In vitro effects of hypertonic saline solution on whole blood clotting

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Background: hypertonic saline solution is frequently used to in the management of hemorrhagic shock.
Objective: To determine the effects of hypertonic (7%) saline solution on whole blood coagulation (clotting) in vitro.
Patients and methods: Ten healthy volunteers (5 males and 5 females with age range of 28-44 years) with no previous medical history of bleeding tendency and no medical history of drug intake were studied after their consent. Hypertonic (7%) saline was prepared immediately before use. For each patient, 4 ml of venous blood sample was obtained and investigated as follows:
Undiluted 450 µl of whole blood for baseline coagulation tests [prothrmbin Time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT)]. After that, the same above tests were done after dilutions of whole blood (i.e whole blood replacement by hypertonic saline and isotonic saline for each dilution). An Isotonic (0.9%) saline dilution serves as control. Blood volume replacements at 2.5%, 5%, 7.5% and 10% were done and studied.
Results: The baseline coagulation tests (PT, APTT, and TT) were normal for all 10 (100%) of subjects. At 2.5% and 5% of blood volume replacement PT, APTT and TT were slightly prolonged, but showed no statistically significant difference between hypertonic (7%) saline and the control (isotonic saline) while PT, APTT and TT were prolonged in hypertonic (7%) saline with statistically significant difference ($p<0.05$) from isotonic (0.9%) saline at 7.5% and 10% of whole blood volume replacement in 10, (100%) of subjects.
Conclusion: The study showed that hypertonic (7%) saline disturbs coagulation in vitro when it replaces 7.5% or 10% of whole blood volume.
Key words: Hypertonic saline, isotonic saline, blood clot.

الخلاصه

الخلفية :غالبا ما يتم العلاج الاولي للمرضى المصابين بتغير او فقدان الوعي بسبب نزف الدم باستخدام المحلول عالي الملوحة عن طريق الوريد.
الهدف : لمعرفة تاثير المحلول عالي الملوحة على عملية تخثر الدم.
الطرق: تمت دراسة 10 اشخاص (5 من الاناث ؛ 5 من الذكور)متبرعين أصحاء ليس لديهم اي مرض ولا يتناولوا اي نوع من الادوية بعد اخذ الموافقة منهم. تم سحب 4 ملليتر من الدم الوريدي من كل شخص وتم اجراء الفحوصات المختبرية الخاصة بتخثر الدم (زمن البروثرومبين وزمن الثرومبوبلاستين وزمن الثرومبين) لكل شخص قبل وبعد اجراء التخفيفات(تخفيف الدم باضافة المحلول عالي الملوحة مرة ونفس التخفيف باضافة محلول متساوي الملوحة والمقارنة بين نتائج زمن التخثر). تم اجراء تعويض (تخفيف) حجم الدم بالمحلول عالي الملوحة والمحلول متساوي الملوحة بالنسب 2.5%,5%,7.5% ونسبة 10% واجراء اختبارات زمن التخثر.
النتائج: تبين ان تعويض حجم الدم بنسبة 2.5% ونسبة 5% بمحلول عالي الملوحة لا يؤدي الى اضرار بعملية تخثر الدم بينما التعويض بنسبة 7.5% ونسبة 10% يؤدي الى احداث اضرار وعرقلة عملية تخثر الدم بشكل مهم احصائيا لدى جميع الاشخاص.
الاستنتاج: يوجد تاثير مهم احصائيا باحداث عملية عرقلة تخثر الدم عندما يتم تعويض حجم الدم بالمحلول عالي الملوحة بنسبة 7.5% ونسبة 10%.

