determining streptokinase production in three different E. coli host strains.

Expression of skc was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. coli. However, expression in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to skc expression. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. coli streptokinase. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for streptokinase activity revealed the substance to be present in all three principal locations, indicating that E. coli cells were capable of releasing substantial amounts of streptokinase into the culture medium **[3-6].**

We studied clot-lysis properties of streptokinase in blood samples of normal subjects (positive and negative control). The control clot (negative control) to which water was added. No clot lysis was observed in tube no.1; a black arrow indicates the intact clot. Tube no. 2–5 (positive control) was lysed by four different concentrations of streptokinase with decreasing order. After dissolution of the clots, tubes were inverted and fluid (blue arrow) along with the remnants of clots (red arrow) could be clearly seen**.**

The work of Prasad S; et. al. were similar to our study. He suggested, Thrombolytic drugs are widely used for the management of cerebral venous sinus thrombosis patients. Several in vitro models have been developed to study clot lytic activity of thrombolytic drugs, but all of these have certain limitations. There is need of an appropriate model to check the clot lytic efficacy of thrombolytic drugs. Whole blood from healthy individuals (n = 20) was allowed to form clots in a preweighed sterile microcentrifuge tubes; serum was removed and clot was weighed. After lysis by streptokinase fluid was removed and remnants of clot were again weighed along with the tube. Percentage of Clot lysis was calculated on the basis of the weight difference of microcentrifuge tubes obtained before and after clot lysis. There was a significant percentage of clot lysis observed when streptokinase was used. On the other hand with water (negative control), minimal (2.5%) clot lysis was observed. There was a significant difference between clot lysis done by streptokinase and water. This study could be a rapid and effective methodology to study clot-lytic effect of newly developed drugs as well as known drugs **[26].**

Our study did correlate to streptokinase treatment and the effect of creatine kinase-MB level. Streptokinase is fibrinolytic enzyme used in cardiovascular disease. During myocardial infarction (cardiovascular disease), creatine kinase-MB level is increased in blood. Streptokinase indirectly regulate the level of creatine kinase-MB. The level of creatine kinase-MB was reduced gradually with time after treatment of different doges of streptokinase. Statistical analysis reveals all treatment of streptokinase are not significantly different (P=**0.9785**) to each other but treatment of streptokinase were associated with cardiovascular disease.

Acute myocardial infarction is the most important and feared consequence of coronary artery disease. STEMI is the one of that kind and it occurs by formation of a vulnerable plaque from atherosclerosis and transforms into coronary artery thrombosis in the end. Streptokinase has been used as a thrombolytic agent in patients with acute STEMI. It combines with the circulatory plasminogen to form an activator complex and it converts into plasmin, where, the plasmin breaks the fibrin complex in the blood

clot. Streptokinase can be given as 1,500,000U a slow I.V infusion over 30 min. Each STEMI subject is different with the aetiology, pain, ST segment elevation. Streptokinase has been shown to have significant effect on pain resolution and ST segment resolution in subjects with acute ST segment elevation myocardial infarction. Acute myocardial infarction (AMI) is one of the main leading causes of mortality and morbidity. Despite the progress in the treatment of AMI, streptokinase is still being used. Because of the critical condition of patients with AMI and complications of streptokinase therapy, this study was performed to evaluate the pattern of adverse drug reaction (ADRs) induced by streptokinase and its associated risk factors in patients with acute ST elevation MI **[25].**

Enzyme kinetics for creatine kinase (CK), CK-MB, aspartate aminotransferase (AST), and lactate dehydrogenase (LD) in serum were followed in 14 patients who had suffered acute myocardial infarction and who were given intracoronary streptokinase shortly (mean 4.9 h, SD 2.6 h) after onset of symptoms. In the 10 patients for whom thrombolysis was successful, CK activity peaked earlier (12.8 vs 21.6 h) and at higher values (3548 vs 2436 U/L) than in the four patients for whom the treatment was unsuccessful. The mean maximum rate of increase in CK was threefold greater in the former group (574 vs 169 U/L per hour), but the total amount of CK released into the circulation and the fractional disappearance rates were similar for both groups. The profiles for AST and CK-MB for successfully treated patients closely resembled those for CK. LD, however, peaked significantly later than CK (25.7 vs 12.8 h). Early peaking of CK or CK-MB after nonsurgical repertusion can be potentially useful as a noninvasive in vitro index to the success of therapy of myocardial infarction with thrombolytic agents **[24].**

Blood clots are a necessary response to prevent bleeding due to mechanical injury. These clots must be both elastic enough to resist the sheering forces of blood but also stiff enough to not rupture before the underlying tissue has healed. Sometimes blood clots form in the wrong location, such as within a vital blood vessel, and block blood flow to important organs. For example, a blood clot forming in the coronary vessels that feed the muscles of the heart leads to a heart attack. If a clot forms in the lungs or a major vessel in the brain, these lead to pulmonary embolism and stroke (images courtesy Medicinenet.com, Hariton & D'Angelo, and Seniorark.com). Together, these conditions comprise a class called "thromboembolic disease" which is literally defined as a piece of a blood clot (the thrombus) blocking flow in a vessel (embolism), and represents the leading cause of death in industrialized nations. **[28-30].** Hemostatic clot formation entails thrombin-mediated cleavage of fibrinogen to fibrin. Previous *in vitro* studies have shown that the thrombin concentration present during clot formation dictates the ultimate fibrin structure. In most prior studies of fibrin structure, clotting was initiated by adding thrombin to a solution of fibrinogen; however, clot formation *in vivo* occurs in an environment in which the concentration of free thrombin changes over the reaction course. Elevated fibrinogen levels are associated with increased risk of incident cardiovascular disease (CVD).1,2 Healthy mice infused with unfractionated human fibrinogen and subjected to FeCl3-mediated carotid artery injury have a shortened time to vessel occlusion and increased resistance of thrombi to acute thrombolysis, suggesting elevated fibrinogen independently contributes to thrombosis **[23].**

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