Introduction

Hypertonic saline solution is recommended for the initial management of patients with hemorrhagic shock and burns[1]. The mechanism of action of hypertonic saline solution is explained by it's ability to increase plasma osmolarity which will lead to subsequent transcellular fluid movement toward plasma[2]. Hypertonic saline solution can be used alone or together with plasma expanders; however, hypertonic saline solution is better than colloids by being cheap, not associated with allergic reactions and free of risk of infection[3]. Several studies showed that hypertonic saline solution is effective for the treatment of cases with increased intracranial pressure due to traumatic cerebral oedema[4]. The major goal of treatment of injured patient with hemorrhagic shock is lifesaving which will not be accomplished only by fluid or blood replacement, but with maintaining sufficient coagulation parameters[5]. Hypertonic (7%) saline is recommended for the treatment of hemorrhagic shock with generally small volumes (4 ml/kg body weight) since larger volumes will lead to marked dilutional effects on platelets and coagulation factors[6]. Following the use of small volumes of hypertonic saline hydrxyethylstarch for resuscitation of cases of porcine hemorrhagic shock, only small effects on coagulation system were observed[7]. Hypertonic (7%) saline solution is considered mucoactive agent and can be used to hydrate mucus or thick secretions for expectoration while hypertonic (3%) saline solution is used in the treatment of acutely raised intracranial pressure or severe hyponatremia[8].Inhalation of hypertonic saline solution is of benefit for children with brochiolitis[9] and recommended for treatment of cystic fibrosis[10]. Hypertonic (23.4%) saline solution is shown to be safe and effective treatment of elevated intracranial pressure in patients after traumatic brain injury[11].

Patients and methods

Ten healthy volunteers (5 males and 5 females with age range of 28-44 yeays) with no previous medical history of bleeding tendency and no medical history of drug intake were studied after their consent. The study was done in a private legal fully equipped specialized medical laboratory for hematological investigations (Iraq, Karbala, Al Mualimeen Sector, Zaid Ibn Ali laboratory). Hypertonic (7%) saline was prepared immediately before use by readily available 20% hypertonic ampoule (Braun medical industries, Malaysia) with water for injection at ratio of 3.5:10 (3.5 ml hypertonic saline with 10 ml water for injection) to obtain hypertonic (7%) saline solution. For each patient, 4 ml of venous blood sample was obtained and divided as follows:

1. Undiluted citrated (i.e with the equivalent sodium citrate, 50 µl at ratio of 1:9) 450 µl of whole blood for baseline coagulation tests [prothrmbin Time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT)]. After that, the same above tests were done after dilutions of whole blood (i.e whole blood replacement) with either hypertonic (7%) saline or isotonic (0.9%) saline solution as a control.
2. Diluted citrated (i.e with the equivalent sodium citrate, 48.75 µl at ratio of 1:9) whole blood 438.750 µl + 11.250 µl (2.5% of blood volume replacement) hypertonic (7%) saline solution.
3. Diluted citrated (i.e with the equivalent sodium citrate 48.75 µl at ratio of 1:9) whole blood 438.750 µl + 11.250 µl (2.5% of blood volume replacement) isotonic (0.9%) saline solution.
4. Diluted citrated (i.e with the equivalent sodium citrate 47.5 µl at ratio of 1:9) whole blood 427.500 µl + 22.500 µl (5% of blood volume replacement) hypertonic (7%) saline solution.
5. Diluted citrated (i.e with the equivalent sodium citrate 47.5 µl at ratio of 1:9) whole blood 427.500 µl + 22.500 µl (5%

of blood volume replacement) isotonic (0.9%) saline solution.
6. Diluted citrated (i.e with the equivalent sodium citrate 46.250 µl at ratio of 1:9) whole blood 416.250 µl + 33.750 µl (7.5% of blood volume replacement) hypertonic (7%) saline solution.
7. Diluted citrated (i.e with the equivalent sodium citrate 46.250 µl at ratio of 1:9) whole blood 416.250 µl + 33.750 µl (7.5% of blood volume replacement) isotonic (0.9%) saline solution.
8. Diluted citrated (i.e with the equivalent sodium citrate 45 µl at ratio of 1:9) whole blood 405 µl + 45 µl (10% of blood volume replacement) hypertonic (7%) saline
9. Diluted citrated (i.e with the equivalent sodium citrate 45 µl at ratio of 1:9) whole blood 405 µl + 45 µl (10% of blood volume replacement) isotonic (0.9%) saline solution.
statistical analysis was based on social science version 10.0 (SPSS, Chicago, IL, USA). Results were analysed using the general linear model to detect intergroup difference. Data were displayed as mean and standard deviation. Statistical significance was determined as $P<0.05$.

Results

The baseline coagulation tests (PT, APTT, and TT) were normal for all 10 (100%) of persons. As shown in table 1 and 2: at 2.5% and 5% of blood volume replacement PT, APTT and TT were slightly prolonged, but showed no statistically significant difference between hypertonic (7%) saline and the control, isotonic (0.9%) saline while PT, APTT and TT were prolonged in hypertonic (7%) saline with statistically significant difference ($p<0.05$) from isotonic (0.9%) saline at 7.5% and 10% of whole blood volume replacement in 10, (100%) of persons.

Table 1. Results of PT, APTT and TT at 2.5% and 5% of blood volume replacement mean (second) ±SD

Test	2.5% replacement		5% replacement	
	hypertonic saline	isotonic saline	hypertonic saline	isotonic saline
PT*	1 ± 14	1±13	2±15	2±13
APTT*	1±34	2±33	3±36	3±33
TT*	2±18	1±18	1±21	1±19

N.B: *= ns, **= P<0.05

Table 2. Results of PT, APTT and TT at 7.5% and 10% of blood volume replacement mean (second) ±SD

Test	7.5% replacement		10% replacement	
	hypertonic saline	isotonic saline	hypertonic saline	isotonic saline
PT**	2 ± 27	1±15	2±28	1±15
APTT**	3±57	3±34	3±58	2±35
TT**	2±28	2±19	2±28	2±19

N.B: *= ns, **= P<0.05

Discussion

The study showed that hypertonic (7%) saline affects whole blood coagulation in vitro by causing prolongation of PT, APTT and TT with statistically significant effects ($p <0.05$) at 7.5% and 10% of whole blood replacement by hypertonic (7%) saline while there was no effects from similar isotonic (0.9%) saline dilutions. This finding agrees with Reed

RL, Johnston TD, et al study that showed that there is significant deterioration in clotting tests in vitro when 10% of normal plasma was replaced by hypertonic (7.5%) saline[6]. Similar studies were done in Singapore by T. S. Tan, K. H. S. Tan and H. P. Ng which showed that hypertonic (7%) saline when given at volumes between 7.5% and 10% of whole blood will delay clot formation; however, the quality of final clot is not affected[12]. A dose of 4 ml/kg of hypertonic (7%) saline for resuscitation will result in about 6% blood volume replacement in patients with normal blood volume while in patients with hemorrhagic shock a similar dose may result in greater dilution of blood volume with more pronounced coagulopathy. Hypertonic saline exerts it's effects by causing an osmotic load (hyperosmolarity) leading to significant transcellular fluid movement toward plasma. For every milliliter of hypertonic saline infusion about 7 ml of free water mover toward intravascular compartment after equilibrium[6]. Other potential problems associated with hypertonic saline are central pontine myelinolysis, convulsions, subdural hematoma and rebound malignant cerebral oedema[13]. Other possible complications include hypotension, metabolic acidosis, hypokalemia and congestive heart failure[14]. In vitro studies on coagulation are generally limited because complex hemostasis pathways cannot be exactly simulated in a complete natural way. Patients presented with trauma frequently develop alterations in coagulation sysyem. The potential exacerbation of coagulopathy with hypertonic saline in such patients needs to be studied further in future clinical trials. Moreover, hypertonic saline causes increase in cardiac output and regional blood flow and may increase hemorrhage and increase mortality in patients with uncontrolled hemorrhage.

Conclusion

The study showed that hypertonic (7%) saline disturbs coagulation in vitro when it replaces 7.5% or 10% of whole blood volume. So that a careful management of patients who need much fluid replacement should include follow up for the whole blood coagulation state.

References

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